

# Methods of obtaining products from generative organs of *Sambucus nigra* L. taking into account the biological properties of the plant cell

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**Abstract.** In Armenia, the species *Sambucus nigra* L. is found in the undergrowth of the lower and middle forest belts of the northern (Lori, Tavush) and southern regions (Zangezur). Black elderberry is almost never used in Armenian folk medicine. In Armenia, research into obtaining beverages from the generative organs of the black elderberry plant is at an early stage. The aim of the study is to identify methods for obtaining products from the generative organs of black elderberry (*Sambucus nigra* L.), taking into account the biological properties of the plant cell. Thus, our proposed method for obtaining beverages from the generative organs of black elderberry plants has a number of advantages: a wide range of beverage applications with comparative product safety; lower risk of changes in taste over a long period of time under the influence of environmental factors; the pH level of the resulting liquid product does not change for three years, which is the most important indicator of the stability of the quality and safety of the beverage; no artificial additives; no heat treatment; the beverage production process is simplified, one-stage; beverages are available for the general public to prepare; the finished beverage has a low cost price.

## 1 Introduction

At the end of the 20th century, due to the energy crisis in Armenia, particularly in the Lori region, illegal logging of forest-forming species became widespread. As a result, in a short period of time, the least valuable species, undemanding to growing conditions, settled in the clearings. One of such species is Black elderberry (*Sambucus nigra* L.). The species belongs to the genus *Sambucus* L., family *Adoxaceae* [1]. Black elderberry is widespread in the Caucasus, Western Europe, North Africa, and Crimea. Shade-tolerant and moisture-loving plant. In the lower and middle mountain zones, there is also a variety of black elderberry *S. nigra* f. *laciniata* (L.) Zabel, which is a beautifully flowering park plant [1]. In the city of

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Vanadzor, Lori region, this variety is found on the territory of the Armenia sanatorium in a single specimen [2, 3].

Since the end of the 19th century, numerous studies have been devoted to the study of the distribution of the species *Sambucus nigra* L. [4]. In central Europe, Holland, Germany, the species is considered aboriginal [5, 6]. In the Baltic countries, there are scattered populations of the species: throughout Latvia (usually in the southern and western parts of the country), western Estonia [7]. In Russia, the plant was first described and introduced into cultivation in the 80s of the 19th centuries. Later, natural populations were formed [8, 9]. In Central Asia, it is cultivated as an ornamental plant [10].

Black elderberry is a medicinal plant. All generative organs have medicinal properties [11]. Also, drinks prepared from the leaves, roots, and bark of the plant have medicinal value [12]. Black elderberry flowers contain sambunigrin, amygdalin, which when broken down form acids, benzaldehyde, glucose, vitamin C, rutin, resin, essential oils (up to 32%), choline, calcium salts. The fruits contain pigments from the anthocyanin group, ascorbic acid, carotene, rutin, etc. [11].

An analysis of scientific literature has shown that studies of obtaining drinks from generative organs and studying their properties have acquired a large scale abroad [13, 14, 15, 16]. Foreign scientists have studied the medicinal properties of black elderberry, during which they determined the antioxidant, antibacterial, antiallergic properties of the plant [17, 18, 19], and studied the properties of drinks obtained from generative organs [15]. During COVID-19, foreign scientists have conducted interesting studies to study the immunomodulatory and antioxidant properties of the extract obtained from the organs of the black elderberry plant. Use of the extract for 30 days by patients with coronavirus infections helped to reduce the symptoms of the disease [20, 21, 22].

In studies by some authors, the antioxidant properties of extracts obtained from fresh and dried generative organs of the plant have been compared [23]. Some works are devoted to the study of the chemical composition of the generative organs, including the content of rutin [24], anthocyanin in flowers [25] and the sugar content in the tissues of the black elder plant, as well as the histological and morphological properties of the plant [26, 27].

The methods of preparing drinks from the generative organs of black elderberry known abroad differ from each other in the complexity of the process due to the use of many components, temperature treatment, and multi-stage nature (Patents RU 2579878, RU 2579893). Thus, to prepare diabetic tea (Antaram), it is suggested to weigh 20 g of raw material, add 200 ml of hot water and boil for 30 minutes. Infuse for 10 minutes, strain, bring to 200 ml with boiled water. However, when boiling, the useful substances contained in the drink decompose. Therefore, the method of obtaining drinks proposed by us does not involve boiling the raw materials.

There are various methods of preparing water-based drinks in the literature, where black elderberry and immortelle flowers are mixed in a 1:2 ratio, infused in red wine, to which a 50% alcohol solution is added, filtered and ethyl alcohol is added (Patent RU 2579893). The disadvantage of this method of obtaining is that it is multi-stage and multi-component. There are other methods of obtaining drinks from black elderberry flowers, for example, fresh flowers are mixed in a 1:10 ratio with dry white grape wine powder, kept at a temperature of 20...25°C for 5 days. After which, they are mixed with a 50% alcohol solution and infused again for 5...7 days (Patent RU 2597286). All methods are multi-stage, expensive, and an alcohol solution is used at all stages. In Armenia, similar studies are at an early stage. An analysis of literary data showed that since 2015, studies of bioecological properties [28, 29] have been conducted in Lori Marz, the distribution of life forms of black elderberry is being studied, and methods for obtaining drinks from the generative organs of the plant are being developed [2]. Research is also being conducted in the republic to study the antibacterial

activity of drinks obtained from flowers and fruits of black elderberry, and biometric indicators of the plant [30].

The aim of the study is to identify methods for obtaining products from the generative organs of black elderberry (*Sambucus nigra* L.), taking into account the biological properties of the plant cell.

## 2 Materials and methods

The object of the study is plants of the species *Sambucus nigra* L. Some properties of products obtained from the generative organs of black elderberry collected from three different experimental sites were studied: from the territory of Lori region (Vanadzor 1326...1600 m above sea level and Stepanavan 1400...1830 m above sea level), Tavush region (surrounding forests of the city of Dilijan 1240...1612 m above sea level).

Considering the difference in climatic conditions, it was necessary to compare some ecological parameters. On each experimental site, 10 black elderberry trees were selected, from which generative organs were collected: flowers and fruits. Later, products (tincture, juice, syrup, tea) were obtained from them, some physicochemical parameters were determined (pH was determined by a device (Milwaukee MI-150), sucrose content was determined by a refractometer (Digital Brix Refractometer Milwaukee MA871). Statistical processing of the obtained data was performed using the Microsoft Excel 2010 program. The measurement results are presented as averages and their standard errors.

## 3 Results and discussion

The results obtained showed significant differences between the methods of obtaining beverages that we proposed. In our proposed method, preference is given to 17% white wine, without adding water (Patent RU 2597286). The production mechanism is the most simplified, providing for the production of environmentally friendly products. The process takes place at room temperature, in one stage. The beverages do not contain strong alcoholic additives, consist only of natural ingredients, are not subject to heat treatment, and the taste qualities of the finished product of black elderberry are preserved (The ABC clinical guide to Elder Berry European Elder Berry).

When developing a method for obtaining drinks (tincture, juice) from the generative organs of black elderberry, similar methods of foreign and domestic authors were previously studied (Patents RU 2579878, RU 2579893, Antaram Diabetic Tea, The ABC clinical guide to Elder Berry European Elder Berry). The methods of processing the raw materials were pre-selected: without lemon, with lemon, with water, without white wine, with white wine, for a period of 5, 10, 15, 20...25 days (Table 1 and Table 2).

In all variants, souring, mold formation, and an unpleasant odor of the resulting product were observed. After studying numerous variants, we chose the following method for obtaining the drink: production temperature of 18...22°C, using white wine "Ijevan" and lemon. After 21 days of aging the raw materials, a product was obtained without subsequent changes in organoleptic properties. For 3 years (2021...2023), the liquid product is stored in a glass container, without changing transparency, color, taste, smell. The quality of the finished product is determined by the fact that it is obtained without preliminary sterilization, at a relatively stable temperature. Many years of experience in obtaining a liquid product have shown that the best ratio for preparing 1 liter of the drink is 1 part flowers : 0.7...0.8 lemon : 1.7...1.8 wine (Patent RU 2597286).

When preparing the raw materials, the biological characteristics of the fruits (tender pericarp) and inflorescences (early petal fall) are taken into account. The inflorescences of

the plants are collected in dry, sunny weather. The flowers should be fully open. The peduncles of the inflorescences are carefully cut with scissors, since the flowers fall off from overripe inflorescences. The cut inflorescences are placed in dry paper bags to avoid losses during transportation. The flowers spoil quickly, so they are dried in the shade, at a temperature of +15...18°C, in a well-ventilated room, laid out in one layer on filter paper, stirring to speed up the drying process. During this time, the bitter taste disappears.

**Table 1.** Changes in organoleptic properties during the preparation of flower products.

| Organoleptic properties | Raw material without lemon, with white wine |          |              |              | Raw material with lemon without white wine |                 |              |         | Raw materials with white wine and lemon |           |           |           |
|-------------------------|---|----------|--------------|--------------|--|-----------------|--------------|---------|---|-----------|-----------|-----------|
|                         | Duration, days                              |          |              |              | Duration, days                             |                 |              |         | Duration, days                          |           |           |           |
|                         | 5   | 10       | 15           | 20...25      | 5  | 10              | 15           | 20...25 | 5                                       | 10        | 15        | 20...25   |
| Smell                   | Strong                                      | Low      | Putrefactive | Putrefactive | Strong                                     | Low             | Putrefactive | Moldy   | Strong                                  | Mixed     | Good      | Berry     |
| Color                   | Yellow                                      | Dark     | Dark yellow  | Brown        | No   | No              | Light brown  | Brown   | Yellowish                               | Yellowish | Yellowish | Yellowish |
| Transparency            | Clear                                       | Clouding | Turbid       | Turbid       | Transparent                                | Not transparent | Turbid       | Turbid  | Clear                                   | Clear     | Clear     | Clear     |
| Sediment                | No  | No       | Weak         | Strong       | No   | No              | Weak         | Strong  | No                                      | No        | No        | No        |
| Suspension              | No  | Weak     | Yes          | Yes          | No   | слабая          | Yes          | Yes     | No                                      | No        | No        | No        |

It should be noted that during the preparation period (21 days), white wine and lemon help improve the taste. After this, the inflorescences are placed in layers in a 3-liter glass container. Each layer contains 5 inflorescences. Three lemon slices are added to the layer of inflorescences. Then the following layers are laid until the container is full. Dry white grape wine is poured in until the mass is completely covered, and the lid is closed. Store for three weeks in the light, at room temperature +18...22°C. After this, the resulting mixture is filtered (Patent RU 2597286).

The method of making drinks that we propose is based on the semi-permeability of the plasma membrane of the plant cell. From the cell juice of plant tissues, due to diffusion, useful substances pass into the extract. Various types of drinks can be obtained from the extract.

To prepare drinks, if necessary, add water and sugar to taste to the resulting extract. First, put the extract on low heat, stir, add sugar, do not bring to a boil. Heat treatment of the extract is unacceptable, since the biologically active substances in its composition are destroyed. The resulting solution must be cooled to room temperature, filtered and poured into a glass container, tightly sealed with a cork, stored in a cool place. Various drinks (juice, syrup, tincture, tea) can be prepared not only from fresh, but also from dried flowers of *Sambucus nigra* L., using the method described above.

To dry the flowers, remove all the peduncles, as they take longer to dry than the petals. After 3...4 days, the flowers are collected and stored in paper or gauze bags. The bags are kept in a dry, ventilated room throughout the winter (Patent RU 2597286).

Fresh and dried fruits of black elderberry plants can also be used as raw materials for making drinks. The fruits are collected after full ripening (August and September). Depending on the type of finished product (juice, syrup), a certain amount of water and sugar is added to the extract, as indicated above.

**Table 2.** Changes in organoleptic properties during the preparation of a fruit product.

| Organoleptic properties | Raw material without lemon, with white wine |           |                |              | Raw material with lemon without white wine |                  |              |            | Raw materials with white wine and lemon |       |       |           |
|-------------------------|---|-----------|----------------|--------------|--|------------------|--------------|------------|---|-------|-------|-----------|
|                         | Duration, days                              |           |                |              | Duration, days                             |                  |              |            | Duration, days                          |       |       |           |
|                         | 5   | 10        | 15             | 20...25      | 5  | 10               | 15           | 20...25    | 5                                       | 10    | 15    | 20...25   |
| Smell                   | Strong                                      | Low       | Acet-mentation | Putrefactive | Strong                                     | Low              | Putrefactive | Moldy      | Strong                                  | Mixed | Good  | Berry     |
| Color                   | Red   | Dark red  | Pronounced     | Pronounced   | Dark red                                   | Bur-gundy        | Pronounced   | Pronounced | Dark                                    | Dark  | Dark  | Bur-gundy |
| Transparency            | Clear                                       | Clou-ding | Turbid         | Turbid       | Trans-parent                               | Not trans-parent | Turbid       | Turbid     | Clear                                   | Clear | Clear | Clear     |
| Sediment                | No  | No        | Weak           | Strong       | No   | No               | Weak         | Strong     | No                                      | No    | No    | No        |
| Suspension              | No  | Weak      | Yes            | Yes          | No   | слабая           | Yes          | Yes        | No                                      | No    | No    | No        |

*Method of drying fruits.* Fruits are dried for 10 days at a temperature of +30...35°C. Dry raw materials are stored in paper bags for up to six months. Fresh fruits have a bitter taste, when used they cause unpleasant sensations, and with proper drying of fruits the unpleasant taste disappears (Patent RU 2597286).

The method of obtaining drinks that we offer has advantages: they contain only natural ingredients, are not subjected to heat treatment during preparation, can be consumed both warm (without bringing to a boil) and cold.

Local raw materials obtained from the generative organs of black elderberry have a low-cost price, are environmentally friendly, and are the basis for obtaining various drinks. The products have a tonic and anti-stress effect, are used to improve well-being, increase endurance, and strengthen the immune system [14].

Below are specific examples of obtaining environmentally friendly natural products from the generative organs of the black elderberry plant. Based on our experimental studies, we offer several options for obtaining drinks without heat treatment.

*Example 1.* 300 g of black elderberry flowers, 2 lemons and 0.5 l of dry white grape wine. Infuse at a temperature of 18°C for 20...25 days. The mass ratio of flowers, lemon and wine is 1.0 : 0.5 : 1.6. Obtain 800 ml of filtrate.

*Example 2.* 300 g of black elderberry flowers, 2 lemons and 0.7 l of dry white grape wine. Infuse at a temperature of 22°C for 20...25 days. The mass ratio of flowers, lemon and wine is 1.0 : 0.8 : 1.8. Obtain 1000 ml of filtrate.

*Example 3.* 500 g of black elderberry berries, 2 lemons and 0.6 l of dry white grape wine. Infuse at a temperature of 20°C for 20...25 days. The mass ratio of fruits, lemon and wine is 1.0 : 0.8 : 1.8. Obtain 1000 ml of filtrate.

We also studied the refractive indices of the solution, sucrose content (%), and pH values of the drinks. Test samples of drinks from black elderberry inflorescences and fruits were prepared in the laboratory of biological and ecological research at Vanadzor State University. Using a refractometer, the amount of sucrose in the drink for the first example was determined. The results are presented in Table 3.

**Table 3.** Determination of sucrose and pH in beverages prepared from fruits and flowers of the plant *Sambucus nigra* L.

| Experimental plot   | Drinks made from samples of fruits and flowers |                     |             |
|---------------------|--|---------------------|-------------|
|                     | Fruit drinks                                   |                     |             |
|                     | Refractive index                               | Sucrose content (%) | pH          |
| 1. Vanadzor         | 1.352  | 14±0.26             | 5.13±0.04   |
| 2. Stepanavan       | 1.346  | 11±0.48             | 5.14±0.05   |
| 3. Dilijan          | 1.356  | 17±0.53             | 4.78 ± 0.11 |
| Drinks from flowers |  |                     |             |
| 1. Vanadzor         | 1.356  | 17±0.48             | 5.08±0.03   |
| 2. Stepanavan       | 1.368  | 24±0.58             | 5.06±0.04   |
| 3. Dilijan          | 1.362  | 20±0.52             | 5.10±0.02   |

The drink obtained from the fruits of the third experimental plot had the highest sucrose content: 17%. It exceeds the sucrose values obtained from the first plot by 1.21 times, from the second by 1.54 times. The deviation from the standard is ±3. Based on the data obtained, the arithmetic mean error was also determined. It was 1.73.

The highest sucrose content was determined in the flower drink obtained from the second section: 24%. It exceeds the sucrose values obtained from the first section by 1.41 times, and from the third section by 1.2 times. The deviation from the standard is ±2. Based on the data obtained, the arithmetic mean error was also determined. It is 1.66.

The peculiarity of the method of the drink we obtained is that the average sucrose content in the liquid product remains unchanged for three years (in flowers – 20.3%, in fruits – 14%).

The highest pH value was determined in the drink obtained from fruits from the second experimental plot: 5.14. It exceeds the pH values obtained from the first plot by 1.001, and from the third – by 1.075 times. The deviation from the standard is ± 0.205. Based on the data obtained, the arithmetic mean error was also determined. It was 0.118.

The highest pH value was determined in the drink obtained from the flowers of plants from the third plot: 5.10. It exceeds the pH values of drinks obtained from the first plot by 1.003 times and from the second – by 1.007 times. The deviation from the standard is  $\pm 0.02$ . Based on the data obtained, the arithmetic mean error was also determined. It was 0.012.

The peculiarity of the method of the drink we obtained is that the average pH in the liquid product remains unchanged for three years (in flowers – 5.08, in fruits – 5.01). The constancy of the pH level of the product indicates the stability of the quality and safety of the drink [31].

## 4 Conclusions

Thus, our proposed method for obtaining beverages from the generative organs of black elderberry plants has a number of advantages: a wide range of beverage applications with comparative product safety; lower risk of changes in taste over a long period of time under the influence of environmental factors; the pH level of the resulting liquid product does not change for three years, which is the most important indicator of the stability of the quality and safety of the beverage; no artificial additives; no heat treatment; the beverage production process is simplified, one-stage; beverages are available for the general public to prepare; the finished beverage has a low cost price.

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