# CONTRIBUTIONS TO THE STUDY OF SPECIES *SAMBUCUS NIGRA* OF SPONTANEOUS BIHOR COUNTY

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***Abstract***

*In this paper there were made microscopic and macroscopic determinations and were identified components from different parts of the plant Sambucus nigra, harvested from the spontaneous flora of Bihor County, Oradea area. Harvesting of the plant product took place during the flowering period, between May-June 2018. Sambucus nigra L., is a shrub/tree that can grow up to the height of 2-7 m. Grows next to houses and fences or on the edge of forests.*

*Determinations of components of parts of the plant product (leaf, flower) were performed by means of spectrometry UV-VIS and thin layer chromatography of the alcoholic extract obtained with a type device called Soxhlet.*

*From the leaf and flower of the Sambucus nigra L., oligoelements were determined by means of X-ray fluorescence spectroscopy. The total amount of polyphenols in the leaf and the shock flower was determined using the method Folin-Ciocalteau and the amount of flavonoids, using a colorimetric method, in both using a UV-VIS spectrophotometer.*

**Keywords:** Sambucus nigra, microsopic, polyphenols, flavonoids

## INTRODUCTION

*Sambucus nigra L.* known under the popular names elder, elderberry, black elder, European elder, European elderberry, european black elderberry, part of the *Adoxaceae* family, the *Sambucus* genus. *Sambucus nigra* L. is a shrub / tree that can grow up to a height of 2-7 m. (https://plantemedicinale.site/plante-medicinale/soc-sambucus-nigra). It grows next to houses and fences or on the edge of forests (Antal D.S., CM Dobrea, 2011; Maugnini E., 1988; Nemeth T., et al., 1998; Dinu M., M. Stamanichi, 2000; Pallag A., 2015).

For medicinal purposes, flowers, fruits and bark are used from elderberry, rich in volatile oil, tannin, mucilage, flavonoides, amines, ethylamine, vitamin C and complex B vitamins, isobutyl amine (Álvarez et al, 2018; Azari et al, 2015; Raafat and El-Lakany, 2015).

One of the aims of the paper is to carry out a more in-depth study of the species of *Sambucus nigra L.* (elderberry), as well as the need to know and identify the natural medicinal resources. This objective is justified by the emergence of particularities within the populations of the same taxonomic unit depending on various factors (area, climate, pollution etc.).

## MATERIALS AND METHODS

*Macroscopic and microscopic analysis of the Sambucus nigra plant product*

The species *Sambucus nigra* L. (elderberry) was harvested from the spontaneous flora of Bihor County, Oradea. Vegetable parts were harvested during flowering in May-June 2018.

The macroscopic examination was performed according to the Romanian Pharmacopoeia, X-th edition, and the Genevez reactive was used for the microscopic exam, consisting of a Congo red ammonia solution and an alcoholic solution of chrysoidine. The sections were examined using an Optika B350 optical microscope, to which a digital camera and software has been attached dedicated to Coolingtech measurements.

*Analysis of bioactive compounds*

Identification and dosing of plant pigments, polyphenols and flavonoids from flowers and elderberry leaves was performed by thin layer chromatography and by UV-VIS spectrophotometry with a Beckmann DU-64 spectrophotometer. The alcoholic extract subjected to analysis was obtained by extraction with a Soxhlet device and rotavapor.

*Sambucus nigra* metal content was determinate with Niton X-Ray fluorescence analyzer (Marian E., et al, 2015).In the identification of plant pigments by TLC, silica gel plates GF 254 (Merk) of 10x10 cm and 0.25 mm thickness were used as stationary phase, and the mobile phase is a mixture of cyclohexane: acetone: petroleum ether (10: 6: 4). Detection was done by spraying the chromatographic plate with a mixture of 15 mL 3% boric acid solution with 5 mL 10% oxalic acid solution.

Determination of the total flavonoside content of the leaf and *Sambucus nigra* flower was performed using a colorimetric method described in numerous published articles as well as by TLC (Badescu et al, 2015; Alvare. et al, 2018).

Determination of the total content of polyphenols in leaf and *Sambucus nigra* was carried out by the Folin-Ciocalteu method, with some modifications (Knudsen et al, 2015; Edwards , 2015, Marian E. at al, 2017; Marian et al, 2018; Vicas et al, 2016, Jurca T. et al, 2015, Vicas et al, 2015).

**RESULTS AND DISCUSSION**

*Macroscopic exam*

The results of macroscopic analysis on vegetal products made up of: radix, caulis, folium, flos, pollinis are shown in Table 1.

*Table 1*

*Sambucus nigra L.* (elderberry) - the results of the macroscopic analysis

| Plant form | Shape Look | Surface Fracture | Dimensions | Color | Smell Taste |
|---|---|---|---|---|---|
| **Root (*Sambuci radix*)** | Branched roots | Irregular fibrous surface | Long, up to cm | Light brown exterior, yellow interior | No smell |
| **Stem (*Sambuci caulis*)** | Erect, cylindrical, in the form of a crown, with oppositely arranged buds | Smooth, marrow more developed compared to other woody species | Straight from the bottom long and straight ramifications up to 2-3m | | Characteristic smell and taste |
| **Leaves (*Sambuci folium*)** | Imparipenate compound, with 3-7 foil, elliptical, sharp | The jagged edge, with the bristles on the dorsal side at the ribs | Long up to 5-15cm | Dark green | Characteristic smell and taste |
| **Flower (*Sambuci flos*)** | Inflorescences such as flat umbeliform cims | Smooth surface with pedicles | type 5, 5 petals and 5 stamens | White petals and stamens with yellow anthers | Characteristic, strong, pungent smell |
| **Pollen (*Sambuci pollinis*)** | Fine powder | Smooth surface | 100-200 micron | Yellowish green | Characteristic |

*Microscopic Exam*

Transversal sections were made through stem, leaf, floral peduncles, and pollen grains were studied from a morphological point of view. In cross-section (Figure 3), the elderberry stem (Sambuci caulis) has a round-oval sinuous outline, with 5-6 rib bites, without bristles. Also, patch cells

are disordered, both in the cortical parenchyma and in the medullar parenchyma.

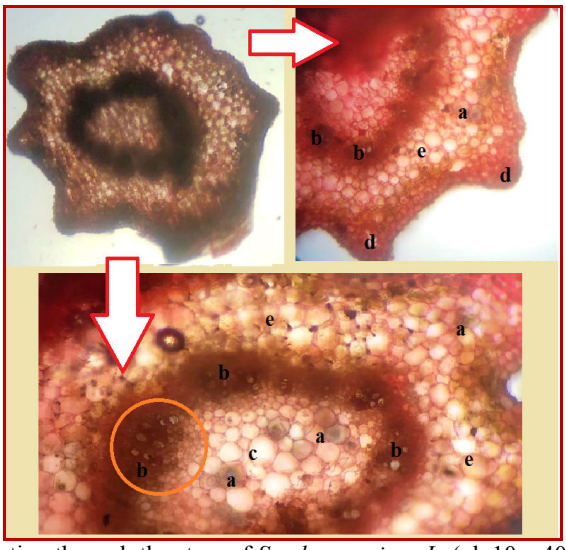


Fig. 1. Cross section through the stem of *Sambucus nigra L.* (ob.10x, 40x). (a – petrified cells, b – free-wood bundles, c medullar parenchyma, d – angular collenchymas, e –cortical parenchyma)

*Sambucus nigra* L. presents the bifacial structure leaf with two epidermis (upper and lower), which housed the mesophile and the leading beads. The upper epidermis consists of a single row of cells, closely joined together, with the outer walls provided with a cut. Under the upper epidermis, palisade parenchyma is formed, consisting of 2-3 layers of elongated cells, closely linked together, rich in chloroplasts. In the lower epidermis, there is long, filamentous, unicellular, tear beads.

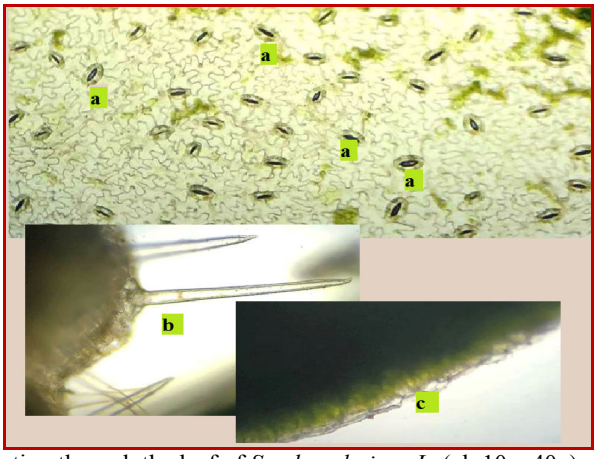

Fig.2. Cross section through the leaf of *Sambucul nigra L.* (ob.10x, 40x). a - stomata, b – filamentous unicellular peristals, c – upper cutin epidermis

At this level it is possible to emphasize the unicellular, shorter filamentous tear brushes compared to the tear brushes presenting the lower surface of the leaf. Pollen grains are round and brown in color. There were no changes in size or morphological changes of the apertures and sculptural elements of the exine, within the species.

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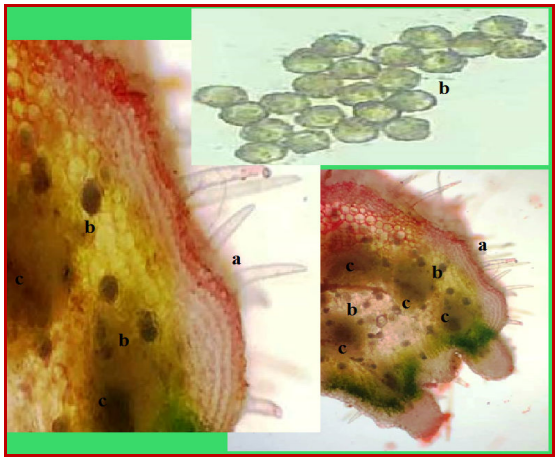

Fig.3. Cross section of floral peduncles of *Sambucus nigra L.* and highlighting pollen grains (ob. 10x, 40x) a – tector bristles, b- pollen grain, c-free-wood bundles

*Determination and representation of the most important trace elements by X-ray fluorescence spectrometry*

The content of representative metals in leaf and flower of *Sambucus nigra* was analyzed. All data were recorded with an error of +/- 2% and are expressed in milligrams/kilogram of plant product (Table 2).

*Table 2*

The content of representative metals in leaf and flower of *Sambucus nigra*

| Part of the plant product / micro-nutrient content (mg / kg of dried plant product) | Sr | Zn | Cu | Fe | Mn | Cr | Ca | K | S |
|---|---|---|---|---|---|---|---|---|---|
| Leaf | 33 | 164 | 84 | 505 | 124 | 143 | 27900 | 87500 | 2569 |
| Flower | 7 | 95 | 68 | 116 | 0 | 112 | 6362 | 101000 | 2406 |

From the analyzes carried out, it was observed that the largest amount of oligoelement (calcium, strontium, iron, copper, manganese, chromium, zinc, sulf) is found in the elderberry leaf, compared to the elderberry flower, except for potassium oligoelement.

*Identification and dosing of bioactive compounds*

The UV-VIS spectrum has four absorption peaks: 1-peak due to chlorophyll A and carotenoids, 2-peak due to chlorophyll B and carotenoids, 3-peak due to chlorophyll A, 4-peak due to chlorophyll B.

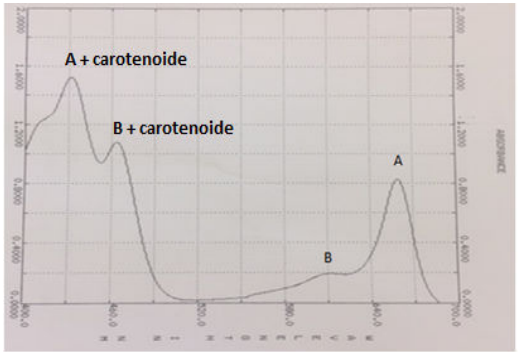


Fig. 4The UV-VIS spectrum of the alcoholic extract of *Sambucus nigra* leaves

From the Rf analysis for the spots found, it can be concluded that thin layer chromatography of the leaf extract or *Sambucus nigra* flower can be identifiedchlorophyll A (light green Rf -0.46), chlorophyll B (dark green Rf - 0.53) and carotenoids (yellow Rf - 0.49) .role (Rf - 0.31-0.35) (Rf -0.4), quercetin-3-O and kempferol-3-O-rhamnozide (Rf -0.8), quercetin-3-0-arabinosides (Rf 0.85) , 9-0.94).

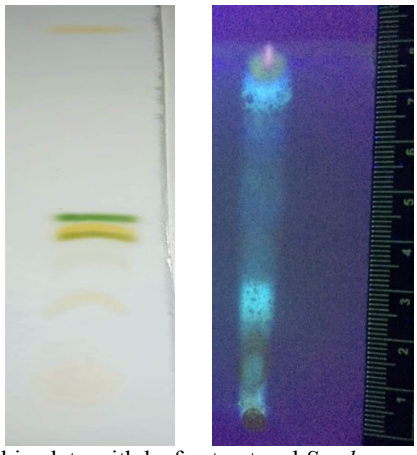

Fig. 5 Chromatographic plate with leaf extract and *Sambucus nigra* flower

*Table 3*

Content of bioactive compounds

| Extracts | *Sambuci nigras* Leaves | *Sambuci nigras* Flowers |
|---|---|---|
| **TFLAV (mg QE/g)** | 0,4050 | 4,0975 |
| **TPh (mg GAE/g)** | 0,1463 | 0,3516 |

**CONCLUSIONS**

The species *Sambucus nigra* L. (elderberry) was harvested from the spontaneous flora of Bihor County in the Oradea area in May-June 2018.

From the microscopic analysis of the plant product no changes in size or morphological changes were observed compared to the data from the existing literature. With X-ray fluorescence spectrometry, it was determined that most of the trace element (calcium, strontium, iron, copper, manganese, chromium, zinc, sulfur) is found in the leaf of elderberry compared to the flower of elderberry, except for potasium oligoelement. The components of the elderberry leaf were identified by UV-VIS spectrophotometer. From the UV-VIS spectrum for elderberry leaf extract chlorophylls A and B and carotenoids have been identified. The same leaf components were also determined by thin layer chromatography. From the elderberry flower has been identified: caffeic acid, chlorogenic acid, rutin, quercetin-3-O-rhamnoside, quercetin-3-O-arabinosides. The flavonoid content was performed by UV-VIS spectrophotometry. The concentration in flavonoids is 10 times higher in the flower than in the elderberry leaf. The determination of total polyphenols was carried out using the Folin-Ciocalteu method and it was concluded that the total concentration of phenols is 2.5 times higher in the flower than in the elderberry leaf.

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