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BIOMORPHOLOGICAL AND BIOCHEMICAL PECULIARITIES OF THE GROWTH AND DEVELOPMENT OF THE SPECIES *Elsholtzia ciliata* (Thunb.) Hyl. UNDER THE PEDOCLIMATIC CONDITIONS OF THE REPUBLIC OF MOLDOVA

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The paper presents a detailed study on the biology and chemical composition of the essential oil produced by the species *Elsholtzia ciliata* (Thunb.) Hyl., which has been researched in the Botanical Garden as a medicinal, aromatic and spicy plant. It is a new and promising species in the field of aromatherapy and phytotherapy. The essential oil has antibacterial, antiviral, astringent, carminative, diuretic and stomachic effects. *E. ciliata* is used as flavouring in food, as an ornamental plant or as a natural air freshener.

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

PARTICULARITĂŢILE BIOMORFOLOGICE ŞI BIOCHIMICE DE CREŞTERE ŞI DEZVOLTARE ALE SPECIEI *Elsholtzia ciliata* (Thunb.) Hyl. ÎN CONDIŢIILE PEDOCLIMATICE ALE REPUBLICII MOLDOVA

Lucrarea prezintă un studiu vast privind biologia şi compoziţia chimică a uleiului volatil din specia *Elsholtzia ciliata* (Thunb.) Hyl., care se cercetează în Grădina Botanică ca plantă aromatică, medicinală şi condimentară. Este o specie nouă, de perspectivă în domeniul aromaterapiei şi fitoterapiei. Uleiul volatil are acţiune antibacteriană, antivirală, astringentă, carminativă, diuretică şi stomahică. Se utilizează în ameliorarea efectelor excesului de alcool, în tratarea răcelilor, febrei, durerilor de cap, diareei. Deţine proprietăţi culinare, decorative și de purificare a aerului.

Cuvinte-cheie: *plantă, ulei volatil, componenţi, cromatografie, aromaterapie, proprietăţi, compuşi.*

Introduction

In the Republic of Moldova, a significant number of aromatic plant species are cultivated, and this number tends to increase as the demands from the cosmetic, chemical-pharmaceutical and food industries also increase. Thus, because more plant raw material is needed, aromatic plants are cultivated now on larger areas and their assortment has become wider.

The need to cultivate aromatic plants also results from the fact that in the wild flora, the species grows over large areas, sometimes difficult to access, so that their detection, collection and transportation are difficult and unprofitable, and the cost of raw materials becomes very high. Another drawback is the fact that the occasional pickers who search for these plants in the wild do not always know them very well and may mistake them for similar species.

Some valuable species are not found in the spontaneous flora of our country, so they are brought from other geographical areas, they adapt to our climate and are successfully researched and then cultivated. In the Republic of Moldova, particular attention is paid to aromatic plants that have passed the period of acclimatisation, adaptation and testing and have stood out as promising species for implementation and use in various sectors of the national economy.

In this context, the present article aims at presenting a comprehensive study on a promising species for Moldova – *Elsholtzia ciliata* (Thunb.) Hyl. (Vietnamese balm), which has been researched in the NBGI since 2019. The plants were obtained from the seeds received by international exchange from the "Jardin botanique de la Ville de Rennes", France, and later studied in detail in order to identify the biological peculiarities of development, to determine the essential oil content, to establish the optimal propagation techniques and to analyse the chemical composition of the essential oil.

Materials and Methods

The research was conducted between 2019-2021, in the experimental field of the Collection of Aromatic Plants of the Plant Resources Laboratory. The plants were grown in open field, under ecologically balanced

conditions, on a general agrotechnical background. The subject of study was the species *Elsholtzia ciliata* (Thunb.) Hyl.

Phenological observations were made on 25 sample plants, every three days, throughout the growing season [1]. During the growing season, observations were made on the reaction of plants to late spring frosts, torrential rains, resistance to low temperatures, the influence of light, drought, rainfall shortage and excess, plant resistance to diseases and pests.

The content of the essential oil, obtained from the aerial parts of the plants harvested in the full flowering stage (middle of September), was determined by the method of steam distillation [2].

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. The chromatography was performed under the following conditions: DB5 column – exterior dimensions 30 m x 0.25 mm – interior dimensions 0.25 µm (5% Phenylmethylsiloxane); Mobile phase: Helium – flow rate: 1 mL/min; Injector temperature: 220°C; Detector temperature: 250°C; Temperature regime: from 60°C initially (3 degrees/min.) to 246°C (constant 8 min); Injected volume: 0.1-0.3 µl; Split ratio – 1:100. The identification of the peaks in the chromatogram was performed using NIST 2008 databases and by confirming the mass spectrum and the retention time according to Adams [3,4].

Results and Discussions

Elsholtzia ciliata (Thunb.) Hyl. (Vietnamese balm) is an annual species in the family Lamiaceae, one of the most numerous families in the order Lamiales, which includes 236 genera and over 6000 species. It is widespread in China, where it grows on river banks, in vegetable gardens and as a weed in all provinces. There are data that indicate that *E. ciliata* occurs in Nepal at altitudes between 1,500 and 3,400 m above sea level. *E. ciliata* occurs throughout East Asia, the Indochinese Peninsula and Japan. The species has been found in most regions of India, as well as in Malaysia and Mongolia, Korea and Europe. It has been naturalised and cultivated in Europe, North America and most of Asia.

It has been proven that the essential oil and phenols belong to the largest group of secondary metabolites in the Lamiaceae family and have multidirectional biological activity. The crude extract of *E. ciliata* contains phenols, essential oil, flavonoids, steroids and triterpenes. In folk medicine, *E. ciliata* has been used in the treatment of headache, fever, diarrhoea, oedema, blood clotting, gastralgia, dysphonia, nephritis and throat infections [5]. According to the scientific literature, *E. ciliata* is a valuable bioactive source of natural anti-oxidants. *E. ciliata* extracts have anti-inflammatory, antiviral, antibacterial, antioxidant, anticancer and vasorelaxant effects.

E. ciliata is also used in the food industry as a flavour for soft drinks. The essential oil with mild aroma and citrus-like scent was appreciated with a perfumery note (4.6 points), being included in the formula of cosmetics and perfumery products [6]. The spicy aroma, reminding of the smell of verbena and lemon, makes this species valuable in gastronomy. Buds and flowers are used as a spice, tested and approved as a flavour for fish dishes. The fatty oil identified in the seeds is used in the paint industry. It is also of interest as a honey plant, being visited by bees from morning to evening [7]. Temperature fluctuations have a small impact on the secretion of nectar from flowers. Up to 200 kg of honey nectar can be obtained from one hectare of flowering Vietnamese balm. Due to the dense, unilateral inflorescence, looking like bright purple comb, it is appreciated as an ornamental plant, planted separately or in groups, usually in the background of a flower garden.

Under the climatic conditions of the Republic of Moldova, it grows as an annual plant, up to 50-80 cm tall and 40 cm in diameter, developing shoots up to 40-45 cm long. The stem is erect, branched, and slightly pubescent, with thicker pubescence below the nodes and in the upper part, just below the inflorescence. The leaves are ovate-elliptical and serrated-toothed, 1.5-10 cm long, 1-3.5 cm wide, narrower towards the base, with a long thin petiole. The area of the leaf blade reaches up to 27 cm. There may be up to 480 leaves on a plant. The inflorescence is dense, pointed, cylindrical and more or less unilateral. The floral axis is pubescent. The bracts are green, herbaceous, round or broadly ovoid, 4-5 mm long and 5-6 mm wide. The calyx is ovoid, with almost identical teeth, glandular, densely pubescent. The corolla is purple, slightly pubescent on the outside, with ciliated lobes. Usually, a plant produces 160-180 inflorescences. The fruit is an ovoid, dark brown nut. The plant blooms in August-September. The aerial part of the plant contains essential oil; its concentration varies from 1.5 to 2.5%. The maximum oil content is found in the full flowering stage. The essential oil is a transparent yellow liquid with a special smell.

The weight of 1000 seeds is 0.32 g. The germination capacity of *E. ciliata* seeds is 70-80% under laboratory conditions and 60% – under greenhouse conditions. The seeds stored for three months began to germinate at a temperature of +20-22°C on the 3rd day after the start of the experiments. The germination energy was observed on the 5th day, the maximum number of germinated seeds was found on the 6th day. The germination period of seeds, under laboratory conditions, lasted 10-11 days.

The seeds are sown in the field as early as possible. Plants emerge on average 6-8 days after sowing, depending on soil temperature and moisture. They are small, characterised by slow initial growth in the first 2-3 weeks. The growing season lasts on average 80-110 days and is characterised by a long period of growth and development of vegetative organs (75-80 days) and a relatively short period of growth and development of generative organs (30-35 days). *E. ciliata* grows fast, especially in the period between 20 June and 30 August. The most intense growth of the generative shoots is observed in the vegetative stage (before the buds appear), when the average daily growth is 1.42 cm. In the budding stage, the growth of the shoots slows down (their growth on average does not exceed 0.55 cm) and stops in the full flowering stage. The largest inflorescences are found on the main shoot and on the first-order lateral shoots. Budding begins in late July-mid-August and lasts for 10-12 days. Flowering begins in early August and is characterised by non-simultaneous flowering in the same inflorescence. At the end of September, the fruit ripening stage is recorded. The plant prefers sunny areas with moist, slightly fertile soils. The load of the plant should not exceed 35-40% of the shoots, with a nutrition area of 60 x 30 cm.

The biologically active substance of the plant is its essential oil, the content of which begins to increase in the early flowering stage, reaching maximum amounts in the full flowering stage, and then it begins to decrease. The essential oil of *E. ciliata* 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 in petals and sepals, less in leaves.

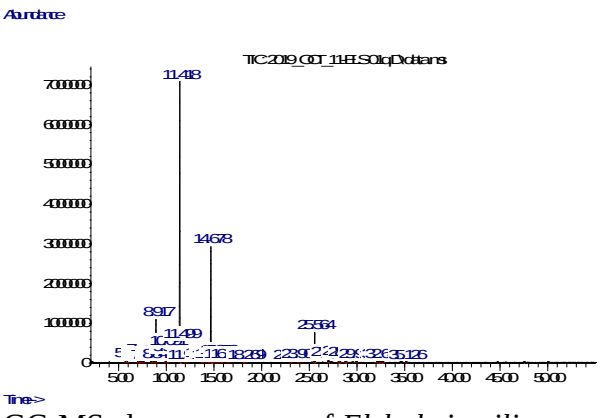
In the reference years, the essential oil content was determined for each organ (flowers, leaves, stems) and development stage. The results of the research indicate that the maximum essential oil content is produced during the full flowering stage, obtaining 1.12% per dry matter, in inflorescences – 2.3% per dry matter, respectively.

The use of essential oils in medicine, cosmetics or food as biologically active substances, flavours and natural preservatives requires a phytochemical study. The essential oil extracted from *E.ciliata* plants is of a clear yellow colour, which perfectly conveys the aroma of the plant. By organoleptic assessment, it belongs to a type with citrus scent. The investigations carried out at the "Stejarul" Biological Research Centre, Piatra Neamț (Romania), allowed the identification of 17 main chemical compounds in the essential oil extracted from *E. ciliata* plants (Table).

Table

The chemical composition of the *Elsholtzia ciliata* (Thunb.) Hyl. essential oil determined by GC-MS and the GC-MS chromatogram

RT(min)	Compounds	Area%
5.92	α-Pinene	0.3
7.02	Sabinene	0.2
7.08	1-Octen-3-ol	0.3
7.15	β-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**	**6.3**
9.94	γ-Terpinene	1.2
10.09	Acetophenone	2.2



GC-MS chromatogram of *Elsholtzia ciliata* (Thunb.)Hyl.

11.42	**(Z)-Cinerone**	**50.8**	
11.50	Linalool	2.7	
14.68	**Rosefuran epoxide**	**20.6**	
15.36	α-Terpineol	1.0	
25.56	β-Caryophyllene	6.2	

The *E. ciliata* essential oil is characterized by a high content of (Z)-cinerone (50.8%) and rosefuran epoxide (20.6%). Other important compounds are: eucalyptol (6.3%), β-caryophyllene (6.2%) and acetophenone (2.2%).

The identification of phytochemical compounds makes it possible to determine the most suitable field of application, in the case of *E. ciliata* species, the essential oil can be directed to investigations on its antimicrobial, antibacterial and fungistatic actions. The presence of rosefuran is an indicator of the value of the oil as a flavouring, therefore it is recommended to be used in the production of cosmetics and perfumery, alcoholic and soft drinks.

Conclusions

Based on the research conducted by us and the information from the specialised literature, we have found that the soil and climatic conditions of Moldova are favourable for the growth and development of *Elsholtzia ciliata* (Thunb.) Hyl. plants (Vietnamese balm), originating from France, which grow as annuals and are able to complete the ontomorphogenetic program.

The research on the accumulation of essential oil in *Elsholtzia ciliata* plants has proven that it depends on the phenological stage and the organ of the plant. The maximum amount of essential oil is produced during the full flowering stage, namely 1.12% per dry matter, and the highest concentration is in inflorescences – 2.3% per dry matter.

In the *E. ciliata* essential oil, 17 compounds were identified, the main ones are (Z)-cinerone (50.8%) and rosefuran epoxide (20.6%). Other important compounds are: eucalyptol (6.3%), β-caryophyllene (6.2%) and acetophenone (2.2%). Due to the identified compounds, the essential oil possesses several therapeutic properties, namely: antibacterial, antiviral, anti-inflammatory and antitumor.

E. ciliata is also used in the food industry as a flavouring for soft drinks. The essential oil with mild aroma and citrus-like scent was appreciated with a perfumery note (4.6 points), being included in the formula of cosmetics and perfumery products. The spicy aroma makes this species valuable in gastronomy. *E. ciliata* is recommended in the economic circuit as an aromatic and medicinal plant, which will contribute to expanding the range of cultivated species in our country.

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