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Comparative study of the chemical composition and antioxidant properties of extracts of various cornel (*Cornus mas* L.) organs

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Abstract: The paper presents the results of study of the chemical composition and the antioxidant activity of fruits, leaves and stones of dogwood (*Cornus mas* L.), growing in Qabala district of Azerbaijan. The antioxidant activity of the ethanolic extracts was tested using the 2,2 - diphenyl - 1 – picrylhydrazyl stable radical. It was found that various parts of dogwood differ in the content of biologically active substances. The content of anthocyanins, varies within the range of 0.053-0.434%, flavonoids 0.135-0.678 % total phenols 1.257-2.543%. The study of the qualitative composition of anthocyanins revealed the presence of derivatives of cyanidin, pelargonidine and delphinidin. The greatest content of anthocyanins are recorded in fruits, flavonoids and common phenols in leaves, and catechins in stones. As a result of the study of antioxidant activity, it was found that the extract from various dogwood organs does not react equally and inhibit free radicals. It was found that the antioxidant properties of organs change in the following increasing sequence: fruits (extraction with 90% ethanol), stones (70% ethanol), leaves (40% ethanol), leaves (70% ethanol), stones (40% ethanol). The greatest inhibitory activity was shown by the extract obtained from stones with 40% ethanol (0.75 ml of a concentration of 93%). The smallest - fruit extract obtained with 95% ethanol (2.5 ml concentration of 30%). In the leaves, the greatest inhibition was shown by the extract obtained with 70% ethanol (89%). As a result of the study of the antioxidant properties of extracts obtained from various dogwood parts, it was revealed that leaves, especially stones, which are waste in the production of juice, can become a complete raw material for concentrates, food and cosmetic additives in the production of various products.

Key Words: *dogwood, anthocyanins, catechins, flavonoids, total phenols, fruits, leaves, seeds, antioxidant activity.*

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INTRODUCTION

The flora of Azerbaijan is rich in wild fruit and berry plants [Mustafayeva, 2015]. Among them, a special place is taken by C*ornus mas* L., belonging to the Cornaceae family [The Plant List]. The plant is widespread in the forests of the Great and Lesser Caucasus, in the steppe plateaus from lowlands to the middle zone.

In Azerbaijan and some Asian countries, fruits are used as an antidiabetic agent and are recommended for metabolic disorders, gout, anemia, and skin diseases [Jayaprakasam et al., 2005; Shukurov, 1981; Lima et al., 2014]. The use of fruit jam for colds and stomach diseases is very popular [Bijelic et al., 2011]. Juice is used in fever as a restorative, tonic and appetizing stimulant.

The composition of cornel fruits revealed the presence of anthocyanin glucosides, derivatives of cyanidin, pelargonidine, and dolphinidin [Novruzov, 2010; Andelkovic et al., 2015; Du and Francis, 1973; Seeram et al., 2002], flavonoids derivatives of quercetin, kempferol, aromathedirin [Pawloska et al., 2010], phenolic acids [Moldovan, David, 2017], catechins [Mustafayeva, 2013], tannins, vitamin C [Kostecka et al., 2017; Hashempour et al., 2010], iridoid substances [West et al., 2012], carotenoids [Horvath et al., 2007].

It has been established that anthocyanins from cornel fruit stimulate the work of C cells producing insulin by the pancreas [Jayaprakasam et al., 2005]. Iridoid components found in cornel fruit, participating in human lipid metabolism, bind to serum lipoproetin (LDL-C) and cholesterol lipoprotein (HDL-C) and thereby prevent the occurrence of atherosclerosis [Asgary et al., 2013]. The content of anthocyanins, flavonoids, carotenoids, tannins allows in fruits and other organs attributing cornel to medicinal plant materials [Seeram et al., 2002; Novruzov, 2010; Pawlowska et al., 2010; Kostecka et al., 2017].

Dogwood fruits and other organs were used as a medicine for various diseases in the form of tea, balms, and therapeutic creams [Ercisli et al., 2008]. In Azerbaijan and some Asian countries, fruits are used as an antidiabetic agent and are recommended for metabolic disorders, gout, anemia, and skin diseases [Jayaprakasam et al., 2005; Shukurov, 1981; Lima et al., 2014]. The use of fruit jam for colds and stomach diseases is very

popular [Bijelic et al., 2011]. Juice is used in fever as a restorative, tonic and appetizing stimulant.

In the last decade, great interest has been shown in establishing the antioxidant activity of medicinal plants, food products and drinks containing various groups of biologically active substances [Lapin et al., 2007; Kajshev et al., 2009; Hajrullina et al., 2005]. In metabolic processes, various forms of oxygen are formed in the body: superoxide (O_2-), hydroxide (OH), nitrate oxide (NO-), hydrogen hydroxide (H_2O_2), hypochlorate (HOCl), nitrite peroxide (ONOO-), oxygen singlet (O) and others. The increase of the active forms of oxygen in the human body leads to the destruction of proteins, fats, enzymes, as a result of which cells and tissues are damaged. All these give an impetus to the development of cancer, cardiovascular diseases (hypertension, ischemia), Parkinson's disease, hypertension, ischemia, cirrhosis of the liver, diabetes and other diseases [Ercisli et al., 2008; Carocho et al., 2013; Faridah et al., 2006; Olas, 2018]. Secondary plant metabolites - carotenoids, flavonoids, aromatic hydroxyacids, anthocyanins, catechins, vitamin E, C and other biologically active substances are the main natural antioxidants. Among these substances, biflavanoids, which have anti-carcinogenic, anti-sclerotic, anti-inflammatory, immuno-strengthening, anti-allergic and other properties are of particular importance. Interest to substances of polyphenolic nature is due to the prospect of obtaining new highly active drugs based on them that have antioxidant, anticancerogenic, anti-inflammatory effects.

Now it has been established that the fruits of various species of wild plants contain substances that have antioxidant, antiradiant, antimutagenic, anti-stress effects [Pyrkozs-Biardzka et al., 2014; Zeynalova et al., 2019]. Consequently, the use of fruits and berries, as well as their products, reduces the incidence and mortality from cancer, cardiovascular and other diseases [Milenkovic-Andjelkovic et al., 2015]. Dogwood is highly valued for its medicinal, nutritional and other biological characteristics. In this regard, to prevent and treat these diseases, it is very important to find new sources of plants with a high content of antioxidant substances and develop new drugs, nutritional supplements, and functional foods based on them.

Thus, in spite of sufficient number of research materials devoted to the study of the chemical composition, medicinal, and nutritional properties of dogwood, there are very few works related to the study of antioxidant activity (AOA) of fruits, there is no information on the study of AOA of leaves and stones. In this regard, the aim of this work was to study the chemical composition and antioxidant activity of various dogwood organs growing in Azerbaijan.

MATERIAL AND METHODS

Plant material. The objects of investigation were various organs of *Cornus mas* L. of the genus *Cornus* L., which grows in Qabala district of Azerbaijan. Materials for the study were collected from June to October 2018. The leaves were collected after full formation, and the fruits and stones during biological maturity.

Determination of the total polyphenols. Phenolic compounds were extracted with 70% ethanol and their total content was determined by the method of Folin-Ciocalteu [Denisenko et al., 2015]. The total polyphenol content was expressed in mg gallic acid equivalent per gram of dry weight.

Extraction and identification of anthocyanins. Plant material was extracted with 95% ethanol containing 1% HCl. The extract was filtered. The content of anthocyanins was determined spectrophotometrically at a wavelength of 510-520 nm on spectrophotometer (Spekol 1500, "Analytik Jena AG", Germany). The content of anthocyanins was calculated by cyanidin-3-glycoside [Chapukhina et al., 2016]. The qualitative composition of anthocyanins and the ratio of individual components were determined by the chromato-spectrophotometric method [Novruzov, 2005].

Extraction and identification of flavonoids. Plant material (leaves) was extracted with 80% ethanol 3 times in a ratio of 1: 3 in a water bath (at 60-70°C) for 30 minutes. The extracts were combined, filtered and evaporate in a rotaru evaporator Rova-N2L apparatus. The total flavonoid content was determined spectrophotometrically at 420 nm [Endeneva et al., 2014]. The flavonoid content was calculated on the basis of rutin (SSS). The qualitative composition and content of individual components was determined by the chromato-spectral method [Novruzov, 2004].

Extraction and identification of catechins. The plant material was extracted with methanol in a ratio of 1:20 for 60 minutes. The extract was centrifuged at 5000 rpm for 10 minutes. The catechin content was determined spectrophotometrically [Ivanov et al. 2014]. The quantitative composition and content of individual catechins was determined by the chromatographic method [Novruzov et al. 1983].

The study of antioxidant activity. The concentration of anthocyanins was determined spectrophotometrically in a hydrochloric acid aqueous solution at wavelength of

510-520 nm on spectrophotometer (Spekol 1500, "Analytik Jena AG", Germany). The content of anthocyanins was calculated by cyanidin-3-glycoside [Chapukhina et al., 2016]. Phenolic compounds were extracted from plant material with 70% ethanol and their total content was determined by the method of Folin-Ciocalteu [Denisenko et al., 2015]. The total polyphenol content was expressed in mg gallic acid equivalent per gram of dry weight. Flavonoids were extracted with 70% alcohol. The total flavonoid content was determined spectrophotometrically [Bulatova et al., 2012]. The content of the amount of flavonoids was established in terms of rutin / GSO. The content of catechins according to the method of N.E. Novruzov [1983] and M.N. Zaprometov [1993].

Extracts for determining AOA of the fruit were obtained from freshly collected samples, from dry and crushed leaves and stones using 40%, 70% and 95% ethanol, antioxidant activity was determined by standard methods [Brand-Williams et al., 1995]. 2,2 - diphenyl - 1 - picrylhydrazyl (DPPH) is used for determining the antioxidant activity.

The optical density of the solution was determined at 734 nm in a UV–VIS spectrophotometer (Jenway 7305). Trolox solutions (6 - hydroxy - 2,5,7,8 - tetramethylchroman - 2 - carboxylic acid) were used as a standard solution for measuring of antioxidant activity. The results of the analysis are expressed in % on dry weight (μmol / g). Concentrations were calculated from a calibration curve in the range from 1 to 10 μM Trolox. Free radical inhibition was calculated by the following formula:

$$AOA\ (\%) = [(A\ DPPH - A\ Sample) / A\ DPPH] \times 100$$

where, A Sample is the absorbance of the solution containing the extract after 30 min and A DPPH is the absorbance of the DPPH solution devoid of extract.

Statistical Analysis. The obtained data were processed using Statistica Version 12 (Station Inc, USA).

RESULTS AND DISCUSSION

This paper presents the results of the analysis of the quantitative content and qualitative composition of anthocyanins, flavonoids, catechins, and the total content of phenols from various dogwood organs, as well as the antioxidant activity of extracts obtained from them.

Phenolic compounds are an essential component of plant materials which are not synthesized in human and animal cells and enter the organism mainly due to food, especially by medicinal plants. They are highly regarded as antioxidants. As known, plant phenolic compounds are characterized by a wide structural diversity and, accordingly, various mechanisms of utilization of free radicals and other active forms of oxygen in cells. So, among flavonoids, some (rutin, catechin) act as a trap of hydroxyl radicals, while others, aglycon rutin and quercetin do not reduce the hydroxyl content, but inhibit the production of the superoxide anion of the radical [Chapuhina et al., 2016]. Plant extracts, consisting of a variety of biologically active compounds of a phenolic nature have a complex effect on organism. Anthocyanins, flavonoids, catechins refers to phenolic compounds and have antiradical and antioxidant properties investigated by us in various organs of dogwood. In pharmacological studies, special accusation is given to anthocyanin compounds that have antioxidant, antiradical, as well as vasodilator, antimicrobial, anticarcinogenic, antidiabetic and interferon inducer properties [Jayaprakasam et al., 2005; Deyneko et al., 2018; Novruzov et al., 1987; Asadullaev et al., 1987].

Data on the content of biologically active components of various organs of dogwood are presented in the figure 1.

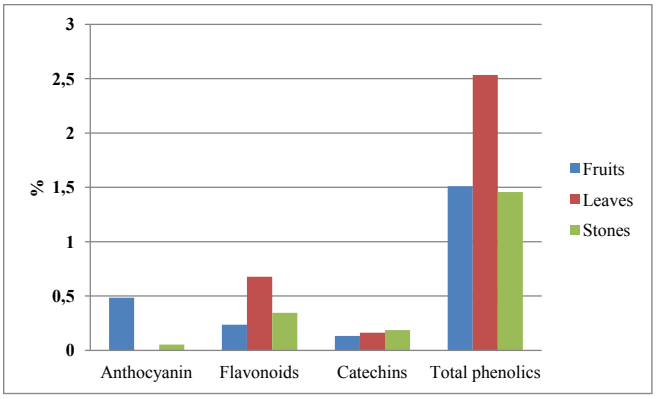

Figure 1. The content of biologically active substances in various parts of dogwood (mg%).

As can be seen from the figure the content of anthocyanins varies from 0.053 to 0.484 %; flavonoids 0.235 to 0.678 %; catechins from 0.133 to 0.185 %, total phenols 1.511 to 2.534 %. The greatest number of anthocyanins was found in the fruits of 0.484 %, flavonoids, total phenols in the leaves 0.678 %; 2.534 %, respectively, catechins in the stones 0.185 %.

Determination of anthocyanins. The results of our analyzes on the content of anthocyanins in dogwood fruits show that they accumulate more in fruits from Azerbaijan (484 mg%) than those growing in Iran (106.81-

442.11 mg%) [Hassanpour et al., 2011], in Poland (235.7 mg /%) [Sozanski et al, 2014]. The content of anthocyanins in dogwood fruits is higher than in cherry fruits - 82-298 mg% [Gao, Mazza, 1995], red grape varieties - 6.9-15.1 mg% [Cantos et al., 2002], black currant - 152-281 mg% [Benvenuti et al., 2004], blackberries - 126-152 mg% [Pantelidis et al., 2007], blueberries - 314-515 mg% [Moyer at al., 2002]. Comparing our results with the data above, we can conclude that dogwood is reach with anthocyanins among other fruit species. A significant content of phenolic substances in dogwood fruits confirms the antioxidant and nutraceutical potential of this plant.

The results of chromatographic analysis of anthocyanidins obtained after hydrolysis of the sum of anthocyanins showed the presence of cyanidin, pelargonidine and dolphinidin. A total of four anthocyanins were found, of which three were isolated individually. According to the results of physico-chemical and spectral analyzes and their comparison with known samples, the isolated anthocyanins were identified as pelargonidin-3-O-β-glycoside (67.5% of the total) cyanidin-3-O-β-glucoside (18.0 %) and delphinidin-3-O-β-galactoside (14.2%).

Determination of flavonoids. A total of eight flavonoid substances are found. Of these, four are classified as flavonols. Three of them are highlighted individually. According to the result of physicochemical and spectral parameters and their comparison with witness data, the substances were identified as quercetin - 3-O – β-rutinoside (with 54.8% of the total), quercetin - 3- O-β-galactoside (20.8%) and kempferol - 3- O-β-galactoside (24.4%).

Determination of catechins. In the total amount of catechins, the presence of catechin (26.3%), epicatechin (21.4%) and gallocatechin (52.3%) was found. The main part of the total amount of catechins is gallocatechin (stones).

Antioxidant activity. Recently, the use of antioxidants in the treatment of various diseases has been increasingly debated [Rajendran at al., 2014]. Most of the doubts are related primarily to the use of synthetic drugs [Baytop, 1991]. In addition, despite the fact that many *in vivo* studies have shown the ineffectiveness of the use of antioxidants in the treatment of a number of diseases, it was also pointed out that the diet of products introduction into the daily, especially of plant origin, rich in compounds with antioxidant properties, significantly reduces the risk of cardiovascular, neurodegenerative and oncological diseases [Olas, 2018]. This is specific to vitamin E, C, flavonoids and carotenoids. Numerous biochemical reactions are known that take place and not directly related to the antioxidant properties of these compounds [Hasanov et al., 2004]. The results of the study of the antioxidant activity of various organs (fruits, leaves, stones) are presented in figure 2.

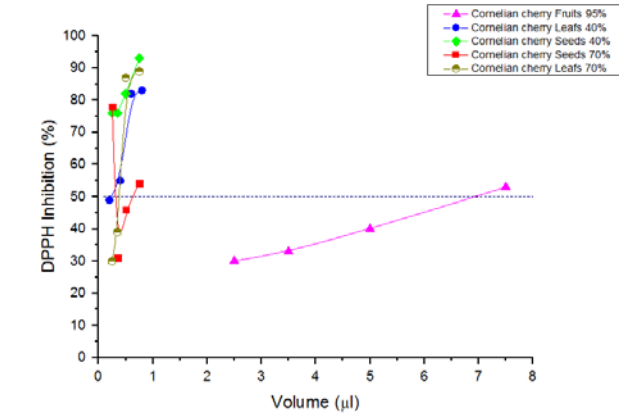

Figure 2. Density of dependence on the DPPH reagent (in%).

From the data in the figure it can be seen that various organs differ greatly in antioxidant activities. The antioxidant activity of the studied dogwood organs varies from 30-93%. The greatest antioxidant activity was shown by the extract obtained from the stones (93%), and the smallest from the fruits (30%). Study of the effect of various extracts on the antioxidant activities of various organs, it was revealed that the concentration of the solvent markedly affects AOA. It was found that AOA of extracts obtained by various solvents from various organs changes according to the following sequence: fruits (extraction with 95% ethanol) → stones (70% ethanol) → leaves (40% ethanol) → leaves (70% ethanol) → stones (40% ethanol). The highest antioxidant activity was shown by the stone extract of 40% ethanol, 0.75 ml concentration (93%), and the smallest fruit extract obtained by 95% ethanol, 2.5 ml concentration (30%). In the leaves, the greatest inhibition was observed in the extract obtained with 70% ethanol (89%). The extracts obtained from leaves with 40% ethanol at various concentrations (0.2, 0.4, 0.6, 0.8 mg) showed 49%, 55%, 82%, 83% inhibitory properties, respectively.

CONCLUSION

The content and composition of biologically active substances show that various parts of dogwood can become raw materials for antioxidant preparations. It was established that various dogwood organs differ in anti-

oxidant activities, the stones and leaves have the greatest activity, and the fresh fruits have the least activity. The obtained data allow us to recommend the stones of this plant, which are waste in the production of juices, as raw materials for food concentrates and additives, as well as to stabilize and increase the biological activity of food, cosmetic products and use as a medicine.

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Zoğalın müxtəlif orqanlarının kimyəvi tərkibi və antioksidant xüsusiyyətinin müqayisəli tədqiqi

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İşdə Azərbaycanın Qəbələ rayonunda yayılmış müxtəlif orqanlarının - meyvə, yarpaq, çəyirdəyinin kimyəvi tərkibi və antioksidant xüsiyyətləri verilir. Etanol çıxarışlarının antioksidant fəallığı 2,2 – difenil – 1– pikril hidroliz (DPPH) reaktivinin sərbəst radikalları udmaq xassəsinə görə müəyyən edilmişdir. Meyvə ekstraktı təzə yetişmiş meyvələrdən, yarpaq və çəyirdəklərdən isə qurudulmuş materialdan alınmışdır. Zoğalın müxtəlif orqanlarının kimyəvi analizi nəticəsində müəyyən edilmişdir ki, onlar bioloji fəal maddələrlə zəngindir. Antosianların miqdarı 0.053 - 0.484, flavonoidlər 0.135 - 0.678%, ümumi fenol birləşmələri 1.257 - 2.543% arasında dəyişir. Antosianların keyfiyyət tərkibinin öyrənilməsi tərkibində sianidin, pelargonidin və delfinidin törəmələrin mövcudluğunu aşkar etdi. Antosianların maksimum miqdarı meyvələrdə, flavonoid və ümumi fenolların miqdarı yarpaqda, katexinlər çəyirdəklərdə olmuşdur. Alınmış ekstraktların antioksidant fəallığının tədqiqi nəticəsində müəyyən edilmişdir ki, onlar sərbəst radikallara qarşı eyni reaksiya vermirlər. Tədqiq edilən ekstraktların antioksidant fəallığının dəyişilmə yüksəlişi belədir: meyvə (90% etanol), çəyirdək (70% etanol), yarpaq (40% etanol), (70% - li etanol), çəyirdək (40% etanol). Ən yüksək inqibirləşmə fəallığı 40% etanolla alınmış çəyirdək ekstraktı (0.75 mm qatılıqda 93%), ən aşağı (2.5 mm qatılıqda 30%) meyvə ekstrakti göstərdi. Yarpaqlarda ən çox inqibirləşmə fəallığını 70 – li etanolla alınmış ekstrakt (89%) göstərdi. Zoğalın müxtəlif orqanlarından alınmış ekstraktların antioksidant xüsisiyyətinin tədqiqi göstərdi ki, zoğal şirəsi istehsalında əmələ gələn çəyirdək tullantısı qida və kosmetik məhsullar istehsalında istifadə edilən antioksidant konsentratı almaq üçün xammal ola bilər.

Açar sözlər: *zoğal, antosian, katexin, flavonoid, ümumi fenol, meyvə, yarpaq, çəyirdək, antioksidant fəallığı*

Сравнительное исследование исследование химического состава и антиоксидантных свойств экстрактов разных органов кизила (*Cornus mas* L.)

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В работе приведены результаты исследования химического состава биологически активных веществ и антиокислительных свойств плодов, листьев и косточек кизила (*Cornus mas* L.), произрастающего в Габалинском районе Азербайджана. Антиоксидантная активность этанольных экстрактов исследованана способность улавливать свободные радикалы 2,2 – дифенил – 1 – пикрилгидразила (ДФПГ). Анализы проводили с использованием экстрактов свежих зрелых плодов и высушенных листьев и косточек. В результате химического анализа установлено, что различные органы кизила отличаются по содержанию биологически активных веществ. Содержание антоцианов в зависимости от органов изменяется в пределах 0,053-0,434 %, флавоноиды 0,135-0,678 %, общие фенолы 1,257-2,543 %. Изучение качественного состава антоцианов выявило наличие производных цианидина, пеларгонидина и дельфинидина. Наибольшее количество антоцианов отмечены в плодах, флавоноиды и общие фенолы в листьях, катехины в косточках. В результате исследования

антиоксидантной активности было установлено, что экстракт из различных органов кизила не одинаково реагируют и ингибируют свободные радикалы. Выявлено, что антиокислительные свойства полученных экс- трактов изменяются по следующей возрастающей последовательности: плоды (экстракция 90% эта- нолом), косточки (70% этанол), листья (40% этанол), листья (70% этанол), косточки (40% этанол). Наибольшую ингибирующую активность показал экстракт полученный из косточек с 40% этанолом (0,75 мл концентрации 93%). Наименьшую - экстракт из плодов полученный 95%этанолом (2,5 мл концентрации 30%). В листьях наибольшее ингибирование показал экстракт полученный 70% этанолом (89%). В результате исследования антиоксидантных свойств экстрактов, полученных из различных органов кизила выявлено, что листья, особенно косточки являющиеся отходом при производстве сока может стать полноценным сырьем для получения концентрат, пищевых и косметических добавок при производстве различных продуктов.

Ключевые слова: *кизил, антоцианы, флавоноиды, общие фенолы, фрукты, листья, косточки, антиоксидантная активность*