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Comparative Study of Flavonoids in Licorice Fibers Growing in Various Soil Conditions

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Abstract
The content of quercetin in the fibers of licorice plant growing in different regions has been investigated by the method of high-performance liquid chromatography (HPLC). The soil composition of the territories and the amount of Cl- and SO42- ions were determined using a spectrophotometer of the DR-3900 brand (Germany). Using the example of quercetin, it was found that the amount of flavonoids in plant tissues changes under the influence of external factors. As a result, the increased content of phenolic compounds in the plant under the influence of various environmental factors of the plant organism performs the function of protecting the plant from environmental factors.

Keywords: flavonoid, HPLC, licorice, sulfate and chloride ion, quercetin, soil.

Introduction

Phenol is one of the largest groups of physiologically active substances in nature, currently more than 9,000 of their species are known [1]. According to the composition, benzene-ring phenol has one or more hydroxyl groups, which are found in plant tissues mainly in the form of compounds, glycosides.

Phenol derivatives in plants are classified into two groups: derivatives of oxybenzoic acid (N-oxybenzoic, protocatechin, vanillin, Gallium and cyrene), derivatives of oxycoric acid (N-Kumar, caffeine, ferule and synapse). In addition to simple phenolic compounds, plants contain their derivatives of complex structure. Derivatives of phenolic compounds of complex structure are found in plants in the form of monomers and polymers, as well as carbohydrates. Monomeric phenolic compounds are divided into 3 main groups. The first group includes phenolic compounds with the structure C_6-C_1, the second-phenolic compounds with the structure C_6-C_3, the third-phenolic compounds with the structure C_6-C_3-C_6.

Phenolic compounds are one of the actively studied classes of substances that protect plants. In addition to the fact that many of these substances are of purely practical importance, the study of the dynamics of the content of these substances is also of great scientific interest [1-2].

Phenolic compounds have many biochemical properties, but the most characteristic property of almost every group of flavonoids is that they have antioxidant properties. The antioxidant activity of flavonoids depends on the location of functional groups in the nuclear structure. The configuration, substitution, and total number of hydroxyl groups significantly affect several mechanisms of antioxidant activity, such as radical removal and chelation of metal ions [3-5].

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The complex mechanisms of regulation by external conditions of biosynthesis of certain classes of phenolic compounds have not been sufficiently studied. This often leads to a lack of understanding. In general, why does a particular plant at this stage of its development synthesize exactly such a qualitative and quantitative composition of biologically active substances in various natural and climatic conditions. As a result, it is almost impossible to predict the situation with the content of substances accumulated by plants in response to changes in external conditions in the natural area [4-6]. This requires a deeper study of the amount of biologically active substances in plants.

The most common group of phenolic compounds are flavonoids, which are of great practical importance for medicine [3-6], and the importance of flavonoids in plant life is being actively studied. Their importance in protecting plants from various biogenic and abiogenic stresses has been established [3-7], in allelopathic relationships [3-8]. In addition, the multifaceted participation of flavonoids in the formation of generative organs of plants and in the process of pollination of plants has been well studied [5-9]. The antioxidant role of flavonoids is great, and often under the influence of external stressors, the amount of their formation increases. Their antioxidant properties ensure stable growth and development. Similar properties of flavonoids have been well studied in both animals and human organisms [7-10].

Flavonoids play an important role in the metabolism occurring in plant cells. The biological role of flavonoids lies in their participation in redox processes in plants. Flavonoids protect plants from the effects of stressors. Flavonoids act as filters in plants, protecting tissues from the harmful effects of ultraviolet rays and other similar biotic (pathogens) and abiotic factors [11]. Flavonoids, plant pigments, give flowers a bright color and attract these insects [12].

The research of the content of flavonoids formed in plants not only allows us to determine important information about the original place of origin of the species and the ways of its distribution, but also allows us to determine the degree of resistance to stressors [7,13,14]. Accordingly, in this work, fragmentary studies were carried out with the determination of flavonoids in licorice tissues growing in various soil conditions.

MATERIALS AND METHODS OF RESEARCH

The main object of the study was a licorice (*Glycyrrhiza glabra L.*) leaf growing in various soil conditions of Uzbekistan.

Appropriate methods were used for the qualitative and quantitative determination of flavonoids contained in licorice leaves [14-15].

We used 96% ethyl alcohol to extract quercetin from the leaves of the plant. To do this, the dry mass of the licorice plant was crushed to a size of 0.1 cm and extracted at a temperature of 20 ° C for 75 minutes, mixing licorice leaf and alcohol in proportions of 1:10, 1:20 and 1:30. As a stationary phase, we used HPLC to determine the amount of quercetin in samples using Shim-Pack GIST-HP C18 150x4,6 mm 3 μm C_{18} sealant (Shimazu, Japan). To do this, solutions of different concentrations were prepared and calibrated from 0.5% acetic acid solution in a ratio of 35:65 and standard solutions in acetonitrile (quercetin Q4951-10G, >95% HPLC, Sigma Aldrich): 1 mg/ml 1.25 mg/ml and 2.5 mg/ml, respectively. Based on standard quercetin samples, optimal

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conditions were determined in 9.32 minutes and the result was obtained on a HPLC device (LC 2030 C3D Plus Shimadzu Japan) [16].

As we studied quercetin levels in G.glabra samples grown under various soil conditions, we also studied the composition of the soil from which the plant samples were taken. For weighing soil samples during their analysis, technical scales Europe GmbH Model: SKX6201 (OHAUS), Max 6200 grams are used. First, in order to determine the soil moisture, 100 grams of soil are taken, dried in a drying oven at 106 degrees and its humidity is determined. It is assumed that the influence of moisture content on the result is taken into account [16-19].

For analysis using a spectrophotometer, a 10:1 ratio is taken, i.e. 100 g of soil and 990 ml of distilled water and a solution is prepared.

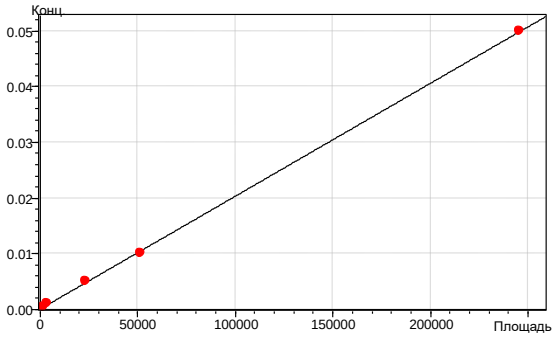
The finished solution in a certain amount was taken from the water suction and through the necessary test cuvettes, a qualitative and quantitative chemical analysis of the suction composition was carried out on a spectrophotometer of the Dr-3900 brand (Germany).

The percentage of quercetin (C_x) in dry raw materials – licorice leaf - was calculated using the formula.

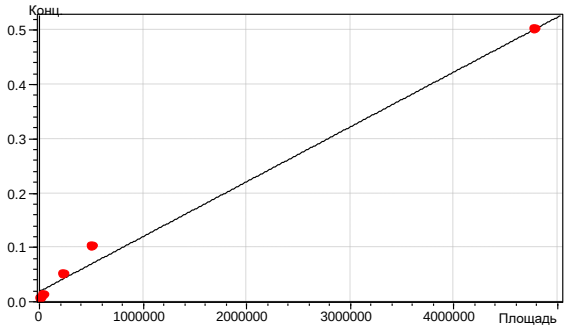
$$C_x = \frac{C_{ст} \cdot S_1 \cdot V_1 \cdot V_2 \cdot 100}{S_2 \cdot M \cdot (100–B)}$$

in this case, C_{st} is the concentration of the corresponding standard flavonoid solution, mcg/ml; S_1 is quercetin in the sample, F.R.P.; S_2 is the standard flavonoid, f.r.p.; V_1 is the volume of eluate after washing the flavonoid from the concentrator cartridge, ml; V_2 is the total volume of the extract, ml; M - the mass of the sample, mg; B is the moisture content of the raw material, %.

After selecting chromatographic conditions for quantitative analysis, calibration graphs were constructed using the LCSolution program (Fig.1-2). Calibration graphs reflect the dependence of the chromatographic indicator area on the amount of substance injected into the chromatograph injector. 3 µl of standard quercetin concentration solutions 0,0005; 0,001; 0,005; 0,01; 0,05; 0,1 and 0.5 mg/ml.



1-rasm - Calibration curve of the quercetin concentration range $5 \cdot 10^{-4}$ -$1 \cdot 10^{-3}$ mg/ml



2-rasm - Calibration curve of the quercetin concentration range $1 \cdot 10^{-3}$ -$5 \cdot 10^{-1}$ mg/ml

The calibration dependences and detection coefficients of flavonoids are shown in Table 1.

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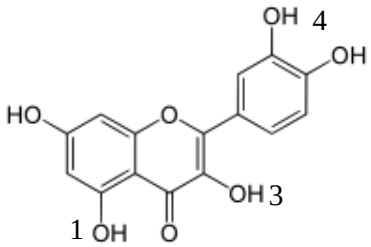
Table 1 Calibration dependencies and detection coefficients.

flavonoid	Concentration range	Calibration dependence	Detection coefficient
Quercetin	$5 \cdot 10^{-4}$-$1 \cdot 10^{-3}$ mg/ml	$y = 2{,}02 * 10^{-7} x + 1{,}09^{-4}$	0,999
	$1 \cdot 10^{-3}$-$5 \cdot 10^{-1}$ mg/ml	$y = 1{,}01 * 10^{-7} + 1{,}90 * 10^{-2}$	0,996

Calibration graphs are based on low concentrations, and the other on high concentrations, because the content of flavonoids in licorice leaf is not the same.
Statistical data was processed using the MS Excel software package.

RESEARCH RESULTS AND ANALYSIS

The accumulation of flavonoids in plants, the most numerous class of natural biologically active phenolic compounds, is significantly influenced by external factors. Flavonoids are involved in plant pigmentation, have antimutagenic activity, protect plants from bacterial, viral and fungal infections, parasite penetration and insect damage, and play the role of inducers (signaling substances) when plants come into contact with microorganisms. They exhibit antioxidant activity, the ability to neutralize hydroxyl radicals and protect cell membranes from oxidative effects. Flavonoid compounds are present in almost all plants, but their distribution varies. Legumes, asters, buckwheat, astrakhan, birch and plants of the Rosaceae families are the most rich in flavonoids [20]. Licorice (Glycyrrhiza glabra L.) belongs to the representatives of the legume family. It is a common perennial plant that grows on almost most continents of the Earth. Flavonoids, including quercetin, are of particular importance in assessing the dependence of licorice on the ecological situation in the place of its growth in terms of quantity in vegetative organs.
Quercetin $C_{15}H_{10}O_7$



That is why it is so important to look for basic methods of detecting, extracting and concentrating quercetin in plant objects. The most popular method for this is high-performance liquid chromatography, which allows determining the content of various flavonoids [16].
The SPE method was used to extract quercetin and purify the sample from matrix components, as well as for preparation and retention. At the next stage of the work, the sorption conditions were selected that fully meet the tasks set.

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Since quercetin is a polar organic compound, they are retained in a hydrophobic sorbent containing complex alkyl groups. Highly polar compounds (organic acids, etc.) pass through the sorption cartridge. The experiment was carried out on sorbents with alkyl groups C_1, C_8 and C_{18}. When sorbents containing C_1 and C_8 in the SPE of oil cake extract are used, quercetin is not sorbed in the sorption cartridge. When using the C_{18} sorption cartridge, all flavonoids are completely absorbed, interfering impurities are not preserved, which made it possible to obtain good high-resolution chromatograms.

During the research, we used its standard sample to identify and compare quercetin in licorice leaf extract (Table 2, Figure 3):

Table 2 Chromatographic indicator for standard quercetin

Mobile phase	buffer in the ratio 35:65:Acetonitrile
Stationary phase	Shim-pack GIST-HP C18 150x4,6 mm 3 μm C_{18} (Shimadzu, Japan)
Wavelength	254 nm
Time of the analysis	11 minut
Flow rate	1 ml/min
Sample size	10 mkl
Temperature	40°C

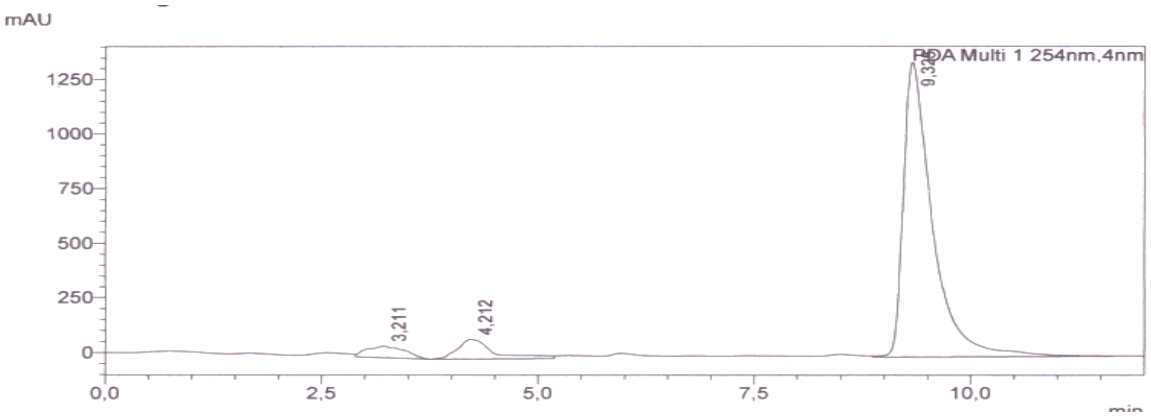

Figure 3. HPLC indicator of the standard sample of quecetin

Methanol is chosen as an eluent, since flavonoids dissolve well in it (Table.1) and have a high elution capacity.

First, the sorption cartridge is cooled with 4 cubic cm of methanol and 3 cubic cm of distilled water. The preparation of the juice sample taken in accordance with paragraph 2.6 was carried out as follows:

After cooling, the extract solution (the first fraction) was passed through the cartridge. Then 2 cubic cm of distilled water is washed (the second fraction). The flavonoid was eluted with 2 cubic cm methanol (the third fraction), and then 1 cubic cm 10:90 L% isopropanol solution: methanol, which has a higher elution capacity than methanol (fourth fraction). At the end of extraction, the cartridge is washed with 4 cubic cm of methanol and 3 cubic cm

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of distilled water. Each of the fractions was analyzed on a liquid chromatograph. Before analysis, all samples taken were filtered through a membrane filter with a syringe with a pore size of 0.45 microns.

Quercetin was found only in the third fraction. Chromatograms of the third part of licorice extract are shown in Figures 19 and 20.

As can be seen from the chromatogram, all indicators of the analyzed flavonoids have a good resolution, which allows for its quantitative analysis, and the analysis time under selected conditions is 15 minutes. Chromatographic performance was determined by comparing the storage time of peaks of the standard substance (quercetin) and the readings obtained on the chromatogram of licorice leaf extract. The coincidence of the parameters of standard solutions and the exposure time of the studied peaks of the extract, as well as the absorption spectra of standard quercetin, allows us to conclude that the compound is identical.

Thus, the use of SPE makes it possible to remove impurities in the sample and concentrate analytes in the eluate.

In the dry mass of licorice leaf grown under various soil conditions, the content of quercetin in relation to the solvent was determined (Tables 3-4, Figures 4-5).

Table 3 Chromatographic parameters of quercetin in licorice leaf samples growing in different areas

Parameters	Bayaut	Gulistan
Time of emergence	9,30 min	9,32 min
Cho‘qqi maydoni	52629	179649
Intensity	702066	2404809
concentration	0.022	0.076

Table 4 Dependence of the amount of quercetin in licorice leaf samples growing in different regions on the ratio of solvents

Sample: solvent	1:10 %	1:20 %	1:30 %
Gulistan	0.006	0.0144%	0.0055%
Bayaut	0.00175%	0.0042%	0.0016%

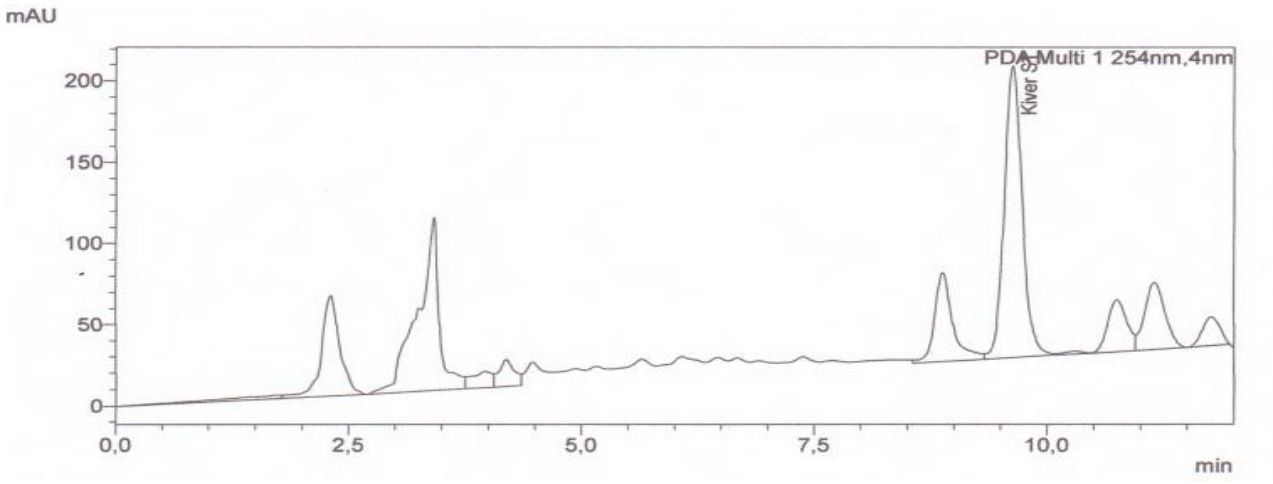

Figure 4. Quercetin indicator on a leaf of a plant sample from the territory of Gulistan city

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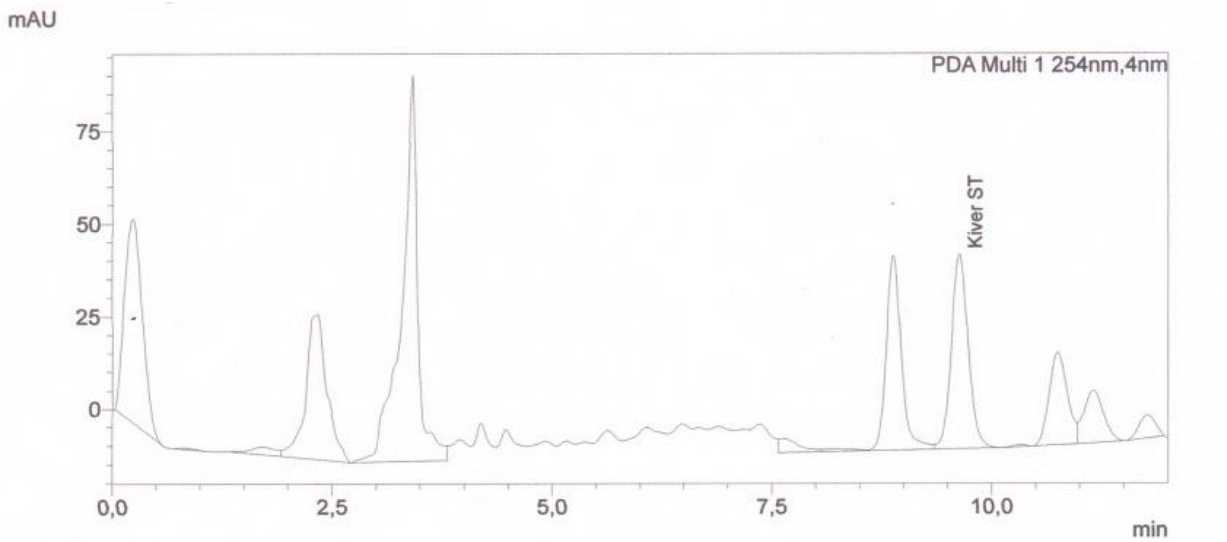
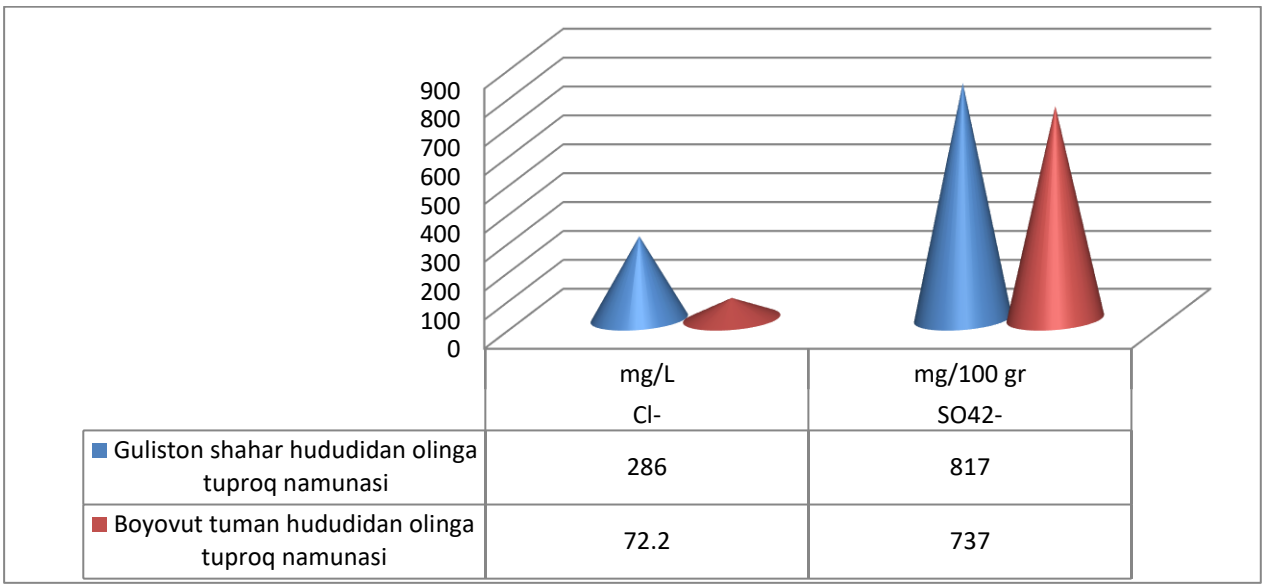


Figure 5. Quercetin indicator on a leaf of a plant sample from the territory of Bayaut district

Based on the studies conducted and the results obtained, it can be noted that the quantitative value of quercetin may be different in hanging plants under different soil conditions. Accordingly, during the research, soil samples were taken from the Syrdarya region from the Bayovut and Gulistan regions, where licorice samples were taken, we analyzed the content of Cl^- and SO_4^{2-} ions in the soil (Fig.6).



	mg/L Cl-	mg/100 gr SO42-
Guliston shahar hududidan olinga tuproq namunasi	286	817
Boyovut tuman hududidan olinga tuproq namunasi	72.2	737

Figure 6. Indicator of salinity in terms of the content of Cl^- and SO_4^{2-} ions in the soil from which licorice samples were taken in Gulistan city district and Bayaut district

From the indicators shown in the figure, it is known that the soils of the city of Gulistan and the territory of the Bayaut district, where licorice samples were taken, differ in salinity in terms of Cl^- and SO_4^{2-} ions. This affects the growth and development of plants.
Based on the data in the above table and the indications in the figure, it can be noted that

studies using RP HPLC have shown that the quercetin content in licorice leaf extract growing in different soil conditions has a different indicator.

Comparing chromatograms and absorption spectra of quercetin with chromatograms of the extract and absorption spectra of licorice leaf samples, it was found that the amount of flavonoid in licorice leaves growing in different soil conditions differs. This is due to the influence of external factors.

Conclusion

To summarize, we can say that the diversity of soil composition in the regions causes a change in the amount of quercetin in the tissues of licorice plants that grow in natural conditions.because the amount of phenolic compounds in plant tissues also increases when plant tissue is stressed due to various external factors. A change in the amount of plant dry samples relative to the solvent leads to a change in the release of flavanoids.

References

1. Олениченко Н.А., Осипов В.И.,. Загоскина Н.В. Фенольный комплекс листьев озимой пшеницы и его изменение в процессе низкотемпературной адаптации растений //Ж. Физиология растений. – Москва. 2006. - №4. -С. 554-559.
2. Nijveldt R.J., et.al. Flavonoids: a review of probable mechanisms of action and potential applications.- Am J Clin Nutr 2001;74: pp. 418–425.
3. Kelly E.H., Anthony, and Dennis J.B., "Flavonoid antioxidants: chemistry, metabolism and structure R.T.-activity relationships," *Journal of Nutritional Biochemistry*. -vol.13, -№ 10. -pp. 572–584, 2002.View at: Publisher Site | Google Scholar
4. Kushiev H. H., Noble A., Abdullaev I., Toshbekov U. Remediation of Abandoned Saline soils using Glycyrrhiza glabra: A study from the Hunger Steppes of Central Asia. //International Journal of Agricultural Sustainability. -Vol.3, -№ 2, -2005. -P. 112-121.
5. Dixon R.A., Pasinetti G.M. Flavonoids and Isoflavonoids: From Plant Biology to Agriculture and Neuroscience.- lant Physiology, October 2010. -V.154. -P. 453–457.
6. Барабой В.А. Фармакология флавоноидов. /МатериалыVII международного симпозиума по фенольным соединениям, Москва, 19–23 октября2009 г. М.: Изд-во Института физиологии растений им К.А.Тимирязева РАН, 2009. С. 26–27.
7. Полякова Л.В., Ершова Э.А. Изменчивость фенольных соединений у некоторых травянистых и древесных растений от межпопуляционного до внутрииндивидуального (эндогенного) уровня //Химия растительного сырья. 2000. -№1. -С. 121–129.
8. Будкевич Т.А. и др. Фенольный статус и аллелопатическая активность почвы в ризосфере агроценозов многолетних трав различного видового состава и режима использования /Материалы YII международного импо-зиума по фенольным соединениям, Москва, 19-23 октября 2009 г. М. Изд-во Института физиологии растений им. К.А.Тимирязева РАН, 2009. С. 38–39.
9. Schijlen G.W.M., et.al. RNA Interference Silencing of Chalcone Synthase, the First Step in the Flavonoid Biosynthesis Pathway, Leads to Parthenocarpic Tomato Fruits. Plant Physiology, July 2007. -V.144. -P.1520–1530.

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8

10. Mierziak J., Kostyn K. Flavonoids as Important Molecules of Plant Interactions with the Environment. // Molecules. - 2014. - V.19. - P. 16240-16265.
12. Koes R.E., Quattrocchio F., Mol J.N.M.The flavonoid biosynthetic pathway in plants: function and evolution. // BioEssays. - 1994. - V.16. - P. 123-132.
13. L.P.Taylor and E. Grotewold, "Flavonoids as developmental regulators," *Current Opinion in Plant Biology*, -vol. 8, -№3, -pp.317–323, 2005.View at: Publisher Site Google Scholar
13. A. K. Jansen, "Ultraviolet-B radiation effects on plants: induction of morphogenic responses," *Physiologia Plantarum*ю -vol. 116 -№ 3, -pp. 423–429, 2002.View at: Publisher Site | Google Scholar
14. Middleton E.Jr., Kandaswami C., Theoharides T.C. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. // Pharmacol. Rev. - 2000. - V.52. -No.4. -P. 673-751.
15. Корулькин Д.Ю., Абилов Ж.А., Музычкина Р.А., Толстиков Г.А. Природные флавоноиды. Новосибирск: Тео, 2007. - 232 с.
16. Füzfai Z., Molnár-Perl I. Gas chromatographic–mass spectrometric fragmentation study of flavonoids as their trimethylsilyl derivatives: Analysis of flavonoids, sugars, carboxylic and amino acids in model systems and in citrus fruits// J. Chromatogr. A. 2005. V. 1073. № 1–2. – P. 201.
17. Rijke E., Out P., Niessen W. M.A., Ariese F., Gooijer C., Brinkman U.A.Th. Analytical separation and detection methods for flavonoids // J. Chromatogr. A. 2006. V. 1112. -№ 1–2. – P. 31.
18. Pyrzynska K., Biesaga M. Analysis of phenolic acids and flavonoids in honey // Trend. Anal. Chem. 2009. V. 28. № 7. – P. 893
19. Naczk M., Shahidi F. Extraction and analysis of phenolics in food // J. Chromatogr. A. 2004. V. 1054. № 1–2. – P. 95.
18. Robards K. Bioactivity and analysis of biophenols recovered from olive mill waste // J. Chromatogr. A. 2003. V. 1000. № 1–2. – P. 657.
19. Кочетова М.В., Семенистая Е.Н., Ларионов О.Г., Ревина А.А. Определение 65 биологически активных фенолов и полифенолов в различных объектах методами хроматографии // Успехи химии. 2007. Т. 76. № 1. – 88 с.
20. Каухова, И. Е. Особенности экстрагирования биологически активных веществ двухфазной системой экстрагентов при комплексной переработке лекарственного растительного сырья //Растительные ресурсы. 2006. -Т. 42. -Вып. 1. – С. 82-91.
21. Kholboev B. E. Amount of Easily Soluble Salts in Water, Type and Level of Salinity in Irrigated Meadow-Gray Soils of Zomin Cone Spread and Its Effect on Soil Melioration. Texas Journal of Agriculture and Biological Sciences. https://zienjournals.com/index.php/tjabs/about/editorialTeam
22. Kholboyev B.E. Reasons for changes in the soil-air regime as a result of irrigation of crops with mineralized water. *American Journal Of Biomedical Science & Pharmaceutical Innovation*, *4*(01), 71–75. 2024 https://doi.org/10.37547/ajbspi/Volume04Issue01-11.

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8

Volume 2, Issue 2, February 2024
ISSN (E): 2938-3781

23. Холбоев Б.Э. Происхождение засоленных почв и солей устойчивость сельскохозяйственных культур в зависимости от степени. Innovations in Technology and Science Education, 2023

24. Raxmonov, K. Isroilovna. (2023). Comparative analytical results of soils seeded with glycyrrhiza glabra and without a control plant (glycyrrhiza glabra). *Academia Repository*, *4*(11), 17–23. Retrieved from https://academiarepo.org/index.php/1/article/view/260

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