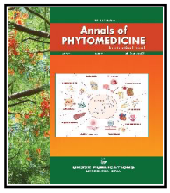
Annals of Phytomedicine 13(2): 1169-1173, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.122

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Morpho-anatomical structure and diagnostic characterization of above-ground organs of *Sambucus nigra* L.

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Article Info

Article history
Received 8 July 2024
Revised 29 August 2024
Accepted 30 August 2024
Published Online 30 December 2024

Keywords
Sambucus nigra L.
Morphology
Anatomy
Lenticels
Crystal salt
Epidermis
Parenchyma
Endocarp

Abstract

Morphological structure, medicinal properties, significance of *Sambucus nigra* L. - black-coral plant in agriculture, pharmaceutical, food industry, anatomical structure of branches, fruits and seeds and diagnostic features in conditions of Uzbekistan were analyzed. As a result of research in the study of the anatomical structure of the cross-section of the branch, fruit and seeds of *S. nigra* it was identified the presence of linear lenticels on the outer surface of the branch, small hairs in the cells of the epidermis, small parenchyma cells and the presence of crystalline salts in them, the fruit consists of pericarp, the absence of intercellular spaces, the seed and it became known that it is located inside the endocarp, the hardness of the seed and the fact that it is not isolated, is a diagnostic sign of the species.

1. Introduction

Sambucus nigra L. - Black coral is a shrub 2-6 m tall (Tsitsin, 1962; Lyubov Nikolaevna Skrypnik and Alina Andreyevna Kurashova, 2019). The stem is thin, white flesh with a soft core, woody and branched. Young branches are green, brownish-grey and yellowish in colour. Leaves on the stem are tapering, 10-30 cm long, consisting of pointed and fleshy oblong-ovoid plates. Flowers are yellowish-white, tumour-shaped, 5-8 mm in diameter. Its fragrant flowers are gathered in brushes, 10-25 cm in diameter. It blooms in May and June. The fruit is a blackberry 5-7 mm in diameter, consisting of two to four seeds. Ripe fruits can be eaten; they taste sour-sweet.

The flowers and fruits of the plant are considered raw materials (Vandyshev *et al.,* 2019). This plant is of great importance and is widely used in the national economy, pharmaceuticals, and food industry. The presence of antioxidants and phenolic compounds and high antioxidant activity have been found in the leaves, branches, flowers and fruits. Also, many studies confirm that black coral is a source of many important bioactive compounds (Barak *et al.,* 2001; Tatvidze and Aleko, 2013; Vrchotova *et al.,* 2019). Therefore, we had a task to study the anatomical structure of the stem, fruits and seeds of black coral, which has not been studied in the conditions of Uzbekistan, and to determine the diagnostic features.

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2. Materials and Methods

Fruits of *Sambucus nigra* L. were collected from June to September 2023. For the study, 30 pieces were selected from 100 collected mature fruits and measurements of length, width, and weight of fruits and seeds were made. The appearance of fruits was studied and sketched. Morphological descriptions and measurements of fruits and seeds were carried out according to generally accepted methods (Morozova *et al.,* 2010). Dimensions were also determined using millimetre paper and a ruler.

Seeds of *S. nigra* were collected in September 2023. Four model plants of black elderberry, which are large shrubs up to 4 m high, were selected for the study. The number of seeds on the model plants was counted without plucking them from the bush. Ten seeds were selectively taken from each shrub. Out of 100 seeds collected, 30 seeds were selected and measurements of diameter, length and width were made. For each parameter, statistical characteristics were calculated according to generally accepted methods (Takhtajyan, 1981).

To study the anatomical structure of the above-ground organs of *S. nigra*, it was fixed in 70% ethanol in the flowering phase of the generative period of the plant. Manual cutting made transverse sections of the above-ground organs of the plant from stem, fruits and seeds. The sections were stained with safranin dyes and sealed with glycerol-gelatin (Barykina *et al.,* 2004). The main tissues and cells of plant organs were described by Kiseleva (1971), epidermis by Zakharevich (1954), and types of mouthparts were described by Baranova (1981). Microphotographs were taken using a computerized photomicroscope, a Canon A123 digital camera, and a Motic B1-220A-3 microscope.

3. Results

The stem is green in color and has linear lenses on the outer surface, which perform the function of gas exchange in the stems with the secondary covering tissue-periderm (Efremov and Shreter, 1996). Gas exchange in the epidermis occurs through the stomata. But after the formation of periderm, the epidermis dies off and falls off, and the function of gas exchange is performed by lenticels. The cross section of the stem is rounded and has a dark color (Figure 1).

A transverse section of a branch of *S. nigra* shows that the bark is covered with a rectangular layer of epidermal cuticle on the outside. Trichomes in the epidermis are rare. Beneath the epidermis is a layer of phellem cells with a thick cortex and thin-walled phellogen cells branch from the base of the phellema. It contains the phelloderm tissue consisting of parenchyma cells. The middle part of the section is occupied by the medullary part, consisting of large and small parenchyma cells. We see that small cells of parenchyma located in the middle and peripheral part of the core are filled with "crystal salts", which is considered to be an anatomical peculiarity of the genus.

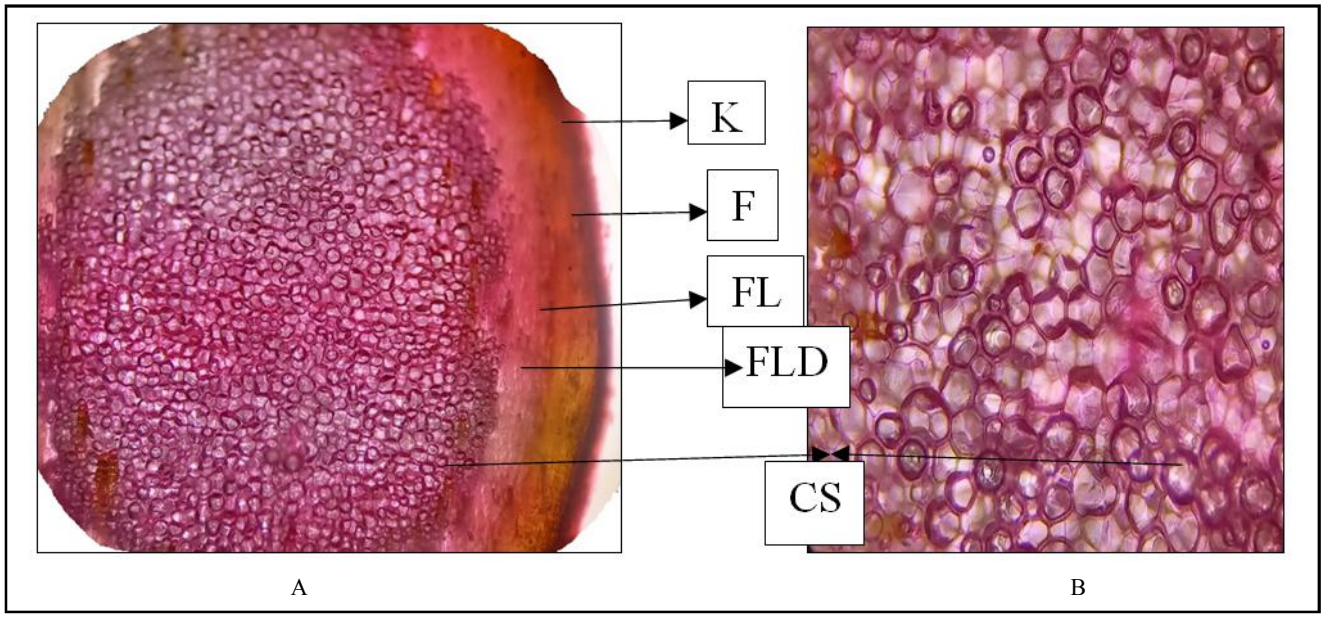

Figure 1: Cross section of a branch of *Sambucus nigra* L.: A - general view, B - enlarged view. K - cuticle, F - phellema, FL - phellogen, FLD - phelloderm, CS - crystal salts.

Table 1: Anatomical parameters of the branch of the plant *Sambucus nigra* L.

S.No.	Parameters	Indicators, µM
1.	Height of the epidermis	11,2 ± 0,3
2.	Width of the epidermis	9,1 ± 0,2
3.	Thickness of the cuticle	2,1 ± 0,2
4.	Thickness of the feloma tissue	13,2 ± 0,4
5.	Thickness of phellogenic tissue	10,1 ± 0,2
6.	Thickness of the phelloderm tissue	12,3 ± 0,4
7.	Core width	11,3 ± 0,2
8.	Length of the parenchyma	6,5 ± 0,3
9.	Width of the parenchyma	4,7 ± 0,2

As it can be seen from the table, the epidermis is small, its height is 11.2 ± 0.3 µM and its width is 9.1 ± 0.2 µM. It can be seen that the epidermis is covered with a cuticle, and its thickness is 2.1 ± 0.2 µM. The sizes of the phellema, phellogen and phelloderm are also shown in the table, they are continued by the nucleus and are 11.3 ± 0.2 µM. The parenchyma tissues are small, 6.5 ± 0.3 µM long and 4.7 ± 0.2 µM wide. Such characteristics, *i.e.,* the smallness of the tissues, indicate that this plant grew in dry conditions. Phellema (tissue) is the outer part of the plant sheath that protects it from the external environment. Phellema is formed from meristematic cells that differentiate into specialized cortical cells. This tissue plays a key role in protecting the plant from mechanical damage, pathogens and water loss. Phellogen (or cambial tissue) is a meristematic tissue that forms the secondary growth of plant stems and roots. The phellogen is arranged as a cylinder inside the bark and root and forms new cells in two directions: Inward (xylem) and outward (phloem). The plant can increase in size and thickness due to the phellogen.

The phelloderm is a layer of cells located between the phloem (vascular tissue that transports organic matter in the plant) and the xylem (vascular tissue that transports water and mineral salts). The phelloderm plays an important role in the transport and storage of substances in the plant.The fruits of *S. nigra* are also widely used. The fruit develops from the lower ovary of the flower. The fruit is black-purple, sometimes greenish-yellow, spherical in shape, 6-8 mm in diameter, and contains 3-5 seeds. The ripe fruits, which have a sweet-sour flavour, can be eaten. The flesh is dark red. It bears fruit in August-September. Unripe fruits contain alkaloids, which may cause stomach upset if eaten. By studying the anatomical structure of black coral fruits, we see that they are composed of pericarp (exocarp, mesocarp and endocarp) (Figure 2).

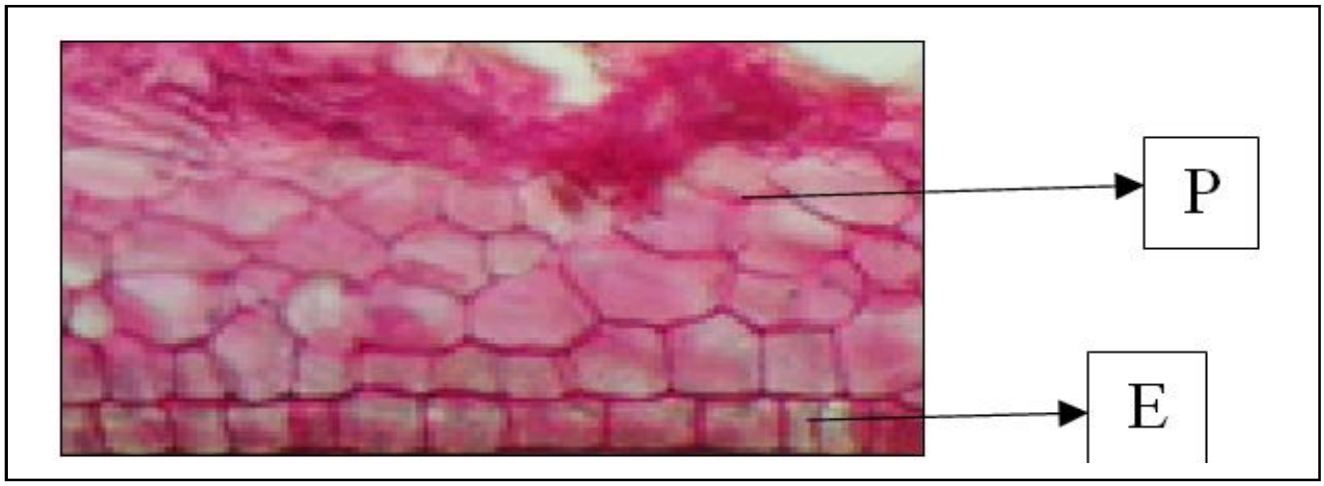

Figure 2: Cross section of the fruit of *Sambucus nigra* L.: E-epidermis, P-parenchyma.

The exocarp consists of 1-2 rows of parenchyma cells. Cells are thin-walled, densely packed, intercellular spaces are almost absent. The mesocarp (watery pulp) consists of 5-8 layers of thin-walled cells, with cells in the outer part of the mesocarp (closer to the skin) slightly different from those in the inner part (closer). The middle part of the endocarp contains the seed, which consists of a hard layer.

Table 2: Anatomical parameters of fruits of the plant *Sambucus nigra* L.

S.No.	Parameters	Indicators, µM
1	Thickness of the exocarp tissue	3,2 ± 0,1
2	Thickness of the mesocarp tissue	9,3 ± 0,3
3	Thickness of the endocarp tissue	13,2 ± 0,4
4	Length of the parenchyma	5,5 ± 0,2
5	Width of the parenchyma	3,3 ± 0,2

Exocarp tissue thickness (3.2 ± 0.1 µM), mesocarp tissue thickness (9.3 ± 0.3 µM), smaller than endocarp tissue thickness (13.2 ± 0.4 µM), indicates that it is characteristic of this species. The length (5.5 ± 0.2 µM) and width (3.3 ± 0.2 µM) of the fruit parenchyma indicate that the stem parenchyma tissue is closed.

The mass of 1000 "seeds" of *S. nigra* is 2.0-4.1 g. The cross-sectional shape of the seed differs from the seeds of other medicinal plants (Figure 3).

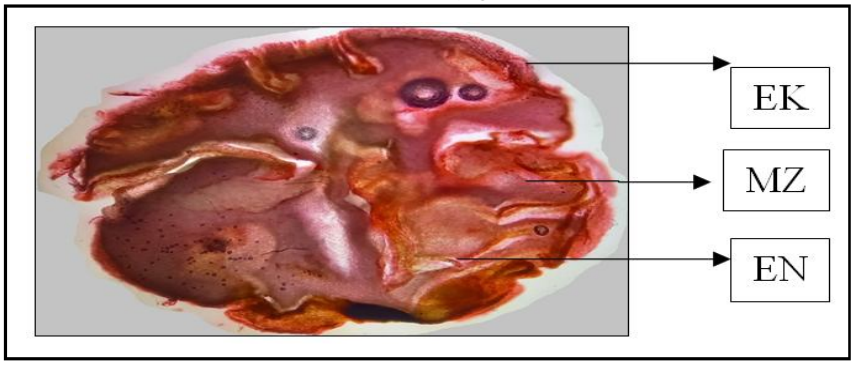

Figure 3: General cross-sectional view of a seed of *Sambucus nigra* L.: EK - exocarp, MZ - mesocarp, EN - endocarp.

4. Discussion

It is often cultivated for fruit production in various parts of Europe. Decoction prepared from wet or dried fruits of black coral is good for treating constipation. Jams and juices are also prepared from the fruit. Sugar in fruits; organic acids; major essential oil components accumulate - methyl vinyl ketone, damascenone; glycosides (sambucin, chrysanthemum); flavonoids (rutin), including various anthocyanin glycosides (cyanidin, pelaronidin, malvinidin, petunidin, peonidin) (Djurenko and Palamchuk, 2006; Burak, 2022). However, immature fruits of black coral may exhibit toxic properties (Lotova, 2010). The accumulation of oils in the pericarp of fruits of some species is known (Medicinal plants and biologically active substances: phytotherapy, pharmacy, pharmacology, 2008; Sorokopudov *et al.,* 2010).

On the cross-section, the seeds are brown in colour. As we know, the fruit of black coral consists of exocarp, mesocarp and endocarp. The seeds of *S. nigra* are located inside the endocarp and it is almost impossible to isolate them due to their hardness. That is why most scientists have studied only the morphology of the seed*, i.e.,* its external structure (Shokrzadeh and Saravi, 2010; G'ulom Sh. Jumaboev *et al.,* 2022).

Microscopic examination revealed that the seeds of *S. nigra* are pointed on one side and slightly flattened on the sides. The color of the seeds is dark brown. Seed length is 2.8 mm, width 1.2-1.8 mm, thickness 0.5-1 mm. The surface of the seed rind consists of irregular lines. The weight of one seed is from 0.013 to 0.020 g.

Morphological and anatomical study of fruits and seeds of *S. nigra* revealed the following characteristics: fruits are juicy fleshy pods, oval-shaped, glossy black-purple colour. Remains of calyx are visible in the upper part of the fruit (opposite the peduncle).

The study of the anatomical structure of the juicy fleshy part of the pericarp (exocarp and mesocarp) showed that the exocarp consists of 1-2 layers of oval-shaped dense parenchyma cells. The cells are thin-walled, densely packed, without intercellular spaces. The mesocarp consists of 5-8 layers of thin-walled cells, the cells of the outer part of the mesocarp are somewhat different from the cells of the inner part. The shape of the former is irregular and oval, intercellular spaces are small. The shape of the latter is irregular, close to rounded, intercellular spaces are delineated. The cell walls have the same thickness.

Fruit and berry production is one of the most important types, which is in high demand among the population and consumers of the Republic of Uzbekistan. Currently, the production of berry products of cultivated medicinal plants lags in its development from the needs of horticulture, pharmaceutical industry and other socially oriented sectors of the economy. The material on observation and morphological study of fruits, flowers and inflorescences of black elderberry *S. nigra* in conditions of Botanical Garden of Tashkent city is studied. Quantitative data recorded on the size of flower parts and the number of flowers in inflorescences of *S. nigra.* The obtained data can be used in obtaining medicinal plant raw materials (Durdona Mirmakhmudovna Alikarieva and Donagul Shavkatovna Nurnazarova, 2022).

The morphological and anatomical description of fresh and dried fruits and seeds of *S. nigra* in the suburbs of Nalchik in the floodplain of the river Cherek, was given for the first time (Vandyshev *et al.,* 2013). They have fruit length 5- 6 mm, width 5-5.9 mm, thickness 5-5.9 mm. The weight of one fruit is from 0.19 to 0.22 g. In our conditions in Uzbekistan, fruit length is 3-4 mm, width 4-4.7 mm, thickness 4-4.7 mm. One fruit weighs from 0.17 to 0.20 g, the dried fruit is shiny, and the skin shows folds running from the top to the bottom of the fruit. This difference, *i.e*., small size of fruits shows that Uzbekistan has more arid habitat. Seeds (endocarp) of *S. nigra* are oval-ovate, acuminate from above and slightly flattened on the sides. The seeds are dark brown. Seed length 3-3.5 mm, width 1.5-2 mm, thickness 0.5-1 mm. There are rough folds on the surface of the skin. The mass of 1 seed is from 0.013 to 0.020 g. The information obtained in the future can be used to create a modern scientific base of black coral fruits.

5. Conclusion

Branches, fruits and seeds of this plant are widely used as raw materials. Ripe fruits of *S. nigra* and flower twigs are harvested by cutting, air-dried, and dried at a temperature of 60-65°C. Then, the fruits are separated from the stems, and the flowers from the branches. The quality of black coral fruits intended for food consumption and export is regulated by the requirements of state standards, which do not contain a description of anatomical features that allow to determine the authenticity of fruits of this plant. In this context, the study of black coral fruits as a possible raw material for the production of new food products and medicines is of both scientific and practical importance.

In conclusion, we report that the study of the anatomical structure of the cross-section of the branch, fruit and seeds of *S. nigra* revealed the presence of linear lenticels on the outer surface of the branch, the cells of the epidermis are covered with sparse hairs, the cells of the parenchyma are small and contain crystalline salts, the fruit consists of pericarp, the intercellular appeared to lack cavities, and the fact that the seed is inside the endocarp, that it is hard, and that it is not isolated are diagnostic signs.

Morphological and anatomical study of the branch, fruits and seeds of *S. nigra* revealed the following features. Black elderberry fruits are juicy cenocarpic bones, oval-spherical in shape, black in colour, and shiny. fruit length is 3-4 mm, width 4-4.7 mm, thickness 4-4.7 mm. One fruit weighs from 0.17 to 0.20 g, the dried fruit is shiny, and the skin shows folds running from the top to the bottom of the fruit. Dried fruits of black elderberry are shiny and wrinkled (some folds on the surface stretched from the top to the base of the fruit), have an elongated egg-shaped shape and are black with a violet tint colour. The study of the anatomical structure of the juicy part of the pericarp (exocarp and mesocarp) showed that the exocarp (skin) consists of 1-2 layers of oval-shaped parenchyma cells. Cells are thin-walled, tightly closed, and intercellular cells are practically absent. The mesocarp (juicy pulp) consists of 5-8 layers of thin-walled cells, and the cells of the outer part of the mesocarp (closer to the rind) are somewhat different from the cells of the inner part of the mesocarp (closer to the pith). The data obtained can be promising for obtaining mature fruits as a source of food and medicinal substances, in the identification of taxonomic characters, in addition, can be used to create modern regulatory documentation for the fruits of *S. nigra*.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **J. Yeschanov, B.Y. Tukhtaev and Dilfuza B. Berdibaeva (2024). Morpho-anatomical structure and diagnostic characterization of above-ground organs of *Sambucus nigra* L. Ann. Phytomed., 13(2):1169-1173. http://dx.doi.org/10.54085/ap.2024.13.2.122.**