



## CONTENTS OF CATECHINS AND ANTHOCYANINS IN THE ABOVE-GROUND ORGANS OF PLANTS OF *PRUNUS SPINOSA* L.

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Carrying out phenological and biochemical studies, the prospect of using the studied varieties of a thorn in breeding programs for obtaining new adaptive, large-fruited varieties and cultivars have been established. The data on the content of catechins and anthocyanins in the bark, leaves, flowers, and fruits of cultivated plants and wild varieties of *Prunus spinosa* L. are presented. As a result of the study, it was found that the wild variety of *Prunus spinosa* has a significantly higher content of anthocyanins and catechins in the above-ground organs than cultural varieties. This indicates a higher adaptive capacity of wild varieties to unfavorable environmental factors in comparison with cultural varieties, as catechins and anthocyanins, as well as other representatives of flavonoids, are used as biochemical markers of species adaptability. According to the content of anthocyanins and catechins can predict the success of the introduction. The highest amount of catechins and anthocyanins found in the leaves. The flowers have the lowest content of anthocyanins and catechins. In the study of the contents of catechins and anthocyanins in fruits of plants cultivated and wild varieties of *Prunus spinosa*. It was found that the wild variety of *Prunus spinosa* (genotype Sp1) has the lowest content of catechins in the presence of the highest content of anthocyanins.

**Keywords:** *Prunus spinosa*, aboveground organs, variety, anthocyanins, catechins

### Introduction

Modern botanical research of cultivated fruit plants is impossible without genetic analysis, selection, and use of the gene pool of their wild relatives (as they are a valuable reserve of genetic material), which provides resistance to biological factors of the environment.

Among the common plants of the family *Rosaceae* Juss. (*Malus* Mill., *Pyrus* L., *Cydonia* Mill., etc.) subfamily *Amygdaloideae* Arn. is one of the most cultivated in temperate climatic latitudes, including the territory of Ukraine (Komarnickij et al., 1975). One of the reasons for this spread was probably the location of ancient human settlements in the area of the historical center of the origin of plants (Lee et al., 2001).

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Today, among wide varieties of species *Prunus* L. significant interest attracts thorns (*Prunus spinosa* L.), which is characterized by high productivity, frost and winter hardiness, resistance to pests and diseases, and is a valuable breeding material for further study. By successive selections and folk breeding among wild thorns were identified large-fruited varieties (Woldring, 2000). Therefore, the comparative ecological and biological, biochemical studies of cultivated and wild representatives of the species *Prunus spinosa* is relevant.

Occupying a fairly large area of distribution of *Prunus spinosa* is marked by a large polymorphism. In the middle of the species are not rare varieties with different ecological fitness, winter hardiness, and drought resistance. That is the last two characteristics are the most important when it is grown in culture and breeding. There are also large-fruited thorns whose average mass reaches 15 g, they grow in the South of the Volga region and Crimea (Eremin, 2003).

Phenological observations have shown that the biological characteristics of the studied representatives are closely related to the climatic conditions of the growth area. Thus, the phase of Bud blooming in the varieties of thorns of large-fruited and wild was observed in the first decade of April at an average daily temperature of +6–8 °C. Flowering varieties of thorns small-fruited and thorns “large-fruited” was observed earlier, for 4–6 days from their wild representative. The beginning of mass flowering occurred at a temperature of +10–15 °C.

Fruiting varieties of thorns cultural variety 1 and cultural variety 2 was noted earlier-in the third decade of August, the first decade of September, and lasted 25–30 days. It is established that the wild-growing thorn begins to bear fruit later, from the second decade of September to the third decade of October, and has a longer period of fruiting, in comparison with the previous ones (Golubkova et al., 2017).

*Prunus spinosa* is a honey plant. Juice from fruits and bark are used to color the tissues, the fruits themselves and the aboveground part of the plant are widely used in folk medicine. Planting of thorns is often used in landscaping, as a hedge. The fruits contain 8.9% sugar, 2.0% organic acids, up to 1.5% pectin. In the nuclei – up to 37% fat, amygdalin, and other substances. Thorn plants serve as a stock for undersized varieties of peach, apricot, and plum (Makarov, 1972; Koch et al., 2016).

*Prunus spinosa* is a valuable genetic resource and is a raw material for the pharmaceutical and agricultural industries, therefore, the main task of the acclimatization Department of fruit plants M.M. Gryshko National Botanical Gardens was the enrichment of biodiversity of stone fruit crops rare plants for wide use in the culture and learning biology.

*Prunus spinosa* has unique medicinal properties. If in many medicinal plants healing power has some one part, the thorn in this regard is unique – for medicinal purposes use not only its berries but also leaves, roots, flowers, branches and bark (Marchelak et al., 2017).

Thorn helps with diseases of the stomach, intestines, kidneys, and liver, as well as metabolic disorders, with a lack of vitamins in the body. It is a good sweatshop and antipyretic. The juice of the thorn berries has antiviral properties, so its purpose makes sense in disorders of the gastrointestinal tract as a result of helminthosis (Yuksel, 2015). Medicinal properties of

the fruit known in dysentery, food poisoning, colitis, non-specific varieties. Pharmacological preparations of blackthorn have anti-inflammatory, astringent, diuretic, expectorant, laxative and antibacterial properties (Chopik et al., 1983).

In homeopathic practice use leaves, young branches, bark, rhizomes, flowers, and fruits of thorns. Water infusion from the flowers of the plant is an effective laxative, soothing the nervous system, a diaphoretic and diuretic (Ozcan et al., 2008). Bark, young branches, roots of thorns have a diaphoretic and anti-sporadic effect (Ahmad et al., 1998). The leaves and flowers of the blackthorn are in the variety of decoction with the aim of increasing the elasticity of blood vessels, improve cell metabolism, increase the protective forces of the body (Marchelak et al., 2017). A major role in the formation of medicinal properties of thorn due to the action of the secondary metabolites of the class of flavonoids – catechins and anthocyanins (Lila, 2004, Horbowicz et al., 2008), which are also considered as environmental markers of plants and participate in adaptive reactions of the plant organism (Minaeva, 1978; Lukner, 1979; Levon et al., 2017).

The aim of this study was to determine the content of anthocyanins and catechins in the above-ground organs of *Prunus spinosa* plants, which will allow evaluating the level of adaptation of its different varieties.

## Material and methodology

### Biological material

For the study, we used a wild variety of *Prunus spinosa* (genotype Sp1), small-fruited variety (genotype Sp2) and large-fruited variety (genotype Sp3). Thorn large-fruited from the collection of M.M. Gryshko National Botanical Garden of NAS of Ukraine. All of them are grafted on the seedlings of cherry plum, which makes it possible to effectively use the genetic material, without the variation of root seedlings, together with other cultural varieties of the subfamily Prunoideae.

### Biochemical analysis

Quantitative determination of the content of anthocyanins and catechins was carried out in the dried leaves and fruits of the middle tier of sprouts dried under shading in natural conditions in a well-ventilated area at a temperature of 20–25 and crushed in an electric mill.

The quantitative determination of catechins is based on the reaction of their condensation with vanillin (4-hydroxy-3-methoxybenzaldehyde). The reaction catalyst was concentrated hydrochloric acid. The optical density of the solution of condensation products was proportional to the initial concentration of catechins. The determination of the optical density was carried out with a green filter (maximum absorption  $520 \pm 10$  nm) (Kriventsov, 1982).

The quantity of anthocyanins was determined by the method of photoelectric colorimetry at a wavelength of 530 nm using an alcoholic extract of plant material homogenate was acidified to 3.5% hydrochloric acid (Kriventsov, 1982).

Measurements were carried out on a photoelectrocolorimeter Zalimp KF 77 (Poland).

Obtained data calculated using formulas:

$$C_{ant} = \frac{D \cdot V \cdot R \cdot H \cdot K}{l \cdot m}$$

$$C_{cat} = \frac{D \cdot V \cdot R \cdot H}{l \cdot m}$$

where:  $D$  – the optical density of the solution;  $V$  – the total amount of the extract and the average sample (ml);  $R$  – dilution ratio of the solution;  $l$  – working length of the cuvette (cm);  $H$  – aliquot part;  $m$  – linkage, the average of the sample (g);  $K$  – conversion factor

The number of parallel measurements was 3. The accuracy of the method was in the range of 2.5–4.8%. The obtained data were presented in mg/100 g of dry matter (DM) in terms of anthocyanidin.

### Statistical analysis

Statistically processed data is shown on histograms as arithmetic means and their standard errors. The significance level was set at  $\alpha = 0.05$ . The statistical analysis was performed with IBM SPSS Statistics, release 23.0.

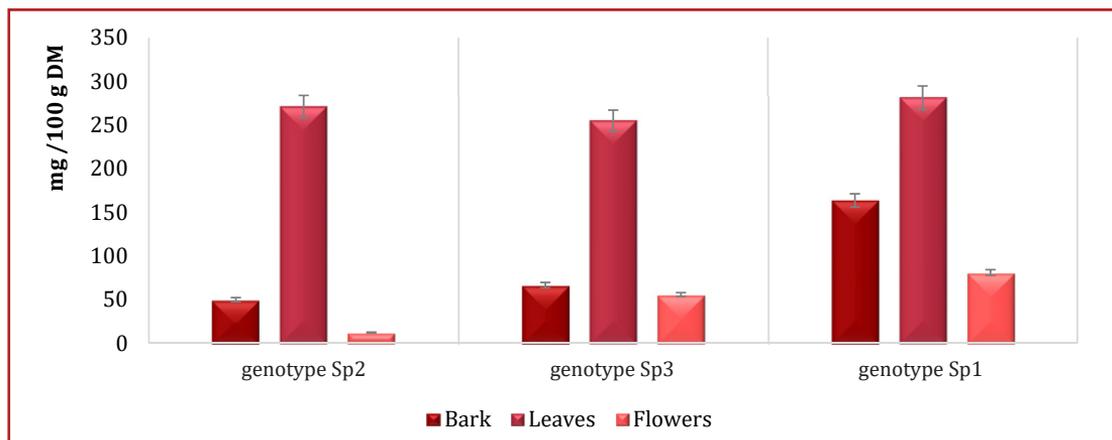
## Results and discussion

According to our previous data on the biochemical properties of *Prunus spinosa*, the aboveground organs of this plant are rich in biologically active compounds. The content of cyanogenic glycoside prunasin in sprouts is in the range of 131–691 mg/100 g DM (Levon et al., 2007), catechin content in fresh fruits – 78.5 mg/100 g DM (Mustafaeva, 2013), vitamin C – 25.5 mg/100 g DM (Celik et al., 2017). Eight flavonoids were isolated from the flowers of *Prunus spinosa*: kaempferol, quercetin, kaempferol 3-O-alpha-L-arabinofuranoside, quercetin 3-O-alpha-L-arabinofuranoside, kaempferol 3-O-alpha-L-ramnopyranoside, kaempferol 7-O-alpha-L-ramnopyranoside, kaempferol 3-O-beta-D-xylopyranoside, kaempferol 3-O-(2"-E-p-coumaroyl)-alpha-L-arabinofuranoside. The last three have been found for the first time in this plant (Olszewska et al., 2001). Steroids and terpenoids are abundant in *Prunus spinosa*: oleanolic acid, alpha-amyrin, sitosterol, beta-Sitosterol-beta-D-glucoside (Wolbis et al., 2001).

*Prunus spinosa* has been found to be a good source of phenylpropanoid esters: 5-hydroxy-6-methoxy-7-O-beta-D-glucosyl coumarin (Crespo et al., 1992). *Prunus spinosa* is also a rich source of epicatechin (Kolodziej), cyanidin-3-glucoside (Werner et al., 1989) and caffeic acid (Olszewska et al., 2000). In the fruits of *Prunus spinosa* was discovered aliphatic components nonacosane (C<sub>29</sub>H<sub>60</sub>) and 10-nonacosanol (Kaminska et al., 1970).

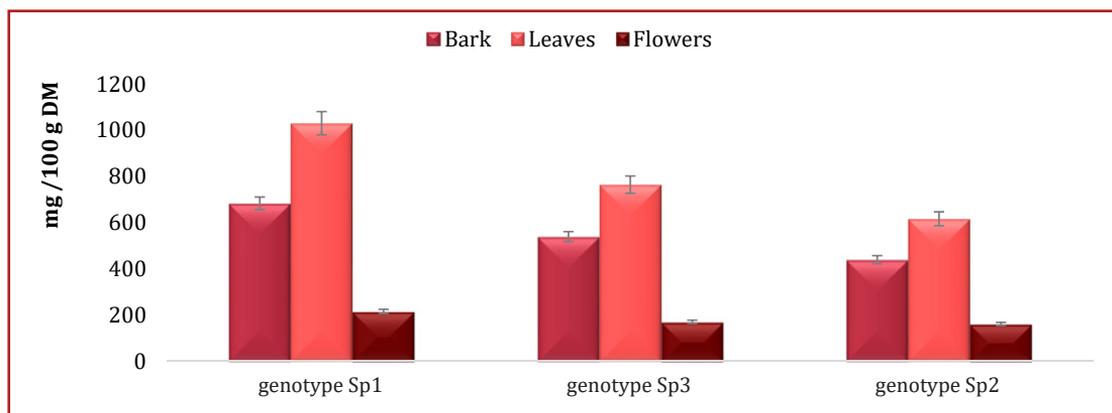
As a result of our research, it was found that among all the studied varieties the leaves had the highest content of anthocyanins (255–281 mg/100 g DM) (Figure 1). The content of anthocyanins in the bark ranged from 50 to 163 mg/100 g DM. In the flowers, the low content of anthocyanins (12–81 mg/100 g of DM) was found. Among the small-fruited (Sp2) and

large-fruited cultural varieties (Sp3) of *Prunus spinosa*, the flowers of the large-fruited variety had a much higher content of anthocyanins than the small-fruited variety.



**Figure 1** The content of anthocyanins in the above-ground organs of plants of *Prunus spinosa* L. (mg/100 g DM)

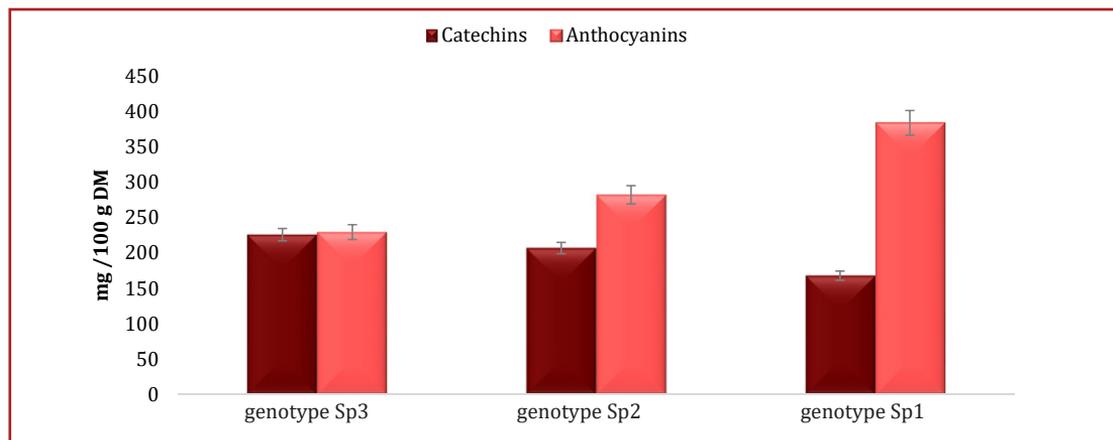
The greatest content of catechins determined in the leaves (617–1031 mg/100 g DM) (Figure 2). The content of catechins in the bark of the studied objects was much lower than in the leaves (440–684 mg/100 g DM). Flowers had a low content of catechins (162–216 mg/100 g DM). Among the small-fruited (Sp2) and large-fruited (Sp3) cultural varieties of *Prunus spinosa*, the flowers of the large-fruited variety had a slightly higher content of catechins (171 mg/100 g DM) than the small-fruited variety (162 mg/100 g DM).



**Figure 2** The content of catechins in the above-ground organs of plants of *Prunus spinosa* L. (mg/100 g DM)

It is interesting to note the fact that the wild variety of *Prunus spinosa* (Sp1) had a much higher content of anthocyanins and catechins in the aboveground organs than cultural varieties. This indicates a higher adaptive capacity of wild varieties to adverse environmental factors in

comparison with cultural varieties, as the accumulation of anthocyanins and catechins is one of the biochemical markers of the level of adaptation of species (Levon et al., 2016, 2017).



**Figure 3** The content of catechins and anthocyanins in the fruits of plants of *Prunus spinosa* L. (mg/100 g DM)

In the study of the content of catechins and anthocyanins in the fruits of cultural and wild varieties of *Prunus spinosa* (Sp2), it was found that among all the studied varieties the greatest content of catechins were the fruits of the large-fruited cultural variety (Sp3) of *Prunus spinosa* (226 mg/100 g DM) (Figure 3). The wild variety (Sp1) of *Prunus spinosa* had a significantly lower content of catechins than the cultural variety and it was 168 mg/100 g DM. According to the content of anthocyanins in the fruits of cultural and wild varieties of *Prunus spinosa*, a radically opposite result observed. The greatest content of anthocyanins had fruits of wild variety (Sp1) *Prunus spinosa* – 384 mg/100 g DM. Low content of anthocyanins had fruits of large-fruited (Sp3) cultural variety *Prunus spinosa* (230 mg/100 g DM). This pattern can be explained by the oxidation-reduction reaction. Catechins are oligomers/polymers which give anthocyanins by cleavage of the C–C bond under strongly acidic conditions in the presence of molecular oxygen (Bilia et al., 1996). Consequently, the oxidation of catechins leads to an increase in the content of anthocyanins.

## Conclusions

The study showed that the above-ground organs of different varieties of *Prunus spinosa* have a high content of anthocyanins and catechins in the conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. The wild variety of *Prunus spinosa* (genotype Sp1) has a higher content of these compounds. The study of the quantitative content of anthocyanins and catechins in the above-ground organs of *Prunus spinosa* varieties showed that they are of interest to the food and pharmaceutical industry and can serve as a raw material for P-vitamin preparations, because the content of vitamin P in the fruit of the wild blackthorn is not inferior rosehip and chokeberry and superior currants.

## References

- AHMAD, S.P., AHMAD, M., RIZWAN, G.H., NAJAM, R., SIDDIQUI, S., AHMAD, S.I., KHURSHID, S.J. 1998. Effect of *Prunus spinosa* and *Amygdalus* extracts on stress induced changes in Mice. In *Pakistan Journal of Pharmacology*, vol. 15(1), p. 27–30. ISSN 0255-7088.
- BAKHTEEV, F.H. 1970. *Vazhnejshie plodovye rasteniya* [The most important fruit plants]. Prosveschenie, 351 p. [In Russian].
- BILIA, A.R., MORELLI, I., HAMBURGER, M., HOSTETMANN, K. 1996. Flavans and A-type proanthocyanidins from *Prunus prostrata*. In *Phytochemistry*, vol. 43, p. 887–892.
- CELIK, F., GUNDOGDU, M., ALP, S., MURADOGLU, F., ERCİŞLI, S., GECER, M.K., CANAN, I. 2017. Determination of phenolic compounds, antioxidant capacity and organic acids Contents of *Prunus domestica* L., *Prunus cerasifera* Ehrh. and *Prunus spinosa* L. fruits by HPLC. In *Acta Chromatographica*, vol. 29(4), p. 507–510. <https://doi.org/10.1556/1326.2017.00327>
- CHOPIK, V.I., DUDCHENKO, L.G., KRASNOVA, A.N. 1983. *Dikorastushchie poleznye rasteniya Ukrainy. Spravochnik* [Wild useful plants of Ukraine. Handbook]. Kiev: Naukova Dumka, 400 p.
- CRESPO, I.A., FERNANDEZ, F.M., GONZALEZ, A.G., GUTIERREZ RAVELO, A. 1992. Constituents of *Prunus spinosa*. In *J. Nat. Prod.*, vol. 55, p. 450–454.
- EREMIN, G.V. 2003. *Sliva i alycha* [Plum and cherry-plum]. Folio, izd-vo AST, 302 p. [In Russian]. ISBN 966-03-1879-0.
- GOLUBKOVA, I.M., LEVON, V.F. 2017. Perspektivi viroschuvannya terenu (*Prunus spinosa* L.) u Nacionalnomu botanichnomu sadu im. M.M. Grishka [Prospects for growing thorns (*Prunus spinosa* L.) in M.M. Gryshko National Botanical Garden]. In *Genofond kolekcij botanichnih sadiv i dendroparkiv – zaporuka stalih fitocenoziv v umovah klimatichnih zmin*. Odesa: ONU, p. 37–40. ISBN 978-617-689-229-8.
- HORBOWICZ, M., KOSSON, R., GRZESIUŁ, A., BSKI, H.D. 2008. Anthocyanins of Fruits and Vegetables – Their Occurrence Analysis and Role in Human Nutrition. In *Vegetable Crops Research Bulletin*, vol. 68(1), p. 5–22. <http://dx.doi.org/10.2478/v10032-008-0001-8>
- KAMINSKA, T., URSZULA, L., RUFINA, S. 1970. Investigations on neutral components of *Prunus spinosa* fruits. I. Aliphatic components. In *Rocz. Chem.*, vol. 44, p. 103–107.
- KOCH, J.A., KOCH, D. A. 2016. Soderzhanie biologicheski aktivnyh veschestv v plodah. *Prunus spinosa* proizrastayushchego na territorii Krasnoyarskogo kraja [Content of biologically active substances in fruits. *Prunus spinosa* grows in the Krasnoyarsk territory]. In *Innovacionnye processy i tehnologii v sovremennom mire*, vol. 1(4), p. 128–132.
- KOLODZIEJ, H., SAKAR, M.K., BURGER, J.F.W., ENGELSHOWE, R., FERREIRA, D. 1991. A-type proanthocyanidins from *Prunus spinosa*. In *Phytochemistry*, vol. 30, p. 2041–2047.
- KOMARNICKIJ, N.A., KUDRYASHOV, L.V., URANOV, A.A. 1975. *Botanika. Sistematika rastenij* [Botany. Systematics of plants]. Izd. 7-e, pererab. M.: “Prosveschenie”, 608 p. [In Russian].
- KRIVENTSOV, V.I. 1982. *Metodicheskie rekomendacii po analizu plodov na biohimicheskij sostav* [Methodical recommendations on the analysis of fruits on biochemical structure]. Yalta, 21 c. [In Russian].
- LEE, S., WEN, J. 2001. A phylogenetic analysis of *Prunus* and the Amygdaloideae (*Rosaceae*) using ITS sequences of nuclear ribosomal DNA. In *American Journal of Botany*, vol. 88(1), p. 150–160. <https://www.jstor.org/stable/2657135>
- LEVON, V., GONCHAROVSKA, I. 2017. Vmist antocianiv ta halkoniv u pagonah krebiv ta gibridiv yabluni ‘Vidubitska plakucha’ [The content of anthocyanins and chalcones in a decorative small-fruited Apple trees and hybrids of Apple trees ‘Vyubetska plakucha’]. In *Agrobiodiversity for improving nutrition, health and life quality*, vol. 1, p. 292–297. <http://dx.doi.org/10.15414/agrobiodiversity.2017.2585-8246.292-297>
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- LEVON, V., KLYMENKO, S., TESLIUK, M. 2017. Dynamics of the content of phenolic compounds in the sprouts of the representatives of the family Cornaceae Bercht. Et J. Presl in the period of winter dormancy. In *Agrobiodiversity for improving nutrition, health and life quality*, vol. 1, p. 298–302. <http://dx.doi.org/10.15414/agrobiodiversity.2017.2585-8246.298-302>
- LEVON, V.F., GOLUBKOVA, I.M. 2016. Nakopichennyya antocianiv u nadzemnih organah predstavnikov rodu *Persica* Mill. u rizni periodi vegetacii [Accumulation of anthocyanins in the aboveground organs of the genus *Persica* Mill. in different periods of vegetation]. In *Visnik problem biologii i medicini*, vol. 3(130), p. 79–83. ISSN 2077-4214.
- LEVON, V.F., VASILISHINA, N.M., KUDRENKO, I.K., MOROZ, P.A. 2007. Dinamika nakopichennyya prunazinu v pagonnah roslin predstavnikov rodu *Prunus* Mill. [Dynamics of accumulation prunasine in sprout of plants of representatives of genus *Prunus* Mill.] In *Introdukciia Roslyn*, no. 4, p. 109–111.
- LILA, M.A. 2004. Anthocyanins and Human Health: An *In-Vitro* Investigative Approach. In *J. Biomed. Biotechnol.*, vol. 5, p. 306–313. <https://doi.org/10.1155/S111072430440401X>
- LUKNER, M. 1979. *Vtorichnyy metabolizm u mikroorganizmov, rastenij i zhivotnyh* [Secondary metabolism in microorganisms, plants and animals]. M.: Mir, 548 p. [In Russian].
- MAKAROV, V.A. 1972. Himicheskoe i farmakologicheskoe issledovanie flavonoidov *Prunus spinosa* L. [Chemical and pharmacological study of flavonoids *Prunus spinosa* L.]. In *Rastitelnye resursy*, vol. 8(1), p. 42–49.
- MARCHELAK, A., OWCZAREK, A., MATCZAK, M., PAWLAK, A., KOLODZIEJCZYK-CZEPAS, J., NOWAK, P., OLSZEWSKA, M.A. 2017. Bioactivity Potential of *Prunus spinosa* L. Flower Extracts: Phytochemical Profiling, Cellular Safety, Pro-inflammatory Enzymes Inhibition and Protective Effects Against Oxidative Stress *In Vitro*. In *Frontiers in Pharmacology 8*: Article 680. <https://doi.org/10.3389/fphar.2017.00680>
- MIKULIC-PETKOVSEK, M., STAMPAR, F., VEBERIC, R., SIRCELJ, H. 2016. Wild *Prunus* Fruit Species as a Rich Source of Bioactive Compounds. In *J. Food. Sci.*, vol. 81(8), p. 1928–1937. <https://doi.org/10.1111/1750-3841.13398>
- MINAEVA, V.G. 1978. *Flavonoidy v ontogeneze rastenij i ih prakticheskoe ispolzovanie* [Flavonoids in the ontogenesis of plants and their practical use]. Novosibirsk: Science, 255 p. [In Russian].
- MUSTAFAEVA, L.A. 2013. Soderzhanie katehinov v plodah nekotoryh dikorastuschih plodovo-yagodnyh rastenij, proizrastayuschih v Azerbajdzhane [Content of catechins in fruits of some wild fruit and berry plants growing in Azerbaijan]. In *Himiya rastitelnogo syrya*, vol. 3, p. 187–193. <http://dx.doi.org/10.14258/jcprm.1303187>
- OLSZEWSKA, M., WOLBIS, M. 2000. Phenolic acids in flowers and leaves of *Prunus spinosa* L. In *Herba Pol.*, vol. 6, p. 249–254.
- OLSZEWSKA, M., WOLBIS, M. 2001. Flavonoids from the flowers of *Prunus spinosa* L. In *Acta Poloniae Pharmaceutica – Drug Research*, vol. 58(5), p. 367–372.
- OZCAN, T., BAYCU, G. 2008. Fatty acid and amino acid profiles in the fruits of *Prunus spinosa* L. subsp. *dasyphylla* (Schur) Domin from Europe-in-Turkey. In *Adv. Mol. Biol.*, vol. 1, p. 39–46. <https://doi.org/10.3109/09637486.2010.533160>
- WERNER, D.J., MANESS, E.P., BALLINGER, W.E. 1989. Fruit anthocyanin in three sections of *Prunus* (*Rosaceae*). In *Hort. Sci.*, vol. 24, p. 488–489.
- WOLBIS, M., OLSZEWSKA, M., WESOŁOWSKI, W.J. 2001. Triterpenes and sterols in the flowers and leaves of *Prunus spinosa* L. (*Rosaceae*). In *Acta Polo. Pharm.*, vol. 58, p. 459–462.
- WOLDRING, H. 2000. On the origin of plums: a study of sloe, damson cherry plum, domestic plums and their intermediate forms. In *Palaeohistoria*, 39–40(40), p. 535–562.
- YUKSEL, A.K. 2015. The Effects of Blackthorn (*Prunus spinosa* L.) Addition on Certain Quality Characteristics of Ice Cream. In *Journal of Food Quality*, vol. 38, p. 413–421. <https://doi.org/10.1111/jfq.12170>
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