UDK: 615.322:581.192.2 **DOI: 10.56292/SJFSU/vol29_iss3/a140**

DETERMINATION OF THE CONTENT OF FLAVONOIDS IN HAWTHORN

DO‘LANA TARKIBIDAGI FLAVONOIDLAR MIQDORINI ANIQLASH

ОПРЕДЕЛЕНИЕ КОЛИЧЕСТВА ФЛАВОНОИДОВ В БОЯРЫШНИКЕ

Askarov Ibrahimjon Rahmonovich[1], Mominova Guyokhon Alidjonovna[2]

Askarov Ibrahimjon Rahmonovich − professor of the Department of Chemistry of Andijan State University, honored inventor of Uzbekistan,chairman of the Academy of Medicine of Uzbekistan.

Mominova Guyokhon Alidjonovna − Associate Professor of the Department of Biological Chemistry of Andijan State Medical Institute, Doctor of Philosophy in Biological Sciences (PhD).

Annotatsiya

O‘zbekistonda keng tarqalgan sariq do‘lana tarkibidagi flavonoidlarni aniqlash. Namuna tarkibidagi flavonoidlarni suyuqlik xromatografiyasi usuli yordamida aniqlandi. Yangi uzilgan sariq do‘lana tarkibida digidrokversetin, kversetin, rutin aniqlandi. Quritilgan do‘lana tarkibida flavonoidlar miqdori kamaygani aniqlandi

Аннотация

Определить флавоноидов в желтом боярышнике, широко распространенных в Узбекистане. Флавоноиды в образце определяли жидким хроматографическим методом. В свежесобранной желтом боярышнике обнаружены дигидрокверти, кверцетин, рутин. Определили снижения количество флавоноидов в высушенном бояришнике.

Abstract

Determination of flavonoids in yellow hawthorn, common in Uzbekistan. Flavonoids in the sample were determined using liquid chromatography. Dihydroquercetin, quercetin and rutin were found in freshly cut yellow hawthorn. It was found that the amount of flavonoids in dried hawthorn has decreased

Kalit so‘zlar: *Flavonoidlar, Sariq do‘lana, xromatografiya, yurak-qon tomir kasalliklari, kversetin, digidrokversetin, rutin.*

Ключевые слова: *Флавоноиды, боярышник, хроматография, сердечно-сосудистые заболевания, кверцетин, дигидрокверцетин, рутин.*

Key words: *Flavonoids, Hawthorn, chromatography, cardiovascular diseases, quercetin, dihydroquercetin, rutin*.

INTRODUCTION

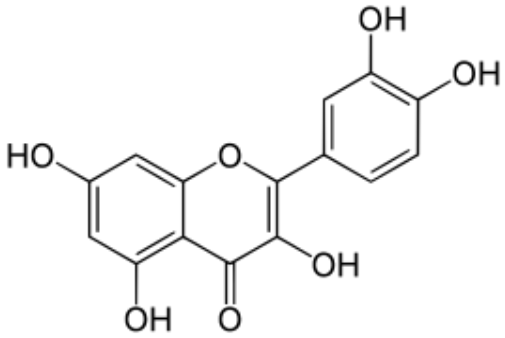
More than 280 species of hawthorn grow all over the world. Hawthorn is an appetizing and digestive aid. Their value is mainly evaluated depending on how many and which types of flavonoids they contain. Hawthorn contains phenolic compounds that provide antioxidant properties. Hawthorn also contains pentacyclic triterpenes, aromatic amines, proanthocyanidins, vitamins and amino acids. Five types of hawthorn grow mainly in Uzbekistan. Among them, Yellow Hawthorn is widespread. We aimed to determine the flavonoids in the Yellow Hawthorn and compare the flavonoids in its fresh and dried state.

LITERATURE ANALYSIS AND METHODS

Hawthorn is used as a medicinal plant all over the world. Hawthorn is used in Europe, China, North America *(Crataegus* spp.) for various heart diseases and hypertension [1;2;3]. *Many species of hawthorn have been studied, including C. Pinnatifida [4;5;6]*, *C. Monogyna [7;8;9]*, *C. Laevigata [1;2]*, *C. Oxyacantha [10;11]. But there are many types of Hawthorn and each type has its own composition.* Hawthorn is a plant rich in flavonoids. Flavonoids are polyphenol compounds that are widespread in the plant world and are divided into 7 subgroups: flavones, isoflavones, flavonols, dihydroflavones, flavans, chalcones and anthocyanidins. Flavonoids are produced by plants as secondary metabolites.

A series of clinical and experimental experiments revealed that flavonoids increase the amount of high density lipoprotein in the body, have antioxidant, anti-inflammatory, and vasodilating effects. Clinical observations with flavonoids showed that taking 10 mg of flavonoids

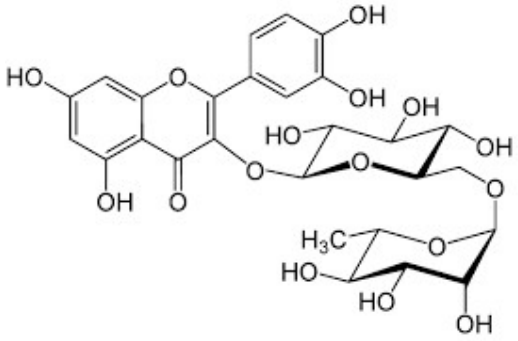
per day reduces cardiovascular diseases by 5% [12]. The lowest risk of cardiovascular disease was found among those who consumed 200 mg of flavonoids per day [13]. In animal experiments, quercetin has been shown to prevent inflammation and atherosclerosis [14; 15]. Flavonoids prevent oxidative stress by regulating NADFH-oxidase subunits. Because NADFH-oxidase plays an important role in preventing the formation of active forms of oxygen in blood vessels [20]. Activated macrophages in inflamed arteries have been observed to be neutralized by flavonoids [16]. Flavonoids are substances that inhibit platelet activity, and the natural flavonoid quercetin has important antioxidant and anti-inflammatory properties. A series of experiments have shown that this compound gives good results in the prevention of inflammation and metabolic diseases. Several experiments using aggregometry and luminescence showed that this compound has antiaggregant properties [17;18]. Flavonoids have antiplatelet activity and can be used in the prevention of cardiovascular diseases. Quercitin has antioxidant properties, increases the activity of peroxidase, catalase and glutathione peroxidase enzymes, and reduces the amount of MDA. It exhibits antioxidant properties through these properties. Quercitin reduces the production of anti-inflammatory cytokines. It was found that the concentration of superoxide dismutase in the liver increased and *the amount of bifidobacteria in the cecum increased when* foods containing quercitin were consumed [19].



Quercetin

Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) is a polyphenolic biflavonoid. Chemically, it is a glycoside composed of quercetin flavonolaglycon and rutinose disaccharide[20].

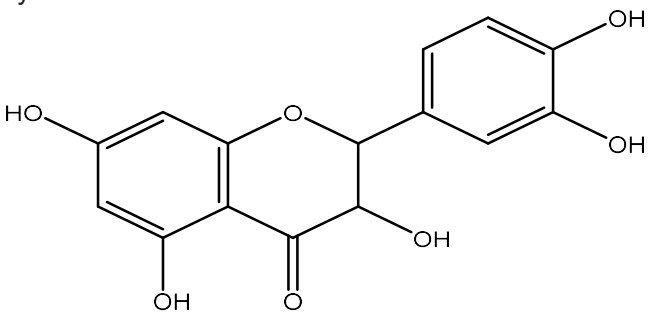
Rutin (quercetin-3-O-rutinoside) is a multifunctional natural flavonoid that can deeply affect cellular functions in pathological conditions. Because rutin and its metabolites can cross the blood-brain barrier, it has been shown to affect cognitive and behavioral symptoms in the treatment of various neurodegenerative diseases. In a series of experimental and clinical experiments, it was shown that this substance affects beta-amyloid aggregation and metabolism, and thus it can be used in the treatment of Alzheimer's disease. Changes in the oxidant-antioxidant balance associated with neuronal loss during Alzheimer's disease have been shown to activate processes that reduce the inflammatory component of neurodegeneration[21]. In some literature, the use of Rutin in acute cerebral ischemia has been shown to have a positive effect on sensorimotor recovery and neurodegeneration[22].



Routine

Osteoarthritis is a chronic disease of the joints, for which complete treatment methods have not yet been found. Treatment is aimed only at reducing pain and improving joint function. Kong Sui et al. (2022) used the flavonoid rutin in osteoarthritis and determined the following effects of rutin: inducible oxide synthase, cyclooxygenase-2 (TsOG-2), α-tumor necrosis factor (TNF- α), matrix metalloproteinase 13 (MMP-13) decreases the level of expression and increases the expression of Col II and aggrecan. In addition, rutin increased the expression of cystathionine- β - synthase (CBS) and decreased the expression of Rho-related helical protein kinase (ROCK) in chondrocytes. Kong Sui et al. thus showed that rutin inhibits the inflammation that develops in osteoarthritis [23].

The flavonoid dihydroquercetin also has important chemical and biological functions. Dihydroquercetin has been used in the treatment of colitis induced by sodium dextran sulfate. Dihydroquercetin has been shown in several experiments to reduce the amount of inflammatory cytokines in the colon. Based on the results of the experiment, it was shown that dihydroquercetin significantly reduces the amount of IL- *1β,* IL - *6* and *TNF - α.* Dihydroquercetin increased the ratio of *Firmicutes / Bacteroidetes in feces, and decreased the amount of Bacteroidetes. Of these, dihydroquercetin has been found to reduce the symptoms of colitis [24]. Dihydroquercetin, like other flavonoids, has been used in various neurodegenerative diseases with positive results. Table Tanaka et al., in their experimental experiment on rats, observed that dihydroquercetin inhibits the formation of* β *- amyloid fibrils, improves blood circulation in the brain, and prevents the reduction of cognitive functions. Many experiments have* shown the possibility of using dihydroquercetin in cerebral amyloid angiopathy and Alzheimer's disease.



Dihydroquercetin

As can be seen from the above information, we think it is appropriate to create natural nutritional supplements containing Quercetin, Rutin, Dihydroquercetin. Therefore, he studied the chemical composition of Hawthorn in order to create a nutritional supplement.

A series of clinical and experimental experiments revealed that flavonoids have a hypolipidemic, hypoglycemic effect on cardiovascular diseases, diabetes and provide effective results in hypertension.

RESEARCH METHODS

We dried Hawthorn for flavonoid determination at 25C for 20 days in a ventilated building, protected from direct sunlight. When we analyzed the literature, A. Urbonaviciute et al. (2006) flavonoids are generally stable substances and can be stored for a long time. They pointed out that flavonoids are substances resistant to high temperatures and can be stored up to 100 C [25]. Flavonoids in the sample were determined using liquid chromatography. 5-10 g of the sample is taken on an analytical scale and placed in a 300 ml flat flask. 50 ml of 70% ethanol solution was added to it. The mixture was heated at 70-80 ^{0}C with intensive stirring for 1 hour and then stirred at room temperature for 2 hours. The mixture was cooled and filtered. 25 ml of 70% ethanol is added to the remaining part and re-extracted 2 times. The filtrates were combined and made up to the mark in a 100 ml volumetric flask with 70% ethanol. The resulting solution is spun in a centrifuge at a speed of 6000-8000 rpm for 20-30 minutes. The resulting solution was taken from the top for analysis.

Phosphorous, acetate buffer systems and acetonitrile were used as eluents in the literature for the determination of flavonoids by YuSSX. We used a phosphate buffer system and acetonitrile.

VEJX " *Agilent-1200* " brand Column *Agilent* C $_{18}$ 5μm, 4.6x250mm. Elution isocratic in mode carried out, as a mobile phase) a mixture of 1% orthophosphoric acid and acetonitrile It was used

in the ratio of 70:30. The eluent pot is large speed - 1.0 ml/ min, sample volume 10 µl. Its length is 254 nm.

Chromatographic conditions:

-Chromatograph Agilent-1200 (equipped with an autodoser)

-Column Exlipse XDB C 18 (obraschenno-faznyy), 5 µm, 4.6 x250mm

-Diode array detector (DAD), 247 nm, 254 nm, 276 nm identified.

-Flow rate 1ml/min

- Eluent phosphate buffer: acetonitrile:

0-5 min 95:5,

6-12 min 70:30,

12-13 min 50:50,

13-15 min 95:5,

thermostat temperature 30 ^{0} C, -10 µl input amount (vkol).

RESULTS AND DISCUSSION

First, working standard solutions and then prepared working solutions were introduced into the chromatograph.

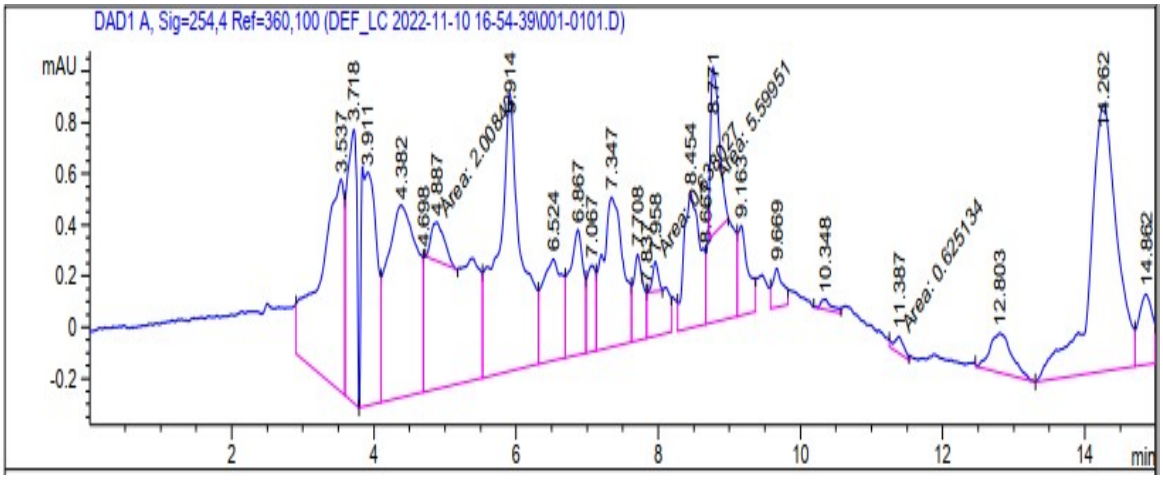
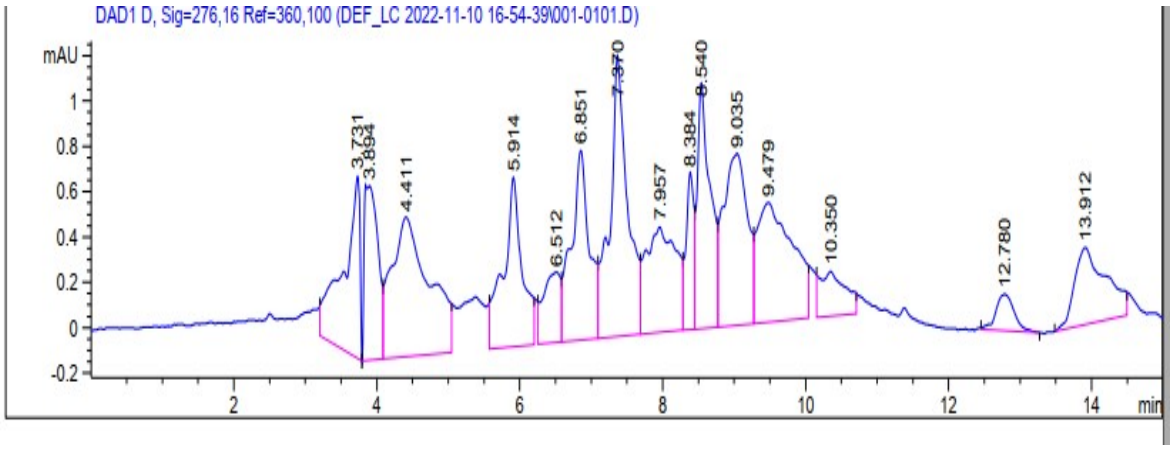


Figure -1. Chromatogram of a standard solution of wet fruit D.



Working chromatogram of hawthorn wet fruit

When we determined the flavonoids in hawthorn, we saw that the amount of dihydroquercetin was 1.25 mg/g. The amount of quercetin was 0.29 mg/g, and the amount of rutin was 0.29 mg/g. But Luthionine was not detected in Hawthorn.

Hawthorn	
Concentration mg/ gr	
Dihydroquercitin	1.25
Luthionine	0
Routine	0.29
Quercitin	0.35

Figure 1. Amount of flavonoids in hawthorn

As can be seen from the results, we saw that the amount of dihydroquercetin in hawthorn is 4.3 times higher than that of quercetin.

CONCLUSION

In summary, The results of the experiment showed that hawthorn is rich in flavonoids and its consumption is beneficial for the body. We think that it is appropriate to create a food supplement from natural means with fewer side effects compared to synthetic drugs and to use it in the prevention and treatment of various diseases. Taking into account that Yellow Hawthorn is rich in flavonoids, we came to the conclusion that it is possible to create a food supplement and use it in the treatment and prevention of cardiovascular diseases, diabetes.

LIST OF USED LITERATURE

1. N Liu, Y. Liu, LL Hu, YL Suo, L. Zhang, F. Jin, XA Feng, N. Teng, Y. Li. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poultry Science . Volume 93, Issue 2 , 1 February 2014, Pages 347-353. https://doi.org/10.3382/ps.2013-03225.

2. Aditya Ganeshpurkar , Ajay K Saluja.The Pharmacological Potential of Rutin. Saudi pharmaceutical journal. 2017 Feb;25(2):149-164. doi : 10.1016/j.jsps.2016.04.025.

3. Yu Duan 1 , No Sun 1 , Min Xue 1 , Xiaolan Wang 1 , Hu Yang 2 Synthesis of regioselectively acylated quercetin analogues with improved antiplatelet activity. Mol Med Rep. 2017 Dec;16(6):9735-9740. doi:10.3892/mmr.2017.7781.

4. Maria Choleva, Vassiliki Boulougouri, Anthy Panara, Eirini Panagopoulou, Antonia Chiou, Nikolaos S. Thomaidis, Smaragdi Antonopouloua, Elizabeth Fragopoulou. Evaluation of anti-platelet activity of grape pomace extracts. Food & Function., 2019, 10, P. 8069-8080. DOI : https://doi.org/10.1039/C9FO02138H

5. Solomon Habtemariam. Rutin as a Natural Therapy for Alzheimer's Disease: Insights into its Mechanisms of Action. Current medicinal chemistry. 2016;23(9):860-73. doi : 10.2174/0929867323666160217124333.

6. Amélia Miranda Gómez Rodrigues 1 , Fralini Dos Santos Marcilio , Michelle Frazão Muzitano , Arthur Giraldi-Guimarães. Therapeutic potential of treatment with the flavonoid rutin after cortical focal ischemia in rats. Brain Research . Volume 1503 , 29 March 2013, Pages 53-61
doi : 10.1016/j.brainres.2013.01.039.

7. Cong Sui , Yichao Wu , Ran Zhang , Tiantian Zhang , Yang Zhang , Jiaojiao Xi , Yanyu Ding , Jiyue Wen , Yong Hu . Rutin Inhibits the Progression of Osteoarthritis Through CBS - Mediated RhoA/ROCK Signaling . DNA and Cell Biology Vol. 41, No. 6 .2022.P.617-630.Doi: https://doi.org/10.1089/dna.2021.1182

8. Fan Wan , Hui Han , Ruqing Zhong , Mengyu Wang , Shanlong Tang , Shunfen Zhang , Fujiang Hou , Bao Yi , Hongfu Zhang. Dihydroquercetin supplement alleviates colonic inflammation potentially through improved gut microbiota community in mice. Food & Function. Issue 12(22), 2021. P.11420-11434 DOI: 10.1039/d1fo01422f

9. Jiajie Chen , Xu Sun , Tingting Xia , Qiqi Mao , Liang Zhong .Pretreatment with dihydroquercetin, a dietary flavonoid, protected against concanavalin A-induced immunological hepatic injury in mice and TNF- a / ActD -induced apoptosis in HepG2 cells. Food Function. 2018 Apr 25;9(4):2341-2352. doi : 10.1039/c7fo01073g.

10. Wang, X., Ouyang, Y.Y., Liu, J., & Zhao, G. (2014). Flavonoid intake and risk of CVD: A systematic review and meta-analysis of prospective cohort studies. *British Journal of Nutrition, 111,* 1–11.

11. Liu, XM, Liu, YJ, Huang, Y., Yu, HJ, Yuan, S., Tang, BW,... He, QQ (2017). Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: A systematic review and meta-analysis of cohort studies. Molecular Nutrition & Food Research, 61

12. Rees, A., Dodd, GF, & Spencer, JPE (2018). The e *ff* ects of *fl* avonoids on cardiovascular health: A review of human intervention trials and implications for cerebrovascular function. Nutrients, 10.

13. Guillermo Gormaz, J., Quintremil, S., & Rodrigo, R. (2015). Cardiovascular disease: A target for the pharmacological effects of *quercetin*. Current Topics in Medicinal Chemistry, 15, 1735–1742

14. Xiao, L., Liu, L., Guo, X., Zhang, S., Wang, J., Zhou, F.,... Yao, P. (2017). Quercetin attenuates high fat diet-induced atherosclerosis in apolipoprotein E knockout mice: A critical role of NADPH oxidase. *Food and Chemical Toxicology, 105,* 22–33

15. **. S Dahmer, E Scott. Health effects of hawthorn - American Family Physician, 2010. 81 (4): 465–468.**

16. Jennifer E. Edwards, Paula N. Brown, Nadia Talent, Timothy A. Dickinson, Paul R. Shipley. A review of the chemistry of the genus *Crataegus*. Phytochemistry. Volume 79 , July 2012, Pages 5-26. https://doi.org/10.1016/j.phytochem.2012.04.006

17. I. Orhan . Phytochemical and Pharmacological Activity Profile of Crataegus oxyacantha L. (Hawthorn) - A Cardiotonic Herb. Current Medical Chemistry. 2018;25(37):4854-4865. doi : 10.2174/0929867323666160919095519.

18. Tunde Jurikova , Jiri Sochor, Otakar Rop, Jiri Mlcek, Stefan Balla, Ladislav Szekeres, Wojtech Adam, Rene Kizek.5 Polyphenolic Profile and Biological Activity of Chinese Hawthorn (Crataegus pinnatifida BUNGE) Fruits. Molecules 2012, 17(12), 14490-14509; https://doi.org/10.3390/molecules171214490

19. Feng Shao , Lifei Gu , Huijuan Chen , Ronghua Liu , Huilian Huang , Lanying Chen , Ming Yang Evaluation of Hypolipidemic and Antioxidant Effects in Phenolrich Fraction of *Crataegus pinnatifida* Fruit in Hyperlipidemia Rats and Identification of Chemical Composition by Ultra-performance Liquid Chromatography Coupled with Quadropole Time-of-flight Mass Spectrometry. Pharmacogn osy Magazine. 2017 Oct-Dec; 13(52): 725–731. doi : 10.4103/pm.pm_402_16

20. H.BardakciaE.CelepbT.GözetcY.KandH.Kırmızıbekmez.Phytochemical characterization and antioxidant activities of the fruit extracts of several *Crataegus* taxa. South African Journal of Botany. Volume 124 , August 2019, Pages 5-13

21. Lillian Barros , Ana Maria Carvalho , Isabel CFR Ferreira . Comparing the composition and bioactivity of Crataegus Monogyna flowers and fruits used in folk medicine. Phytochemical Analysis . Volume 22, Issue 2. P. 181-188. https://doi.org/10.1002/pca.1267

22. Seyed Fazel Nabawi , Solomon Habtemariam , Touqeer Ahmed , Antoni Sureda , Maria Daglia , Eduardo Sobarzo-Sánchez, Seyed Mohammad Nabawi. Polyphenolic Composition of Crataegus monogyna Jacq.: From Chemistry to Medical Applications. Nutrients 2015, 7(9), 7708-7728; https://doi.org/10.3390/nu7095361

23. Kamal Ranjbar, Ebrahim Zarrinkalam, Iraj Salehi, Alireza Komaki, Bayan Fayazi. Cardioprotective effect of resistance training and *Crataegus oxyacantha* extract on ischemia reperfusion-induced oxidative stress in diabetic rats. Pharmacotherapy. Volume 100 , April 2018, Pages 455-460

24. Subha Rastogi, Madan Mohan Pandey, AKSRawat. Traditional herbs: a remedy for cardiovascular disorders. Phytomedicine . Volume 23, Issue 11 , 15 October 2016, Pages 1082-1089. https://doi.org/10.1016/j.phymed.2015.10.012

25. A. Urbonaviciute, V. Jacstas, O. Kornisova, V. Janulis, A. Maruska. Capillary electrophoretic analysis of flavonoids in single-styled hawthorn (Crataegus monogyna Jacq.) ethanolic extracts. Journal of Chromotografy A, 1112(2006). P.339-344

26. Sychev, A. Ya. Homogenynyy kataliz soedineniyam zhila/ A.Ya. Sychev, V.G. Isak - Chisinau: Shtiintsa, 1988. - 216 p.