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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

**BIOECOLOGICAL, PHYTOCHEMICAL AND SOME
PHARMACOLOGICAL FEATURES OF SPECIES OF THE
SAMBUCUS L.GENUS**

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INTRODUCTION

Relevance of the topic. One of the urgent problems posed to botanists is the broad scientifically grounded rational use of plant resources, ensuring their reproduction and improving the environment. In this regard, the study of wild species and forms of natural flora and their introduction into the national economy is acquiring practical significance.

Despite the fact that in the modern medicine uses a wide range of multidirectional drugs, the need for medicinal plants is growing from year to year. The nutritional and medicinal value of medicinal plants is due to their chemical composition. Their advantage over synthetic drugs are explained by their low toxicity, mild and at the same time multicomponent action. At the same time, herbal preparations are devoid of side effects of both allergic and non-allergic nature. Medicinal plants contain a complex of biologically active substances that have a positive effect on metabolism, on the functions of vital organs and systems, such as cardiovascular, nervous, digestive, excretory etc. But the widespread use of medicinal plants due to their poor knowledge is still limited.

The flora of Azerbaijan is rich by various medicinal plants. One of the promising species requiring a comprehensive study is the species of the elderberry genus (*Sambucus* L.) of the *Adoxaceae* Trautv family widespread in Azerbaijan. Flowers, fruits, bark, young branches, and elderberry leaves are used as raw materials. The plant contains flavonoids, glycosides (sambunigrin), essential oil, terpenes, choline, alkaloids (coniine and sanguinarine), carotene, ascorbic acid, and other organic acids, tannins, mucus, pentosans, resins, mineral salts, amino acids (tyrosine).^{1,2} Some biologically active substances in the fruits of the species of the genus *Sambucus*, especially flavonoids, catechins, pectins, have the ability to delay pathological

¹Veberic, R., Jakopic, J., Stampar, F., Schmitzer, V. European elderberry (*Sambucus nigra* L.) rich in sugar, organic acids, anthocyanins and selected polyphenols. // Food Chem., – 2009, 114, – p. 511–515

²Новрузов, Э.Н. Пигменты репродуктивных органов растений и их значение // – Баку, ЭЛМ, – 2010, – 308 с.

tissue growth and thus are effective means in the prevention and treatment of malignant neoplasms was found. There is information about the antitumor effect of drugs from the roots of the plant.³ Drugs from elderberry flowers are used as a diaphoretic, diuretic, anti-inflammatory, disinfectant for colds, flu, diseases of the upper respiratory tract, kidneys, and bladder, for rinsing the mouth, rheumatism, gout, and joint inflammation in folk and traditional medicine.^{4,5}

Raw materials from various parts of the plant are included in many antidiabetic and hepatic preparations.⁶

The fruits and flowers of the plant are used for food. They are used to prepare jams, drinks, juices, add to grape must improve the aroma and taste of wine. Ripe fruits can produce a harmless dye used in the food industry.^{7,8,9,10}

Biological and phytochemical properties of the species of the *Sambucus* genus growing in Azerbaijan in order to expand the local range of food additives and medicines considered expedient to

³Luísa, M. Construction and validation of a *Sambucus nigra* biosensor for cancer-associated STn antigen / M.Luísa, S.Silva, E.Gutiérrez [et al.] // Biosensors and Bioelectronics, – 2014, Vol. 57, – p. 254-261.

⁴Schwaiger, S. Identification and pharmacological characterization of the antiinflammatory principal of the leaves of dwarf elder (*Sambucus ebulus* L.) / S.Schwaiger, I.Zeller, P.Polzelbauer [et al.] // J Ethnopharmacol, – 2011, 133, – p. 704–709.

⁵Kinoshita, E. Anti-infl uenza virus effects of elderberry juice and its fractions / E.Kinoshita, K.Hayashi, H.Katayama [et al.] // Biosci. Biotech. Bioch., – 2011, 76, 1633-1638.

⁶Salvador, Á. C., Król, E., Lemos, V. C. Effect of elderberry (*Sambucus nigra* L.) extract supplementation in STZ-induced diabetic rats fed with a high-fat diet. Int. J. Mol. Sci., – 2017, Vol. 18, – p. 1–19.

⁷Кривченкова, М.В., Клышинская, Е.В. Растительные флавоноиды как функциональные добавки в косметических и пищевых продуктах // Вестник РАЕН, – 2012. № 3, – с. 47-51.

⁸Szalóki-Dorkó, L., Stéger-Máté, M., Abrankó, L. Evaluation of colouring ability of main European elderberry (*Sambucus nigra* L.) varieties as potential resources of natural food colourants // Int. J. Food Sci. Technol., – 2015. №50, – p. 1317-1323.

⁹Senica M. Processed elderberry (*Sambucus nigra* L.) products: A beneficial or harmful food alternative / M.Senica, F.Stampar, R.Veberic [et al.] // LWT-Food Science and Technology, – 2016, Vol. 72, – p. 182-188.

¹⁰Novruzov, E.N. Flavonoid-containing plants of flora of Azerbaijan and prospects of their use / E.N.Novruzov, E.E.Jafarova, M.B.Zulfugarova, A.M.Zeynalova//XIX International Botanical Congress, – China: July 23-29, – 2017, – p. 525-526.

study in view of the above useful properties and the rich chemical composition of the plant. The pharmacological action of species of the *Sambucus* L. genus in some pathologies have been revealed taking into account the high content of biologically active substances, especially flavonoids, in species of the *Sambucus* genus, their antioxidant and antiradical activity, as well as the undeniable role of the participation of free radicals in the pathogenesis of many diseases.

Object and subject of research. The object of the study was various organs of wild-growing elderberry species, the subject of the study was the study of bioecological, phytochemical, and pharmacological properties of the plant.

The purpose and objectives of research. The purpose of this work is to study the bioecological characteristics, the phytochemical composition of species of the *Sambucus* genus, develop technology for obtaining biologically active concentrates (BAC) with a high content of biologically active substances, and also, on an experimental model of diabetes mellitus (DM) and toxic hepatitis, to investigate the pharmacological action of galenic preparations (extracts) obtained from the flowers, leaves, and fruits of the black elderberry plant. According to this goal, we have carried out experimental studies.

The following tasks have been set for this purpose:

- to study the bioecological feature, distribution, productivity of species of the *Sambucus* L. genus
- to study the ontogenesis, the state of populations of species of *Sambucus* L. genus
- to study the qualitative composition and quantitative content of nutrient and biologically active substances of the species of the *Sambucus* genus, to isolate and identify individual components, to reveal the influence of soil and climatic conditions on the composition and content of individual components
- to develop a scientifically grounded highly efficient technology for the production of BAC with a high content of biologically active substances
- to study the pharmacological action of galenic preparations

(extracts) obtained from flowers, leaves, and fruits of the black elderberry plant on an experimental model of diabetes and toxic hepatitis.

Research methods. Geobotanical descriptions of phytocenoses with the distribution of *Sambucus* species were carried out by generally accepted methods and coenopopulations were analyzed on the basis of analysis of ontogenetic spectra. Phytochemical methods were used to study the qualitative and quantitative composition of biologically active substances, and pharmacological analysis methods were used to study the effectiveness against diseases.

The main provisions of the dissertation defence:

➤ The data on the location of the species of the *Sambucus* L. genus and on the fruit stock serves as a basis for planning fruit harvesting in the studied areas.

➤ The obtained data on the quantitative content and qualitative composition of the species of the *Sambucus* L. genus is necessary to establish the value of plants as raw materials for the food and medical industry, as well as for widespread use by the population for dietary and prophylactic purposes.

➤ The positive pharmacological effect of galenic preparations (extracts) obtained from the flowers, leaves and fruits of black elder against the background of experimental diabetes and toxic hepatitis allows the preparation of drugs for this purpose.

The scientific recency of the research. For the first time, the species of the *Sambucus* L. genus have been comprehensively studied both in chemical and biological aspects. 2 species of the genus *Sambucus* L. grow in the flora of Azerbaijan, which belong to the Central Asian geographical element - *S. ebulus* herbaceous-chamephite, *S. nigra* shrub plant-phanerophyte has been established. These species are widespread from lowlands to mid-mountainous zones and have 3 life forms - compact aerosilicous shrubs, diffuse aerosilicous shrubs, and small trees. They are characterized by mesosympodial growth, depending on the course of ontogenesis, changes in the composition of the structure of the shoot system occur.

For the first time, populations with a low and a high degree of

variability in the morphology of the elderberry vegetative organs were identified. The size of clusters on plants tends to decrease as the number of clusters increases. The clusters are composed of over 2,000 small (6 mm) white flowers. All the studied populations - full-member, which indicates the stability of the cenopopulation to self-maintenance was found.

The qualitative composition and quantitative content of various nutrient and biologically active substances in various organs of the species of the *Sambucus* L. genus have been established. Qualitative composition and quantitative content of biologically active and nutrient substances are the characteristics of the species was revealed. In both species, during the period of biological maturity of fruits, the qualitative composition and quantitative content of nutrient and biologically active substances coincide.

Fruits of *S. nigra* and *S. ebulus* contain solids of 17.8, 20.9%; carbohydrates (glucose, fructose, sucrose, rhamnose was found. Rhamnose was discovered for the first time) - 4.80, 5.20 mg%; organic acids (tartaric, acetic, citric, malic) - 1.0%, 1.20%; ascorbic acid 42.4, 382.0 mg%; catechins 200.0, 285.4, respectively.

The species of *Sambucus* L. genus are rich in anthocyanins was revealed. The content of anthocyanins, depending on the place of growth of the plant, varies from 2181,4 mq% to 3124,0 mq%. The sum of anthocyanins contains 3 peak regions of anthocyanins: cyaniding- cyanidin 3,5-diglucoside, cyanidin 3-glucoside, and cyanidin 3-sambubioside. Cyanidin 3,5-diglucoside was found in negligible amounts. The content of the main anthocyanin in elderberry fruits, cyanidin 3-sambubioside, is more than half of all detected anthocyanins (64.4%).

For the first time, the flavonoid composition of various organs of the genus *Sambucus* L. growing in some regions of Azerbaijan has been comprehensively studied using modern chromatographic and spectral methods. The content of flavonoids of *S. nigra* and *S. ebulus* varies within 270,3 mq% and 415,4 mq% per air-dry weight of the raw material was found. Flavonoids in leaves of *S. nigra* have been identified as isoquercetin, hyperoside, rutin, astragalín, and glucoluteolin (luteolin-7-glucoside). Rutin and astragalín constitute

the bulk of the total flavonoids has shown results of chromatographic analysis. Leaves of *S.ebulus* are rich in both content (1.8%) and qualitative composition of components (6 components). In terms of content (1.23%) and component composition (4 components), flowers differ little from leaves have been revealed by the chromatic-spectrophotometric method. The dominant components identified are rutin and narcissin.

For the first time, a highly effective scientifically grounded technology was developed for the use of fruits of the species of *Sambucus* genus for various purposes based on the results of chemical-technological analyzes. The content of total sugar increases by 2.1-2.8, organic acids - 1.4-2.3, vitamin C - 1, 5-2.2, polyphenols - 1.8-2.4, anthocyanins - 1.7-2.6, and catechins - 1.8-2.2 was revealed when processing fruit juice with a pectolytic enzyme and ultrasound, compared with the control juice, depending on the species of the *Sambucus* genus. The obtained data is possible to use in the resulting concentrate as food additives, as well as for the production of soft drinks with high biological properties.

For the first time, extracts of flowers and leaves of black elderberry reduce the content of total cholesterol, glucose triglycerides in the blood of animals, have a pronounced effect on lipid metabolism and hepatic parameters, and also reduce the activity of catalase, which also indirectly confirms a decrease in the effect of lipid peroxide oxidation against the background of experimental DM was found.

Scientific and practical significance of work. Experimental data expand the information on the chemical composition of various organs of the species of the *Sambucus* L. genus, their biological characteristics, resource characteristics, as well as nutritional and medicinal value. The analysis of cenopopulations of species of the *Sambucus* L. genus is possible to gain an idea of the peculiarities of the age structure of cenopopulations.

The revealed patterns of the accumulation of biologically active substances and nutrients provide information about the optimal timing of harvesting and using fruits and berries as raw materials for the production of food, pharmaceutical, and other products. This data

makes it possible to regulate the quality of these products.

The dissertation materials can be used to monitor the state of wild species of the *Sambucus* genus in Azerbaijan, in the educational process when giving lectures on the "Botany", "Environmental Biochemistry", "Resource Studies" disciplines, as well as in the preparation of new editions of "Flora of Azerbaijan" and "Red Book" of Azerbaijan.

Approbation of the dissertation work. Results of the study were presented at the scientific and practical conference dedicated to the principles and methods of biodiversity conservation (Yoshkar-Ola, 2015, 2019); the international scientific-practical conference dedicated to the modern achievements of Azerbaijani medicine (Baku, 2016); the international conference on topical problems of modern chemistry and biology (Ganja, 2016, 2017); the XIX International Botanical Congress (China, 2017); the conference dedicated to the 90th anniversary of academician V. Hajiyeu (Baku, 2018).

21 scientific works on the topic of dissertation was published. Two of them were published in journals abstracted and indexed in international databases.

The name of the organization in which the dissertation work was carried out. The dissertation work is carried out in the Department of Plant Resources of the Institute of Botany of the Azerbaijan National Academy of Sciences and in the Department of Modeling of Pathological Processes on the basis of the Research Center of the Azerbaijan Medical University.

Structure and volume of dissertation work. The dissertation work is published on 224 pages, including 44 tables and 34 pictures. 284 sources of references were used. The dissertation includes introduction, seven chapters, results, recommendations and list of references (the total volume of the sign - two hundred and thirty nine thousand characters: introduction - 14911 characters, Chapter I - 32944 characters, Chapter II - 11487 characters, Chapter III - 15955 characters, Chapter IV - 41089, Chapter V - 41411, Chapter VI - 11753, Chapter VII - 64735, Results - 3730, Suggestions and Recommendations – 1134 characters).

CHAPTER I LITERATURE REVIEW

The literary material concerning the issues of origin, taxonomy, chemical composition, biological activity, and the use of species of the *Sambucus* L. genus in the medicine and the food industry was analyzed.

CHAPTER II MATERIAL AND METHODS

2.1.Object and material of research

The object of the study was species of the elderberry genus (*Sambucus* L.) from the *Adoxaceae* Trautv. family, and the material of the research was the fruits, flowers and leaves of elderberry.

Analyzes of the herbarium materials collected by us, as well as the study of the herbarium material from the fund of the Institute of Botany of ANAS is possible to clarify the species composition of the elderberry common in this region.

To find out the habitats of the studied species, the national navigation system AzNav was used. Expeditionary trips were made to the following botanical and geographical regions of Azerbaijan: to G.C. (Guba) - to Gusar, Guba, to G.C. east - to Ismayilli; Caspian - Shabran; on G.C. east - to Gabala, Gakh; Alazan-Agrichay valley - to Sheki region, Oguz, Belokan, Zagatala; Samur-Davachi lowland - Khazmaz; Lankaran lowland - Lankaran, Astara; Lankaran mountain - Masalli; Kobystan - Shamakha; Small Caucasus (Gadabay, Dashkesan), Western Kura plain – Goranboy districts. Research materials were collected in the process of reconnaissance route, detailed route, and semi-stationary studies.

2.2.Methods of botanical research

Geobotanical descriptions were carried out using generally accepted methods. Based on the analysis of ontogenetic spectra, the

type of the studied cenopopulations was determined. ¹¹The aging index proposed by A.A. Uranov¹² was used to assess the ontogenetic spectrum of the cenopopulation. In addition, the efficiency index, the recovery index, the replacement index, and the aging index were calculated.

2.3. Methods of phytochemical analyses

The phytochemical studies were carried out on freshly collected or fixed materials. The content of anthocyanins, flavonoids, carotenoids and other biologically active substances was mainly determined in freshly collected material, and the qualitative composition, both in fresh and fixed. We used gravimetric, colorimetric and chromatographic methods. Spectrophotometric and chromatographic methods of analysis and approved GOSTs (State standards) (GOST 31663-2012, GOST 5472-50, GOST 32167-2013, GOST 33410-2015) was used when performing the analyzes.

2.4. Methods of pharmacological research

The experiments to study the pharmacological properties of the obtained extracts of black elderberry were carried out on 125 outbred white rats weighing 260-299 g.^{13,14} All experimental animals were used repeatedly in the experiments several times. In this case, the washing period of the injected extracts was taken into account, which was tenfold their half-life and the time required for the animals to recover after the next experiment. All animal experiments were carried out in accordance with the "European Convention for the Protection of Vertebrate Animals".

¹¹Лемеза, Н.А. Геоботаника. Учебная практика / Н.А.Лемеза, М.А.Джус – Минск, – 2008. – 225 с.

¹²Уранов, А. А. Возрастной спектр фитоценопопуляций как функция времени энергетических волновых процессов // Биол. науки, – 1975. № 2, –с. 7-34.

¹³Сравнительное исследование различных моделей аллоксаниндуцированного сахарного диабета // Казанский Медицинский Журнал, –Казань, –2013, 94(6), – с. 915-919.

¹⁴Groza, M. The effects of vegetal extracts on the immune system dysfunction in the diabetes mellitus / M.Groza, M.Ciocoiu, L.Bădescu[et al.] // Annals of the Romanian Society for Cell Biology, – 2010, 15(1), – p. 241-246.

CHAPTER III.

BIOMORPHOLOGICAL CHARACTERISTICS, GROWTH AND DEVELOPMENT OF SPECIES OF THE *SAMBUCUS* L. GENUS

3.1. Botanical characteristics of species of the *Sambucus* L. genus

Works of phenological, geobotanical and morphological character have been carried out, descriptions of the species of the *Sambucus* genus, widespread in this region, have been compiled.

3.2. Biomorphological characteristics of species of the *Sambucus* L. genus

Leaf apparatus on the shoot, as well as the shoot height, the diameter of 1/3 of the shoot height, the length of the inflorescence, the number of internodes, the number of flowers on the apical inflorescence was measured. 10 shoots and measured the shoot height in the counting area was selected, and then deduced the arithmetic mean value.¹⁵ Comparative analysis of the variability of *S. ebulus* showed that the maximum average values for the number of leaves have CP2, the minimum values are CP1. The rest of the shoots in these parameters occupy an intermediate position. Comparative analysis of the variability of the numerical traits of the aerial part of the *S. nigra* plant showed that CP1 has the maximum average values for the number of leaves, while CP2 has the minimum values.

3.3 The growth and development of species of the *Sambucus* L. genus

Beginning of the growing season in species of the *Sambucus* L. genus is observed at average daily air temperatures of 0°C have shown results of the study. The onset of the growing phase, depending on weather conditions, occurs at different times in each year under study (Fig.1).

¹⁵Зульфугарова, М.Б., Новрузов, Э.Н.

Биоэкологическая характеристика из запасов плодов дикорастущих видов рода *Sambucus* L. // Botanika tədqiqatları yəni çağırışlar, – Bakı, – 2018, – s. 140-142.

Район исследования АВМВ	Месяцы											
	Декабрь	Январь	Февраль	Март	Апрель	Май	Июнь	Июль	Август	Сентябрь	Октябрь	Ноябрь
Самарканд												
Сурхандария												
Шарканд												
Ташкент												
Андижан												
Бухара												
Алтай												
Исмаилян												
Этап роста	Развитие почек	Развитие листьев	Развитие побегов	Развитие соцветия	Цветение	Формирование плодов	Созревание плодов	Начало листопада				

Figure 1. Schematic representation of the main growth stages

A direct correlation of the average value was established between the date of the beginning of flowering and the sum of positive temperatures, as well as between the date of the beginning of the phase and the amount of precipitation at the beginning of the phenophase, which indicates a direct relationship between the characteristics of the weather and the entry into this phase of the growing season. The growing season lasts from 160 to 250 days.

CHAPTER IV

DISTRIBUTION, POPULATIONS, ONTOGENESIS, YIELD AND FRUIT STOCK OF SPECIES OF THE *SAMBUCUS* L.GENUS

4.1. Distribution, populations of species of the *Sambucus* L.genus

The areas of distribution of elderberry species are mainly confined to the regions of the Greater Caucasus. On the slopes of southern exposures in the lower belt, communities of species of the genus *Sambucus* with fruit shrubs were established. The dominant vegetation of the phytocenosis with the participation of species of the genus *Sambucus* in different areas and their changes under the

influence of anthropogenic factors and environmental conditions was determined.

4.2. The state of populations of species of the *Sambucus* L. genus

In general, individuals of *Sambucus* are in a satisfactory condition, growing in the second or third tier of the stand, during the generation period. There are few aging individuals, seed productivity is average, seedling undergrowth is observed throughout the site.

4.3. Productivity and fruit stock of species of the *Sambucus* L. genus

Elderberry is distributed from lowlands to 1600 and above m above sea level, but most often found in the lowlands and in the foothill zones in the regions studied by us. They grow in various soil and climatic conditions. The research was carried out in 2015-2019 in the following districts: Belokan, Zagatala, Kakhi, Gusar, Guba, Oguz, Gabala and Ismayilli. For a detailed examination of the thickets, counting plots of 1x1 m in size were laid in the sections assigned to the blank base. In total, 352 registration areas were laid to determine the yield. Every second area was examined. The number of fruiting plants and the number of inflorescences were determined on the counting site. The average weight of one fruit and one fruiting inflorescence was determined by weighing 50 samples and counting the fruit in 30-50 replicates. The biological stock of fruits was determined as the product of the average yield by the value of the reduced area of distribution of the species (i.e., in terms of 100% projective cover).

4.3.1. Stocks of fruits of *Sambucus ebulus* L. in Azerbaijan

The biological stock was determined by multiplying the area of distribution of the species by the average yield, since dwarf elderberry grows in accessible areas for collection, the operational stock of fruits should be calculated as 95% of the biological. It was determined that the biological reserve of fruits was 16.5 tons and the operational reserve was 12.8 tons on 298 hectares of land in the study areas, where dwarf elder is widespread. The average yield of dwarf elderberry fruit depends on the place of growth of the plant and varies from 87.6 to 752.7 g/m².

4.3.2. Stocks of fruitsof *Sambucus nigra* L. in Azerbaijan

Determinations of productivity in the surveyed areas showed that *Sambucus nigra* occupies a rather large territory - 54512.3 hectares, it forms thickets and has a sufficient amount of biological and operational reserves of industrial importance. The biological reserve is 252 tons, the operational one is 182 tons.

4.4. Ontogenesis and different species of life forms of *S. nigra* L. in different ceonotic conditions

The research was carried out in 13 CPs. The main research was carried out in an oak-hornbeam forest, a herb-elderberry association. The study of black elderberry (*Sambucus nigra* L.) was carried out in two different ceonotic conditions (CC).¹⁶

All studied plants were of seed origin. For each individual, the ontogenetic state, absolute age, height of the aboveground part, a diameter of the crown of the bush, the diameter of the base of the stems and their number in the bush, the structure of the shoot system, and the life form were determined. A variety of life forms (compact aërocsil shrubs, diffuse aërocsil shrubs, and trees) contribute to the prolongation of ontogenesis and an increase in the duration of the existence of elderberry specimens under the forest canopy.

4.4.1. Ontogenesis of elderberry growing in well-lit glades and clearings

The first CC is the felled, well-lit areas of the forest, the herb-elderberry-sea buckthorn community, where the black elderberry grows freely. The ontogenetic state of *Sambucus nigra* growing in well-lit areas has good indicators (Table 1).

The age value of the studied ceonopopulations has a strong distribution amplitude, the efficiency indices vary from 0.22 (CP II) to 0.57 (CP III).

¹⁶Зульфугарова, М.Б., Новрузов, Э.Н., Мустафаева, Л.А.

Онтогенезиразличныхвариантыжизненныхформбузинычерной (*Sambucusnigra*L.) вразныхценоотическихусловиях // Műasirkimyaвə biologiyanınaktualproblemləri, – Gəncə, – 2019, – с. 133-135.

Table 1.

**Ontogenetic state of *Sambucus nigra* growing in well-lit areas
(biochronological indicators)**

Ontogenetic state	Bush age	Bush height, cm	Crown diameter, cm	Number of stems, pcs	Stem base diameter, cm
Juvenile (j)	1	30-50	—	1	0,2-0,5
Immature (im)	1-3	45-125	30-40	3-5	0,5-1,4
Virginal (V)	2-4	65-130	140-150	3-7	0,9-1,5
Generative (g1)	3-7	120-160	80-160	3-12	1.1-2,0
Generative (g2)	6-10	150-390	160-200	6-12	2,0-2,5
Generative (g3)	9-16	380-560	200-350	2-3	3,5-7,0
Senile (s)	12-16	50-120	50-80	2	1,0-1,5

Using the delta-omega criterion, three types of populations were obtained: young, transitional, and aging. The recovery index ranges between 0.15 - 2.0. We have not found a threatened condition in the populations. Thus, all studied populations are stable, self-sustaining, full-member, and in a normal state.

4.4.2. Ontogenesis of species of the genus *Sambucus* L. growing in shady places in the oak-hornbeam forest

The second cenotic condition (CC) is shady forest areas, communities with the participation of herb-hawthorn-dogwood-elderberry, where individuals of black elderberry grow undersized, oppressed. Ontogenetic state *Sambucus nigra* growing in shady places in the oak-hornbeam forest have low rates. Unlike plants grown in bright glades and clearings, virginal and generative individuals in small forest clearings have smaller sizes and insignificant seed productivity.

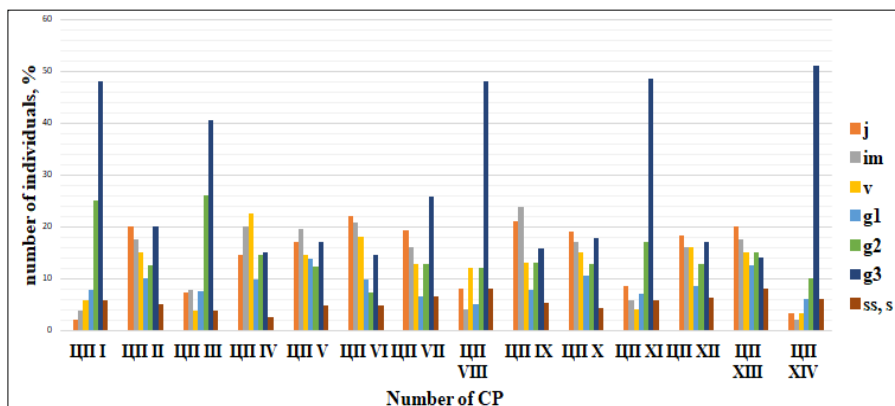


Figure 2. Ontogenetic spectrums of *S. nigra* populations

But the shoot formation of elderberry, apparently, is determined genetically and persists in different cenotic conditions (Fig.2).

CHAPTER V PHYTOCHEMICAL STUDIES OF SPECIES OF THE GENUS *SAMBUCUS* L.

5.1. Phytochemical composition of fruits of species of the genus *Sambucus* L.

The organoleptic and some chemical characteristics were studied, the content of dry and nutritious, as well as biologically active substances was studied (Tab. 2)^{17, 18}

The dry matter content in fruits varies from 17.8 to 20.9%, sugar from 4.80 to 5.20 mg%, organic acids from 1.0% to 1.20%, vitamin C from 42.4 to 382.0 mg%, catechins from 200.0 to 285.4 mg% in different types of fruits .

¹⁷Зульфугарова, М.Б. Исследование биохимического состава видов рода *Sambucus* L. // Azərbaycan təbii və tıbbi bitkiləri haqqında məlumat jurnalı, – 2019, – s.269-271.

¹⁸Зульфугарова М.Б., Новрузов Э.Н.,

Биохимическая характеристика зрелых плодов *Sambucus nigra* L. // Botanika, Ümumi Biologiya, Mikrobiologiya, Aqrar elmləri, – Gəncə, – 2015, – c. 164-168.

Table 2.**Phytochemical composition of species of the *Sambucus L. genus***

Phytochemical composition (% of the wet weight of the fruit). *	<i>Sambucosebulus</i> (number of samples 3)	<i>Sambucusnigra</i> (number of samples 4)
Dry matter	17,8	20,9
Sum of sugars	4,80	5,20
Sucrose	0,10	-
Total pectin	0,70	0,90
Organic acid	1,20	1,00
Ascorbic acid**	382,0	42,40
Carotinoids**	2,19	1,30
Anthocyanins	2181,4	3124,0
Flavonoids	415,4	270,3
Catechins	285,4	200,0
Leukoanthocyanins	808,0	857,0

Note: * - the average value is given, ** - mg% on wet weight

The species do not contain a large amount of flavonoids (270.3-415.4 mg%), but the distribution of flavonoids coincides with the distribution of anthocyanins. The studied species in terms of the content of nutrients and biologically active substances differ in a rather wide amplitude of fluctuations depending on species characteristics and geographic factors.

5.1.1. Influence of environmental factors on the chemical composition of species of the *Sambucus L. genus*

The chemical composition of fruits of the species of the *Sambucus* genus collected from 13 CPs was studied. The content of the chemical composition during the formation and ripening of fruits is greatly influenced by the location and air temperature.

Depending on the CU in the fruits of *Sambucus ebulus*, the amount of vitamin C accumulated in the range of 293-382 mg%; titratable acids 1,00 - 1.20%; dry insoluble substances 16.16-20.47%; soluble substances 29.1-37.7%; sugar 4,5-4,8 %; anthocyanins 2133,2-2181.4 mg%.

In the fruits of *Sambucus nigra*, the amount of vitamin C is 22.65-46.83mg / 100g; titratable acids 0,56 - 1,0 %; dry insoluble

substances 14.26-27.7%; sugar 4,88-5,20 %; anthocyanins 3081,71-3124,0.

5.1.2. Influence of environmental factors on biologically active substances of species of the genus *Sambucus* L.

As a result of studying the influence of soil and climatic conditions on the content of biologically active substances in elderberry fruits, it was revealed that the habitat and meteorological conditions during the period of vegetative development of plants significantly affect the content of biologically active substances.^{19, 20} Of these, illumination, terrain and temperate climate have a beneficial effect on the accumulation of biologically active substances.

5.2. Study of catechins in species of the *Sambucus* L. genus

The species *Sambucus nigra* and *Sambucus ebulus* contains 6 separate catechins. (6 catechins each) of their composition, but they differ in the ratio of the individual components was found at studying the component composition of catechins. The content of catechins, depending on CP in mature fruits of *Sambucus nigra* 185,2 to 200 mg% and *Sambucus ebulus* 282,3, to 285,4 mg% varies from was found.

5.3. Study of flavonoids in species of the *Sambucus* L.genus

The amount and distribution of flavonoids in various organs of species *S.nigra* and *S. ebulus*, the dynamics of accumulation by development phases, and the yield of the ground part were studied during the research. Flavonoids are distributed unevenly throughout the organs was found. The maximum amount is collected in the flowers from the generative organs, and in the leaves from the vegetative organs. In *S. nigra* (in II, V and VIII SP)::in the phase of

¹⁹Zulfugarova M.B., Novruzov E.N. The Study of Composition and Content of Anthocyanins and Flavonoids of Fruits of the *Sambucus nigra* L. (*Sambucaceae* Botsch ex Bork. family) // AMEA-nın Xəbərləri (biologiya və tibb elmləri), – 2016. Cild 71, №3, –s. 30-34.

mass flowering, the mass was 49.4 g, by dry weight in the fruiting phase 10.24 g, *S. ebulus* 45.2 and 9.84 g, respectively.

5.3.1. Flavonoid composition of flowers of *Sambucus nigra* L.

As a result of the research, 3 individual substances were obtained: 1) quercetin, 2) isoquercitrin, 3) rutin (Fig. 3.).²⁰

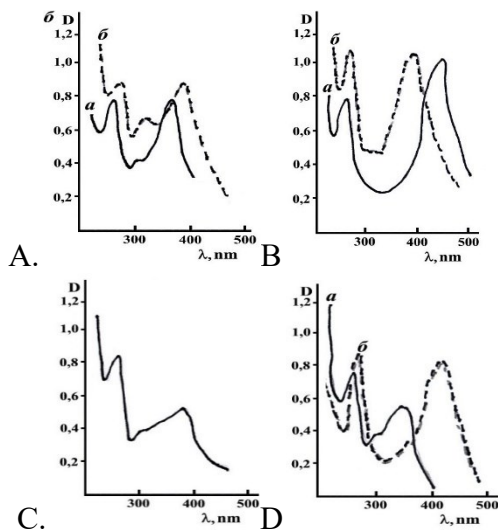


Figure 3. Chromatographic and spectral characteristics of the isolated flavonoids of the flowers of *Sambucus nigra* L.

Note: UV spectra of the isolated flavonoids: A - quercetin, b - quercetin + CH_3COONa ; B) a - quercetin + AlCl_3 , b - quercetin + $-\text{CH}_3\text{COONa} + \text{H}_3\text{BO}_3$; B) a - rutin; b - rutin + AlCl_3 ; D) rutin + CH_3COONa

5.3.2. Flavonoid composition of *Sambucus ebulus* flowers.

Three individual substances were obtained: 1-quercetin, 2-narcissinin, 3- rutin as a result of the research.²¹ Rutin and narcissinin are the main components of all studied organs of dwarf elder. In the

²⁰Зульфугарова, М.Б. Исследование флавоноидов бузины травянистой (*Sambucus ebulus* L.) // Нəyat elmləri və biotibb jurnalı, – 2019, 74(2), – s. 28-32.

²¹Zulfugarova, M.B., Novruzov, E.N. Flavonoids of flowers of *Sambucus nigra* L.// International Conference Innovative Approaches to Conservation of Biodiversity, – Baku, – 2016, – p. 125.

III, VII, IX and XIII SP the largest amount of flavonoids was noted in the leaves (1.82%) and flowers (1.23%), and the smallest in the roots (0.23% of the air-dry weight). The content of flavonoids in leaves and flowers shows that the leaves and flowers of elderberry herb can become a source of raw materials for obtaining flavonoid preparations.²²

5.3.3. Investigation of the dynamics of accumulation of flavonoids in species of the *Sambucus L. genus*

Taking into account the use of *S. nigra* and *S. ebulus* species as a source of raw materials, the main goal is to determine the regularity of accumulation of flavonoids depending on the phase of development, their distribution in various organs, productivity of biodiversity, flavonoid yield, optimal time of accumulation of flavonoids.

The study of the dynamics of the accumulation of the amount of flavonoids in the species of the *Sambucus* genus, depending on the phases of plant development in various organs, showed that during the growing season the amount of flavonoids undergoes a sharp change. The maximum amount of flavonoids accumulates in the flowers I, VI, VII and X SP- (7.34; 5.58%) in the generative organs the minimum amount is in green fruits (1.91; 2.83%, respectively) was found that in the studied *S. nigra* and *S. ebulus* species. The maximum amount of flavonoids in flowers and leaves of the *S. nigra* species accumulates at the beginning of the flowering phase (7.57; 4.94%), and *S. ebulus* - in the phase of mass flowering (4.91; 5.49%, respectively).

The minimum amount of flavonoids in both species accumulates in the leaves (3.03; 3.06%) in the phase of fruit ripening. In comparison with other vegetative organs, flavonoids accumulate in the roots little (0.67%). From the dry ground parts of both species, the flavonoid can be obtained in maximum quantity during the full bloom phase (480g / 100m² and 335.9 g/100m²).

²²Зульфугарова, М.Б., Новрузов, Э.Н. Состав флавоноидов листьев *Sambucus nigra L.* // Műasir kimya və biologiyanın aktual problemləri, – Gəncə, – 2016, – с. 302-305.

5.4. Study of the qualitative composition and quantitative content of anthocyanins of ripe fruits of species of the *Sambucus L. genus*

Chromatographic analysis of the acid hydrolyzate of the sum of anthocyanins showed the presence of one aglyconocyanidin. Three chromatographically pure anthocyanins and designated them as substances A, B, C. During acid hydrolysis of the sugar part of substances A and B, one substance was found – glucose was obtained. Spectral analyzes and acid hydrolysis and their comparison with the authentic literature data, substance A was identified as cyanidin-3-glucoside. Substance B and - as cyanidin-3,5-diglucoside. substance V - as cyanidin-3-sambubioside according to the results of chromatographic (Tab. 3 and 4).²³

Table 3.

Chromatographic characteristics of individual anthocyanins

Substances	Rf values in systems			Staining	
	I	II	III	in visible light	in UV light
Substance A	0,38	0,25	0,06	fuchsin	dull fuchsin
Substance B	0,26	0,41	0,19	fuchsin	gray-fuchsin
Substance C	0,35	0,62	0,16	fuchsin	sulfur-fuchsin

Table 4. Spectral characteristics of isolated anthocyanins

Substances	λ_{\max} нм			E_{440} E_{\max}
	methanol containing 0.1% HCl	ethanol containing 0,1 % HCl	methanol + 3% AlCl ₃	
Substance A	280,525	535	543	22
Substance B	278,524	531	540	13
Substance C	282,526	536	541	25

²³Зульфугарова, М.Б., Новрузов, Э.Н. Состав и содержание антоцианов плодов *Sambucus ebulus* L. // Химия растительного сырья, – 2017, №1, – с. 163-167.

The composition and content of anthocyanins in the fruits of herbaceous elderberry growing in Azerbaijan have been established for the first time.

Comparative chromatographic analysis of the sum of anthocyanins isolated from the fruits of *S. nigra* and *S. ebulus* showed the identity (3 anthocyanins) of their composition, but they differ in the ratio of individual components. It was established by paper chromatography that the fruits of *Sambucus ebulus* contain three anthocyanin substances.²³ The bulk of anthocyanins are cyanidin-3-sambubioside (67% of the total) and cyanidin-3-glucoside. *Sambucus ebulus* and *S.nigra* are highly anthocyanin species (2181.4-3124.0 mg%, respectively). The smallest amount of anthocyanins was found in the fruits of *Sambucus ebulus* (2181.4 mg%).

5.5. The influence of the place of growth, meteorological conditions, soil-ground, hydrological factors on the content of vitamin C.

The relationship between the height of the area of plant growth and the accumulation of vitamin C has been established. The data obtained show that with an increase in the height of the area, the content of ascorbic acid in fruits and berries increases due to its reduced form, with a simultaneous decrease in the oxidized form. Studies have shown that, depending on the place of growth, the content of ascorbic acid varies significantly. In the I, X, vø XII SP the fruits of *Sambucus ebulus* and *Sambucus nigra* accumulate more ascorbic acid (58.4; 382.7 mg%, respectively) than in the II, VIII, vø IX SP (35.3; 225.2 mg%, respectively).

CHAPTER VI

IMPROVEMENT OF INTENSIFICATION OF TECHNOLOGY FOR OBTAINING BIOLOGICALLY ACTIVE CONCENTRATES FROM PLANT RAW MATERIALS

Flavonoids and anthocyanins, have the ability to increase the strength and elasticity of blood vessels (especially capillaries), have a preventive and therapeutic effect in atherosclerosis, hypertension, radiation injuries, capillaries, toxicosis. Therefore, the problem of finding polyphenol-containing sources, the development of a rational method for obtaining biologically active concentrates, food additives, as well as medicinal preparations is very relevant at the present stage.

We have developed a technology for producing biologically active concentrates (BAC) with high biologically active substances.²⁴ In order to intensify the return juice and reduce the viscosity of the juice, as well as reduce the time of the technological process, we used: Enzyme preparation "Frutozim-Color". The results of the study of fermentation show that it is advisable to carry out the enzymatic hydrolysis for 1.5 hours at a dosage of the enzyme preparation of 2.2 units of PCA / g of pectin. At the same time, the yield of juice from elderberries increases to 33%, the viscosity decreases by 85%. The resulting concentrate has good organoleptic and physicochemical characteristics, a high content of biologically active substances, in particular anthocyanins, catechins, vitamin C, which make it possible to use them as food additives, as well as for the production of soft drinks with high biological properties.

²⁴Зульфугарова, М.Б., Новрузов, Э.Н., Мамедова, Ш.М. Совершенствования интенсификации технологии получения биологически активных концентратов из растительного сырья // *Müasir kimya və biologiyanın aktual problemləri, Botanika, Ümumi Biologiya, Mikrobiologiya, Aqrar elmləri*, – Gəncə, – 2015, – с. 133-144.

CHAPTER VII

PHARMACOLOGICAL STUDY OF EXTRACTS OF FLOWERS, LEAVES AND FRUITS OF BLACK ELDER

7.1. On the background of the experimental model of DM, the study of the effect of extracts of flowers, leaves and fruits of black elderberry on some pathologically changed parameters.

The experiment was carried out on 53 white outbred male rats (48 rats with experimental diabetes and 5 rats in an intact state).

7.1.1. Results of determination of glucose in blood

There is a dynamics of a decrease in glucose in the blood of animals under the influence of extracts of flowers, leaves and fruits of black elderberry against the background of alloxan diabetes. Analysis of the data obtained shows that against the background of experimental DM, extracts of flowers and leaves of black elderberry, to a small extent, but statistically significantly, reduce the glucose content in the blood of animals, and the extract of plant fruits does not have a pronounced effect on this indicator.

7.1.2. Results of determination of lipids and triglycerides in blood

Studies have shown that against the background of alloxan diabetes, the use of an extract of black elderberry flowers leads to the normalization of lipid metabolism and the effect increases with time, so all the studied parameters, compared with the 3rd day of using the extract, positively changed on the 7th day. The investigated black elderberry extracts have a pronounced effect on lipid metabolism. They are most effective in reducing blood triglycerides.

7.1.3. Results of determination of liver damage markers in blood

The results of determining the content of liver damage markers in the blood of animals - alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), show that the studied extracts of black elderberry have a significant effect on liver parameters. They all reduce the content of ALT and AST in the blood of experimental animals. The detoxification function of the liver was improved by extracts of flowers and fruits of the plant,

while the leaf extract, on the contrary, by increasing the content of TB, had a negative effect on this function of the liver. Thus, improving the state of the enzymatic system of the liver tissue.

7.1.4. Results of determination of LPO products and catalase activity in blood

The investigated extracts of black elderberry leaves have a significant effect on the severity of oxidative stress. The extracts of flowers and fruits, in contrast to the extract of leaves, significantly reduce the content of DC and MDA in the blood of experimental animals.^{25,26,27}

7.2. Study of the effect of extracts of flowers, leaves and fruits of black elderberry on the functional state of the liver against the background of an experimental model of toxic hepatitis

It was revealed that black elderberry extracts have a beneficial effect on the functional state of the liver in experimental diabetes mellitus.

7.2.1. The results of determining the indicators of the functional state of the liver in the blood

Research results have shown that all black elderberry extracts improve the functional state of the liver against the background of experimental toxic hepatitis. The beneficial effect is associated with an improvement in the state of the enzymatic system, which is confirmed by a significant decrease in ALT and AST in the blood of animals, as well as an increase in the detoxification function of the liver, as evidenced by a decrease in the content in the blood.²⁸

²⁵Зульфугарова, М.Б. Действие экстрактов Бузины черной на содержание глюкозы в крови на фоне экспериментальной модели сахарного диабета// Sağlamlıq, – 2017, №3, – с. 145-149.

²⁶Зульфугарова, М.Б. Перспективныиспользования бузины черной при сахарном диабете // Təbabətın aktual problemləri, – Bakı, – 2017, – с. 182.

²⁷Зульфугарова, М.Б. Выявление гепатопротекторного действия Бузины черной / М.Б.Зульфугарова, Э.Н.Новрузов, Р.Э.Джафарова [и др.]// Təbabətın aktual problemləri, – Bakı, – 2017, – с. 183.

²⁸Джафарова, Р.Э., Зульфугарова, М.Б., Джавадова Г.Ч.Исследование действия экстрактов цветков, листьев и плодов бузины черной на функциональное состояние печени на фоне экспериментальной модели токсического гепатита // Вестник Российской Военно-Медицинской Академии, – 2017, №1(57), – с. 124-128.

RESULTS

1. Two species of the *Sambucus* L. genus grow in the flora of Azerbaijan. *Sambucus nigra* and *S. ebulus* have 3 life forms (compact aërosilicus shrub, diffuse aërosilicidal shrubs and saplings).

2. A high degree of correlation ($r=+0.76$) was revealed between the amount of precipitation at the beginning of the precipitation phase and the date when the black elderberry entered the phase of the start of shoot growth. Species of the *Sambucus* genus are characterized by a mesosympodial growth; elderberry shoot formation is preserved under different cenotic conditions.

3. As a result of a comparative analysis of the morphological characteristics of plants of different populations, populations with a low and a high degree of variability in the morphology of the elderberry vegetative organs were identified.

4. *Sambucus nigra* occupies a rather large territory - 54512.3 hectares, the biological reserve is 252 tons, the operational reserve is 182 tons was revealed. *S. ebulus* occupies 298 hectares of land, the biological reserve of fruits is 16.5 tons, and the operational reserve is 12,8 tons The average yield of elderberry fruit depends on the place of growth of the plant and varies from 87.6 to 752.7 g / m².

5. The phytochemical composition of species of the *Sambucus* L. genus was studied for the first time in 13SP. Fuits of *S. nigra* and *S. ebulus* contain dry matter (17.8 -20.9%), carbohydrates (glucose, fructose, sucrose, rhamnose was found. Ramnose was discovered for the first time, 4.80, - 5.20 mg%. organic acids 1.0%, - 1.20% (tartaric, acetic, citric, malic); ascorbic acid 42.4-382.0 mg% . catechins 200.0-285.4%, as well as leukoanthocyanins 808.0-857.0%, anthocyanins 2181.4-3124.0 mg%, flavonoids 270.3-415.4 mg%, respectively

6. Composition of the sum of anthocyanins includes 3 derivatives of cyanidin, which are identified as cyanidin-3-glucoside, cyanidin-3,5 diglucoside and cyanidin-3-sambubioside was revealed. The bulk of anthocyanins are cyanidin-3-sambubioside (67% of the total) and cyanidin-3-glucoside.

7. For the first time, the presence of (+)-catechin, (-) epicatellate, (-)epicatellate, (-) galatechin, (-) epigallacatechin and (-) - epigallacatellate was found in ripe fruits of *Sambucus nigra* and *Sambucus ebulus*.

8. The flavonoid composition of the *Sambucus nigra* species contains rutin, isoquercitrin, quercetin; in *Sambucus ebulus* - rutin, narcissin, quercetin. The maximum amount of flavonoids accumulates in flowers. flowering (7.34; 5.58%, respectively).

9. The total sugar content increases by 2.1-2.844; organic acids - 1.4-2.3; vitamin C - 1.5-2.2; polyphenols - 1.8-2.4; anthocyanins - 1.7-2.6; catechins 1.8-2.2 times when treated with a pectolytic enzyme and ultrasound, compared to juice without treatment, depending on the type of plants. The obtained data is possible to use them as food additives, as well as for the production of soft drinks with high biological properties.

10. Against the background of experimental DM, extracts of flowers and leaves of *Sambucus nigra* reduce the content of total cholesterol, glucose triglycerides in the blood of animals, and have a pronounced effect on lipid metabolism.

11. Extracts of flowers and fruits of *Sambucus nigra* had a positive effect on the detoxification function of the liver. and the leaf extract, on the contrary, increased the UB content and negatively influenced this liver function, but had a positive effect on the enzymatic system of the liver tissue

12. The studied extracts of leaves of *Sambucus nigra* have a significant effect on the severity of oxidative stress. Unlike leaf extract, flower and fruit extracts significantly reduced the amount of DK and MDA in the blood of animals.

PRACTICAL RECOMMENDATIONS

1. Workshops for the processing of wild fruits are necessary to build and to provide the food, pharmaceutical and perfumery-cosmetic industries with food additives and biologically active concentrates. Industrial plantations with selected species of the *Sambucus* L. genus are necessary to establish.

2. Fruits of the most valuable species of the genus *Sambucus* L. containing a high amount of nutrients and biologically active substances should be used to obtain food additives and biologically active concentrates.

3. Collect plant material in the phase of mass flowering, or technical maturity of the fruit.

4. The results of the analysis of the content of biologically active and nutrient substances of the species of the *Sambucus* L. genus showed the prospect of using the plant for medical purposes in the treatment and prevention of various, as well as viral diseases.

5. Use the research results when giving lectures on botany and pharmacognosy in the biological and pharmaceutical faculties of the respective universities, as well as in the preparation of new publications on medicinal plants and resources of Azerbaijan and the flora of Azerbaijan.

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