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Research Article

Antiradical Activity of Flavonoids from the Fruits of *Crataegus meyeri* Pojark

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Abstract

Hawthorn is an important edible plant belonging to the *Rosaceae* family and has a wide range of uses in traditional medicine. In the 19th century, tea made from hawthorn flowers and leaves began to be used as a blood purifier, and since the early 20th century, hawthorn fruits and flowers have been recommended as a remedy for heart and vascular diseases. The biological properties, medical and traditional uses of the *Crataegus meyeri* Pojark. species selected as the research object were investigated, and the antiradical activity of flavonoids from the fruits against the free radical DPPH was investigated. The relationship between the structure of flavonoids and their radical activity was determined. The results obtained show that the radical neutralizing effect of flavonoids directly depends on the substitution feature of free hydroxyl groups in the flavonoid structure. It was revealed that the presence of 3-OH and 3,4-dihydroxy groups in the flavonoid molecule is decisive for the antiradical activity.

Keywords: *Crataegus meyeri*, environment, DPPH, food reserves, medical use

Introduction

Edible and medicinal plants, which hold a significant place in human nutrition and have been used in the treatment of diseases since ancient times, have become an important field of research. These plants are currently receiving increased research due to their low side effect profile and broad biological effects (Birinci et al., 2025). The study of the environment and its relationship with humans allows people to realize their potential, because a person's life and health largely depend on their attitude towards nature. Acquiring knowledge about medicinal plants is the basis for their proper use; on the other hand, one of the issues underlying the concept of ecological education is the concept of balanced nutrition, because only an ecologically literate person can lead a healthy lifestyle. In this sense, the study and use of medicinal plants that are part of the flora, the investigation of the possibilities of using local medicinal plant sources, and the identification of their reserves are extremely relevant in the modern era (Bahri-Sahloul et al., 2009).

The flora of the Nakhchivan Autonomous Republic is highly represented by a sufficiently diverse range of medicinal, wild vegetable, nectar-pollen, fodder, essential oil, dye, vaccine, glycoside, and other economically valuable plant species. Among such economically important plants, medicinal plants with high therapeutic properties are of great importance. These plants have been used in both scientific and folk medicine from ancient times to the present day, some of them are widely used in scientific medicine for the production of various herbal preparations (Rahimova et al., 2024).

Among the medicinal plants widely distributed in the flora of the autonomous republic, species belonging to the hawthorn genus (*Crataegus* L.) are of particular importance for their medicinal uses. Studies have revealed that this plant offers diverse physiological and pharmacological effects thanks to its various bioactive natural compounds. This genus includes 20 species in the region, most of which have been widely used in folk medicine for many years (Ibrahimov 2021). These plant species include *Crataegus momogyna* Jacg. , C.*orientalis* Pall. Ex Bieb., C.*meyeri* Pojark, C.*pentagyna* Waldst.&Kit., C.*pontica* C.Koch and others (et al., 2018). Among these medicinal plants, the study of the bioecological characteristics, distribution area, phytocenological characteristics, and application areas of the C. *meyeri* Pojark species, which is widespread in the region, will contribute to expanding the possibilities of using this medicinal plant and its recognition as a medicinal plant (Karagül, 2024). Leaves of the most species of *Crataegus* have two leafy bracts; their stalks meet the twig. Leaves are 15 mm-5 cm long, glabrous, broad-ovate or obovate, and have toothed margins with three to seven lobes. The flowers grow in clusters of 5-12 with colour ranges from white to pink, pink to red. They contain both male and female sexes and are mostly fertilized by insects, which are attracted by the perfume released by the flowers (Ibrahimov et al., 2018).

One of the urgent tasks of modern science is the search for raw materials of biologically active substances (BAS) and the development of methods for their complex processing in order to create effective drugs and therapeutic agents. In this regard, special attention is drawn to genera of plants containing numerous species, some representatives of which are used in medical practice. Such genera of plants include hawthorn (*Crataegus* L.), whose representatives are rich in various groups of BAS (Long et al., 2006).

The problem of screening the antioxidant activity of natural compounds and assessing their effectiveness is a pressing task for identifying the mechanisms of their biological action and for creating drugs based on them. One of the methodological approaches within the framework of this task is the study of antiradical activity (ARA) as one of the components of the antioxidant activity of potential antioxidants. Flavonoids are a large class of low-molecular polyatomic phenols of plant origin. Research into their structure, physicochemical properties, functions in plants, and biological activity concerning other organisms began in the 1930s (Amanzadeh et al., 2007).

Material and methods

The aim of the work was to study the antiradical and antioxidant activity of flavonoids obtained from the fruits of *Crataegus meyeri* Pojark species common in the territory of the Nakhchivan Autonomous Republic, to determine the dependence of their antiradical activity on the chemical structure of flavonoids.

Extraction

For the extraction of the fruit, 50 g of fresh fruit was crushed in 250 ml of 60% ethanol, kept in an ultrasonic bath for 2 hours, and then macerated in a blender for 24 hours. After maceration, this mixture was first filtered through a fine cheesecloth and then filtered through Whatman filter paper. The resulting filtrate was stored in aliquots in the deep freezer for further studies. After drying, the leaves were powdered, and then similarly extracted in 60% ethanol, first in an ultrasonic bath and then by maceration. The ethanolic filtrate was saved for study (Birinci et al., 2025).

DPPH radical scavenging activity

The antiradical activity of flavonoids was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. An ethanol solution of the DPPH free radical in the spectral region at 517 nm is characterized by an intense peak. Free radical scavenging activity of DPPH was measured using a spectrophotometric assay. The result was calculated as SC50, defined as the value of the sample concentration causing a 50% reduction in the concentration of DPPH• radicals, with lower SC50 values indicating higher radical scavenging activity. To calculate the SC50 (µg/mL) value, a working curve was prepared with Excel with at least 6 different concentrations of samples. The sample concentration, the SC50 value, which halved the maximum absorbance, was calculated from the graph (Kolaylı et al., 2024). When the unpaired electrons of DPPH combine with electrons and hydrogen separated from phenolic compounds, the absorption at 517 nm decreases. The degree of decolorization of the ethanol solution of DPPH indicates the reducing capacity of flavonoids and thereby determines their antiradical activity (Rahimova et al., 2024). The DPPH neutralizing effect of flavonoids was measured. The final working mixture contained 100 µM DPPH and flavonoid (0-20 µM), both dissolved in ethanol. From a plot of the concentration of neutralized DPPH versus flavonoid concentrations, the number of moles of DPPH scavenged by one flavonoid molecule was determined (Šavikin et al., 2017).

Results and Discussion

Distribution and ecology of species

The genus name *Crataegus*, Rosaceae, is derived from a Greek word kratos, meaning hardness of wood (Akbulut, 2024). The plant's natural range covers the Caucasus, Asia Minor and the northern regions of Iran. It grows well in shrub areas, forests, and forest edges, forest glades, shrubby mountain slopes, in various shrubby plant groups, juniper forests, river banks, valleys and open areas, especially on heavy clay soils. It is well adapted to the harsh continental climate of the Autonomous Republic. It sometimes grows singly or in groups on rocky slopes, at an altitude of 800-2000 m above sea level (Ibrahimov, 2017). Within the forest, the tree-like form, reaching a height of 4-5 m, predominates. Only in shrubby areas are shrub-like forms 1.5-2 m tall found. It acts as an edificator and dominant species in the formation of forests and shrubs. The species' natural distribution area in the Autonomous Republic covers almost the entire middle mountain belt (Ibrahimov et al., 2018).

Figure 1. Distribution of the *Crataegus meyeri* Pojark. species in the Nakhchivan Autonomous Republic

Traditional use

Crataegus species (hawthorn) have been traditionally used since ancient times. Moreover, it has been proposed that its antioxidant constituents account for its beneficial therapeutic effects. A decoction of leaves and unripe fruits from *Crataegus aronia* is used to treat cardiovascular diseases, cancer, diabetes, and sexual weakness in the Arabian traditional medicine system (Kumar et al., 2012).

Hawthorn (*Crataegus*) is a traditional medicinal plant. Consider a "cardiotonic" herb, the hawthorn plant has been used in traditional medicine to treat irregular heartbeat, high blood pressure, chest pain, hardening of the arteries, and congestive heart failure; the antioxidants in hawthorn may help control high blood pressure and high cholesterol (Ekin et al., 2017).

Hawthorn fruits have long been used both in folk medicine and as a healthy food product. Due to its beneficial properties, hawthorn is known as a means of treating and preventing many cardiovascular diseases. The use of hawthorn can also help with other health problems. However, the uncontrolled consumption of the fruits of this plant only worsens the condition of the body (Ebrahimzadeh et al., 2009). The healing properties of hawthorn were successfully used even in ancient China, where doctors used the plant as a means to normalize the digestive system. Today, the fruits of the plant are used in preparations to support the health of many body systems, primarily the cardiovascular system. The antioxidants in hawthorn improve blood flow and repair damaged blood vessels. Therefore, hawthorn-based preparations can be used in cases where a person suffers from arterial hypertension (Bengü et al., 2023).

Chemical composition and use: In the bark of the trunk and branches of hawthorn, vaccinates, and coumarin derivatives, esculin and crategin have been identified. Several preparations are prepared from its fruits and flowers: infusion, tincture, extract, kratezide, etc. and are widely used in functional disorders of the heart, angina, arrhythmia, hypertension, insomnia, cardiac neurosis, as well as as a medicinal plant that regulates heart activity after acute angina and influenza, as well as lowering blood pressure (Yusuf et al., 2016).

Pharmacological activities

Crataegus species possess immense medicinal applications, but a few species have been screened for their biological activities. *Crataegus* may improve coronary artery blood flow and the contractions of the heart muscle, hence used widely in cardiovascular disorders like arrhythmia, myocardial infarction, congestive heart failure (Long et al., 2006). *Crataegus* is a rich source of phytochemical compounds. Molecules such as procyanidins, epicatechin, hyperoside, isoquercitrin, chlorogenic acid, ursolic acid, and oleanolic acid are important bioactive compounds that enhance the plant's biological activity (Karagül, 2024).

Crataegus extract appeared to be capable of inducing rhythmicity in quiescent cardiomyocytes and showed antiarrhythmic activity. The commercial hawthorn preparations also exhibit similar chronotropic activities. Its extract also showed negative chronotropic effects and does not cause β-adrenergic receptor blockade (Long et al., 2006). The study evaluated in vitro antioxidant activities of C. *meyeri* Pojark leaves ethanolic and aqueous extract, in terms of total antioxidant activity, DPPH radical scavenging activity, total phenolic and flavonoid vitamins and trace elements contents. The contents of copper, zinc, manganese, selenium, chromium and Cobalt in C. *meyeri* leaves were found 21,99 ± 2,01, 47,32 ± 2,44, 51,01 ± 2,26, 0,091 ± 0,01, 1,72 ± 0,09 and 1,18 ± 0,05 µg g-1, the contents of α-tocopherol, retinol and ascorbic acid in C. *meyeri* leaves were found 2,55 ± 0,19, 0,41±0,024 and 202,05 ± 12,46 µg g-1 respectively on dry weight basis (Ekin et al., 2017).

Antiviral activity

Previous studies have shown that the polyphenolic profile of other hawthorn fruits and leaves were different and depended on the growth stage. Few reports indicated that the flavonoids were the major markers for the interspecific distinction between *Crataegus* species (Šavikin et al., 2017). Antiviral activity was investigated on leaves and berries of three Crataegus species, including *C. aronia* var. aronia, *C. monogyna* as well and *C. pseudoheterophylla*, and was also evaluated for flavonoid amount and total proanthocyanidin content. Results revealed that the extracts containing these active constituents have been verified to be highly effective against Herpes simplex virus (Orhan et al., 2007).

Flavonoids of natural origin have been used for many years, with increasing attention from researchers. This is largely due to their value for medicine, as sources of capillary-strengthening, anti-inflammatory, choleretic, anti-sclerotic, anti-tumor, and other drugs. In recent years, much attention has been paid to their antioxidant properties. In this regard, it is relevant to search for plant sources for obtaining flavonoids and studying their antiradical and antioxidant activity (Arslan et al., 2011).

Effective and low-toxic natural substances with antioxidant activity are widely used to solve many technological problems and improve the quality of products in the food, cosmetic and pharmaceutical industries (Ebrahimzadeh et al., 2009).

A large raw material base, insufficient study of the chemical composition of a number of representatives of this group of plants, creates prerequisites for their in-depth study as sources of biologically active substances, as promising plants for introduction into the official nomenclature. Flavonoids are natural compounds that are various derivatives of benzo-γ-pyrone (chromone). The basic structure of flavonoids is the flavan core, which consists of 15 carbon atoms (C6 -C3- C6), consisting of two benzene rings (A and B), connected by a pyrone heterocycle (Ekin, et al., 2017).

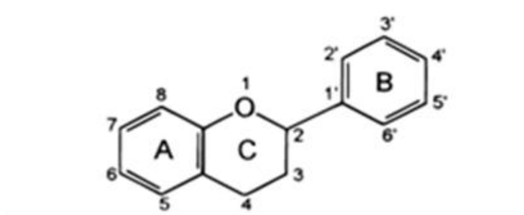

Figure 2. Basic structure of flavonoids

The antiradical activity of flavonoids against the free radical DPPH was studied in the concentration range of 0-20 mM flavonoids by monitoring the absorbance of the solution at 517 nm. The results showing the dependence of DPPH radical-scavenging activity on flavonoid concentration are presented in Fig. 1. The following flavonoids isolated from hawthorn fruits were examined: quercetin (3,5,7,3′,4′-OH), kaempferol (3,5,7,4′-OH), luteolin (5,7,3′,4′-OH), rutin (3-rut., 5,7,3′,4′-OH), catechin (3,5,7,3′,4′-OH), and apigenin (5,7,4′-OH), as well as the commercial compounds flavone, naringenin, and naringin (Ekin et al., 2017).

Flavonoids, except for flavone, apigenin, naringin, and naringenin, exhibit high antiradical activity. Flavonoids that do not contain a hydroxyl group (flavone), one or two hydroxyl groups (5,7-dihydroxyflavone), flavanones containing 5,7-dihydroxy groups in the A ring, but not containing a C2-C3 double bond in the C ring (naringin, naringenin), and a hydroxyl group at position C-3 do not exhibit a neutralizing effect on free radicals. However, all flavonols with a hydroxyl group at C-3 are very effective antioxidants, also characterized by a high neutralizing capacity for DPPH radicals (Birinci et al., 2025).

Table 1. Flavonoids and their free radical inhibitory effect - DPPH•

Class	Compounds	Substituents	C2 –C3 Multiple connection	Number of moles of neutralized DPPH, moles flavonoid
Flavon	Flavon	-	+	0
	Apigenin	5,7, 4’-OH	+	0
	luteolin	5,7,3’,4’-OH	+	2.7
Flavonol	Quercetin	3,5,7,3’,4’-OH	+	3.0 ± 0.1
	Kaempferol	3,5,7,4’-OH	+	2.2 ±
	Rutin	3-rut.,5,7,3’,4’.-OH	+	2.0 ±
	Hyperozide	3-gal.,5,7,3’,4’.-OH	+	2.1±
Flavanol	Catechin	3,5,7,3’,4’-OH	-	1.8±
Flavanone	Naringenin	5,7,4’-OH	-	0
	Naringin	7-ramn., 5,7-OH	-	0

Strong antiradical activity is exhibited by flavonoids having an o-dihydroxy group in the B ring (dihydroflavonols, flavonol-3-O glycosides, and flavones). Their high activity is associated with the strong effect of C-3’ on the activity of the C-4’ hydroxyl. In the molecules of all the studied flavonoids, there is a hydroxyl group at C-4’. The results show that the presence of this hydroxyl group is essential for the manifestation of the antiradical activity of these flavonoid groups.

The presence of the 3-OH group in the C ring is very beneficial for the antioxidant activities of flavonoids. Comparison of the antiradical activity of quercetin (3,5,7,3’,4’-OH) with the values of luteolin (5,7,3’,4’-OH), rutin (3-rut.,5,7,3’,4’.-OH) and hyperoside (3-gal., 5,7,3’,4’) shows that the presence of the 3-OH group in the quercetin molecule significantly increases the antiradical activity.

The luteolin molecule does not have a hydroxyl group at the C-3 position, whereas the glycosides of quercetin, rutin, and hyperoside block the chemically active hydroxyl group (C-3–OH) as a result of glycosylation. The presence of a double bond between the C2 and C3 atoms in the C ring is not a prerequisite for antiradical activity. On the other hand, the presence of a C2–C3 unsaturated bond conjugated with a 4-oxo group is not a determining structural element for DPPH neutralization.

The C2–C3 double bond conjugated with a 4-keto group, which facilitates the delocalization of electrons from the B ring, enhances the radical-inhibitory activity (11–18). However, the absence of this unsaturation is sometimes associated with a decrease in antioxidant potential. Based on these results, the most effective antiradical agents appear to be flavonoids possessing both a 3′,4′-dihydroxy substitution on the B ring and a hydroxyl group at the C-3 position. The presence of an o-dihydroxy structure on the B ring provides greater stability to flavonoid phenoxy radicals through electron delocalization, which is a key feature of antiradical potential (11). Interestingly, the existence of a C2–C3 double bond in the C ring does not seem to be essential for antiradical activity.

This is illustrated by the fact that quercetin and catechin possess the same number and positions of hydroxyl groups, yet catechin lacks both the C2–C3 unsaturated bond and the 4-oxo group. Nevertheless, the C2–C3 double bond conjugated with a 4-keto group, which promotes electron delocalization from the B ring, generally increases radical-scavenging activity.

Determining the antiradical activity (ARA) of medicinal preparations is essential for understanding the mechanisms of action of natural medicinal products (MPs). The therapeutic effects of preparations derived from plant raw materials are determined by the collective presence of bioactive compounds such as flavonoids, saponins, terpenoids, vitamins, and others.

Conclusion

Flavones lacking hydroxyl (–OH) groups in their molecules, as well as flavanones without a C2–C3 unsaturated bond (such as naringin and naringenin), do not exhibit significant antiradical activity. The obtained results indicate that the most effective flavonoids in terms of antiradical properties are those containing a 3′,4′-dihydroxy substitution on the B ring and a hydroxyl group at the C-3 position. The presence of an o-dihydroxy (catechol) group on the B ring confers a high degree of stability to the phenoxyl radicals formed in flavonoids through electron delocalization and represents an essential structural feature of their antiradical potential. Conversely, the absence of both the o-dihydroxy group on the B ring and the hydroxyl group at the C-3 position significantly reduces antioxidant efficiency. Determining the antiradical activity of flavonoids is therefore crucial for understanding the mechanisms of action of natural medicinal products and for assessing the therapeutic potential of plant-derived compounds.

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