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## SOME ASPECTS OF THE INTRODUCTION OF *ELSHOLTZIA STAUNTONII* BENTH. IN THE REPUBLIC OF MOLDOVA

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This article presents the results of a study on the species *Elsholtzia stauntonii* Benth., an aromatic and medicinal plant, introduced and little investigated, but valuable from an economic point of view. The research aimed at the evaluation and characterization of this species from a botanical, phytochemical and agrotechnical point of view, in order to determine the biomorphological features, the composition and content of essential oil, as well as the primary elements of cultivation, respectively. The essential oil has antibacterial, antiviral, astringent, carminative, diuretic and somatic action. Its subtle aroma, is similar to the balsamic smell of lemon, which determines the value of using elsholtzia. It has high potential as an ornamental and melliferous plant. The research highlighted the high adaptive potential and the prospects of cultivation under the new climate and soil conditions, contributing to the widening of the assortment of aromatic and medicinal plants, which could constitute a source of raw material for aromatherapy, perfumery, phytotherapy and gastronomy.

**Keywords:** *plant, essential oil, components, chromatography, aromatherapy, properties.*

### UNELE ASPECTE PRIVIND INTRODUCEREA SPECIEI *ELSHOLTZIA STAUNTONII* BENTH. ÎN REPUBLICA MOLDOVA

În prezenta lucrare sunt expuse rezultatele unui studiu referitor la specia *Elsholtzia stauntonii* Benth., plantă aromatică și medicinală, introdusă și puțin investigată, dar valoroasă din punct de vedere economic. Cercetările au vizat evaluarea și caracterizarea sub aspect botanic, fitochimic și agrotehnic, în vederea determinării particularităților biomorfologice, compoziției și conținutului în ulei volatile, respectiv a elementelor primare de cultivare. Uleiul volatil are acțiune antibacteriană, antivirală, astringentă, carminativă, diuretică și somatică. Aroma subtilă amintește mirosul balsamic de lămâie, care determină valoarea utilizării elsholtziei. Prezintă interes ca plantă decorativă și meliferă. Cercetările efectuate au scos în evidență potențialul adaptiv înalt și perspectiva cultivării în condițiile noi de climă și sol, contribuind astfel la lărgirea sortimentului de plante aromatice și medicinale, care ar putea constitui o sursă de materie primă pentru domeniul aromaterapiei, parfumeriei, fitoterapiei și gastronomiei.

**Cuvinte-cheie:** *plantă, ulei volatil, componenți chimici, cromatografie, aromaterapie, proprietăți.*

### Introduction

The research on aromatic plants makes it possible to widen the range of essential oils, which are main components in the production of perfumes and cosmetics, alcoholic and soft drinks, as well as non-toxic pharmaceuticals. The rational use of aromatic plants opens up possibilities for identifying effective measures to prevent diseases in a natural way and for producing plant-derived medicines, which have a healing effect and exert a complex action on the human body. A very interesting aromatic species is *Elsholtzia stauntonii* Benth. of the family *Lamiaceae* Lindl, order *Lamiales*, genus *Elsholtzia* Willd. Other scientific names: *Aphanochilus* Benth., *Cyclostegia* Beth, *Paulseniella* Briq, *Platyelasma* Kitag, common name – *Chinese mint shrub*. This species is native to China, Pakistan, Japan and Mongolia, but also grows and is cultivated in Crimea, Krasnodar, the Far East and Siberia. Due to its complex chemical composition, the essential oil is appreciated in the cosmetics and perfumery industry as a component in the formula of perfumes, eau de cologne, deodorants and soaps. It has antimicrobial, antifungal, hemostatic and diuretic effects and helps relieving the symptoms of tachycardia [1,2]. As a spice, buds and flowers are used to flavour soft drinks, salads, snacks, sandwiches and soups [3].

### Materials and Methods

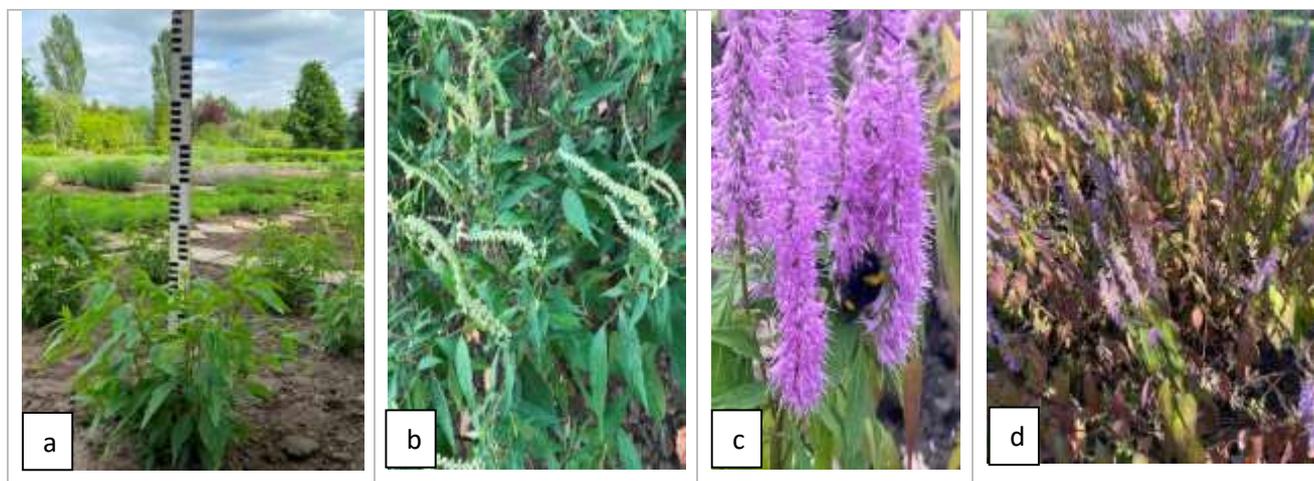
The research was conducted in 2019-2021, the experiments were done in the field of the Collection of Aromatic Plants of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The plants were grown in open field with southern exposure, under ecologically balanced conditions, on a general agrotechnical background. Phenological observations were made according to the method of I.N. Beidemann, with small modifications to make it suitable for this crop, during the entire growing season [4]. The content of essential oil was determined by the method of steam distillation [5]. The chemical composition of the essential oil was

determined by Gas chromatography–mass spectrometry (GC-MS) using the gas chromatograph Agilent Technologies 6890N coupled to the mass selective detector 5975 inert XL MSD. During the growing season, observations were made on the reaction of plants to late spring frosts, the resistance to low temperatures, the influence of light intensity, the atmospheric precipitation deficiency and excess, the resistance of plants to diseases and pests.

### Results and Discussions

The enrichment of the range of aromatic and medicinal plants is achieved by introducing them from the flora of other geographical regions. The research and appropriate use of these plants open up new ways of identifying effective measures to promote health and prevent diseases. In the collection of aromatic plants of the Botanical Garden, which includes about 130 species, forms and cultivars, the species *E. stauntonii* Benth, an introduced plant with a rich content of essential oil, was highlighted.

The results of our research have demonstrated that, under the conditions of the Republic of Moldova, the species *E. stauntonii* Benth. grows as an herbaceous perennial. To begin the cultivation of this species, the germination capacity of the seeds and the methods of propagation were determined. After the seeds germinate, very small and fine plants appear. The hypocotyl is 3-5 mm long, the cotyledons are almost round, slightly cordate and covered with single-celled hairs. At the beginning of stem growth, the size of the cotyledons and hypocotyl increase slightly. The first leaves are petiolate, with oval blade. When 4-5 pairs of leaves develop on the stem, the cotyledons dry out and the root collar deepens to 0.5 cm in the soil. The main root is 5-6 cm long and the lateral roots begin to grow from the middle segment. In the following years, during the growing season, *Elsholtzia stauntonii* Benth. goes consecutively through the following stages of development: leaf development (Figure 1a), budding – beginning of flowering (Figure 1b), full flowering (Figure 1c), end of flowering – fruit development and maturation (Figure 1d). Leaf development begins in spring, after the sap has started to circulate. This stage highly depends on the weather conditions and begins when the average daily temperature is 8-9 °C during 8-10 days, usually in early April. During this stage, the plant begins to develop 35-40 annual shoots, on which about 10 pairs of leaves grow.



**Fig.1.** The general aspect of *Elsholtzia stauntonii* Benth. plants in the generative phase. (a - leaf development, b - budding - beginning of flowering, c - full flowering, d - end of flowering - fruit development and maturation.

A slow growth of the plants was observed until mid-May. In May – June, the high amount of rainfall favoured the development of a typical, vigorous shrub, reaching a height of 70-90 cm and a diameter of 60 cm, with opposite, elongated-oval leaves, reaching a length of 14-18 cm. Budding is the stage when the inflorescences appear and grow, as a continuation of the branches of the shoots that had developed in the previous stage. The internodes of the shoots begin to grow and form flower stalks. Budding lasts over 45 days and ends with the beginning of the flowering stage. Because this species is receptive to the amount of precipitation, in 2021, the flowering stage started earlier than usual, so the beginning of flowering was observed on August 16, when the corolla of flowers started opening. The full flowering stage was observed on September 12-15, being very abundant in the respective year, and lasted for 30-40 days, positively influencing the essential oil content. The spike inflorescences were large, the central one 9-17 cm long. There were more flowers in the

lower spiral and fewer in the upper. The plant had very decorative appearance at the time of flowering, being covered with inflorescences of purple flowers, hanging at the ends of the branches. During this period, the plants were frequently visited by bees, which indicated that it is a wonderful honey plant. Up to 200 kg of nectar can be obtained from one hectare of herba. Chinese mint honey is considered very tasty, transparent with a pleasant aroma. The seed ripening stage began on October 17-19 and lasted for 30 days. During the period of our observations, no damage caused by any pests or diseases was noticed. At the beginning of November, the basal part of the stems lignified and the first pairs of leaves, of a bright yellow-reddish colour, fell. The growing season lasted 125-165 days, depending on the weather conditions. When temperatures below 0°C were recorded, the above-ground organs of plants died and, in this state, they entered the winter season. The weight of 1000 seeds is 0.2 g.

The biologically active substance in *Elsholtzia stauntonii* plants is the essential oil, the content of which depends on the age, phenological phase and organ of the plant. In our investigations, we studied the essential oil that, in the *Elsholtzia stauntonii* species, is synthesized by the single-celled hairs, located on the epidermis of the plant, as well as by the secretory glands located in the epidermis of the above-ground organs, especially the petals and sepals, less so in the leaves. In the reference period, the essential oil content was determined according to the age, phase and organ of the plant, thus a maximum content was found in 3-year-old plants, in full flowering phase (1.35-1.56%), and as for organs – in inflorescences (1.77-1.80%).

The essential oil has an orange color and perfectly conveys the aromas of the plant. By organoleptic evaluation, it refers to a type of balsamic fruit fragrance with hints of dried fruits. The phytochemical research has resulted in the identification of 25 chemical compounds, the main ones being (Z)-cinerone (50.8%), rosefuran (20.6%), eucalyptol (6.3%) and  $\beta$ -caryophyllene (6.2%) (Table). The presence of rosefuran is an index of the quality of the flavoring oil, laying at the basis of its recommendation for the production of cosmetics and perfumery products, as well as flavoring of alcoholic and soft drinks. The evaluation of the fragrance of the essential oil was carried out by the researchers of the Botanical Garden of Yalta, being rated with 5 points.

*E. stauntonii* Benth. can be propagated by seeds sown directly in the field or by seedlings obtained in greenhouses, as well as vegetatively by cuttings. The seeds sown in the field in early spring, sprout in mid-May. It takes 55-60 days to obtain seedlings in greenhouses. After preventive hardening, they are transplanted in the field in early May. The plants, in the first year of vegetation, go through the whole development cycle, but all the phenological stages are delayed, thus, the seeds are not able to reach the maturity stage. In the second and the following years, the plants begin to vegetate at the end of April. A significant role in the development of perennials is played by shaping the bush. Therefore, the plants are pruned each spring, so that the number of annual shoots does not exceed 30-35 over a feeding area of 70 x 30 cm. The plants are pruned starting with the second year of vegetation, at a height of 15-20 cm above the ground. Each 3-year-old bush develops up to 50 annual shoots, which grow about 35-80 long. In warm and rainy years, the plants reach, on average, 90-105 cm in height.

Table

The analysis of the chemical composition of the essential oil produced by the plants of the species *Elsholtzia stauntonii* by GC-MS.

Retention time (min)	Name of the compound	Content of the compounds (%) from the peak area
5.92	$\alpha$ -Pinene	0.3
7.02	Sabinene	0.2
7.08	1-Octen-3-ol	0.3
7.15	$\beta$ -Pinene	0.8
7.31	3-Octanone	0.2
7.51	Myrcene	0.1
7.61	3-Octanol	0.1
8.66	p-Cymene	0.6
8.84	Limonene	0.1
8.92	Eucalyptol	<b>6.3</b>

9.94	$\gamma$ -Terpinene	1.2
10.09	Acetophenone	2.2
11.42	<b>(Z)-Cinerone</b>	<b>50.8</b>
11.50	Linalool	2.7
14.68	<b>Rosefuran epoxide</b>	<b>20.6</b>
15.36	$\alpha$ -Terpineol	1.0
25.56	<b><math>\beta</math>-Caryophyllene</b>	<b>6.2</b>
27.01	$\alpha$ -Caryophyllene	0.7
28.09	$\alpha$ -Curcumene	0.3
28.17	Germacren D	1.0
28.83	Bicyclogermacrene	0.9
29.92	$\delta$ -Cadinene	0.2
32.09	Spathulenol	0.2
32.69	Viridiflorol	0.4
35.13	$\alpha$ -Cadinol	0.1
	<i>Other compounds</i>	2.8

Because they are perennial shrubs, *E. stauntonii* plants can be used for a long period (10-12 years), depending on the way the plantation is maintained and the climatic conditions. For these reasons, it is important to remove the weeds from the plantation. The exposure of the land should be southern, so that the crop would benefit from more light, and the land should be protected from winds; such conditions will favour the accumulation of essential oil and will protect the plants from frost during winter. The Chinese mint shrub prefers light, humus-rich soils. A plot of arable land, well prepared agrotechnically, is recommended for this crop. During the early pregenerative stages, *E. stauntonii* grows slowly and requires special care. Particular attention should be paid to weed control, as young plants are weak competitors. The acute shortage of water in 2020 has proven the need for irrigation, to maintain a constant amount of soil moisture in the root layer, which greatly increases plant productivity. The heavy rains in the spring – summer of 2021, which visibly influenced the biometric indices of the plants, increasing the height of the plants, the number of side shoots and the content of essential oil, are a proof in favour of this statement. The optimal time for harvesting *E. stauntonii* is the full flowering stage, which occurs in September – October. Plant productivity averages 2.05 kg/m<sup>2</sup>. In order to obtain seeds, the harvest is carried out in the complete maturation stage, at the end of November. The harvested raw material is dried in the shade, in clean and well-ventilated rooms.

Based on the biomorphological data, the ontogenetic periods and stages of the *E. stauntonii* plants were revealed, which allowed the argumentation of the successful introduction of the species under the conditions of the Republic of Moldova, the determination of the quantity and quality of the essential oil, the development of the primary cultivation technology in order to create industrial plantations.

In our country, as well as worldwide, there has been a spectacular return to alternative medicine, homeopathy and especially to aromatherapy, phytotherapy with the hope that they will be effective remedies for the harmful effects of various pollutants and risk factors that affect people's health.

The use of aromatic plants in gardening and landscape design is attracting more and more attention as an accessible and relatively inexpensive means of optimizing the environment, with a positive effect on the microclimate, reducing the number of pathogenic microorganisms, as well as improving the work capacity and the psycho-emotional state of people, which creates many new possibilities of usage.

According to data from the literature, the essential oil has powerful antibacterial activity against pathogenic bacteria. The dry raw material contains a number of vital micro- and macronutrients: iron, manganese, molybdenum, which makes it possible to effectively use it in the treatment of anemia, as a diuretic and stimulant for digestion, as well as in the treatment of diseases of the respiratory system. This plant has not yet revealed all its secrets. It is a promising one for study – there are great prospects for researchers and people who like new natural products.

### Conclusions

- The soil and climatic conditions of the Republic of Moldova are favourable for the growth and development of *Elsholtzia stauntonii* Benth. – species native to China. The plants are able to complete fully the ontogenetic cycle.
- Plants are propagated both vegetatively (by cuttings), and generatively (by incorporating seeds in open ground in early winter or early spring and by seedlings grown in a greenhouse).
- The content of essential oil in plants varies depending on the phenological stage and the organ that produces it. The maximum amount of essential oil is produced in the full flowering stage (1.35-1.56%) in inflorescences (1.77-1.80%).
- The essential oil, obtained from the plant herba, is characterized by a content rich in (Z)-cinerone (50.8%) and rosefuran (20.6%), eucalyptol (6.3%) and  $\beta$ -caryophyllene (6.2%).
- *Elsholtzia stauntonii* Benth. is of interest as an aromatic, medicinal and spicy plant, with high melliferous potential.

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