Chemistry and Technology of Plant Substances

Chemical and Biochemical Aspects

Alexander V. Kutchin
Lyudmila N. Shishkina
Larissa I. Weisfeld
Editors

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CHAPTER 15

THE CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM WILD-GROWING AND INTRODUCED PLANTS OF THE ASTRAKHAN REGION

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ABSTRACT

This chapter submits the data on studying the chemical composition of essential oils isolated from land parts of wild-growing plants of the Astrakhan region—European Bugleweed (*Lycopus europaeus* L.), Bugleweed High (*Lycopus exaltatus* L.); four endemic types of wormwood of the Astrakhan region such as *Artemisia lerchiana*, *Artemisia santonica*, *Artemisia arenaria*, *Artemisia austriaca*, as well as two species of the plants are introduced in the Astrakhan region—fennel gianthisson (*Lophantus anisatum* Benth.) and common hyssop (*Hyssopus officinalis* L.). The essential oils were extracted by the use of steam distillation during the vegetation and blossoming periods of the plants. The dependence of the yield of essential oil on plant species, habitat, and vegetation period are studied, the appearance and change range of reflective index are characterized. The quantitative analysis of components in essential oils was carried out by the method of gas–liquid chromatography. The yield of essential oil from *L. exaltatus* was 0.7–0.9%, *L. europaeus* gave 0.5–0.7%, four types of wormwood including *A. lerchiana, A. santonica, A. arenaria,* and *A. austriaca* gave 0.24–0.7% fennel gianthisson; 0.25–0.55%, common hyssop; and 0.1–0.8% in equivalent to air-dry raw materials, respectively, during vegetation and blossoming of plants. The chromatography–mass spectrometry was used to study the chemical composition of essential oils. In the sample of essential oil from European lycopus, 31 components were identified, the essentials ones are α-terpineol, caryophyllene oxide, and isoeugenol methyl ether. In the essential oil from high bugleweed, 12 components are most abundant, of which are 2,4-decadienal, 2,4-hexadien-1-ol, and α-limonene diepoxide are identified. The predominant components of essential oil from four studied types of wormwood are 1,8-cyneol, camphor, isoborneol, terpine-4-ol, α-bisabolol, β-pinene, α-pinene, limonene, *trans*-pinocarvyl acetate, germacrene D, and γ-elemene. The essential oil from fennel gianthisson differs for the high concentration of methyl chavicol, methyl eugenol, caryophyllene, and D-limonene. The main components of the essential oil from common hyssop are isopinocamphone and pinanediol. It is found that the essential oil from fennel gianthisson has a high antifungal activity versus *Microsporum canis*, *Trichophyton rubrum*, and *Candida albicans*.

15.1 INTRODUCTION

The unique character of the biota in the Astrakhan region is explained by the specifics of the geographical location and climate. The natural features make the flora of the Astrakhan region diverse; there are both widespread species and those with a very limited habitat. Here is an area of a big variety of wormwoods (*Artemisia* L.).

The Caspian deserts are the land of suffrutescent sagebrushes among which white wormwood, black wormwood, sand wormwood, and common wormwood are the most widespread. The family of wormwoods is presented in the Astrakhan region by 10 species, many of them have a large habitat and form the considerable phytomass that can explain the prospects of their practical use [1].

As a result of evolution, desert plants developed a number of features that help them to survive the lack of water and the salinity of soil. The leaves of many species have changed—the surface area of a leaf became much smaller. The sprouts of some have strengthened.

As a rule, the underground part of desert plants surpasses their elevated parts in the power of development by 19–20 times. Such salt-loving species of plants as *Salicornia*, *Halocnemum strobilaceum*, *Tamarix ramosissima*, and *Limonium gmelinii* grow here. *Ephedra distachya* L., *Koeleria*, mat-grass, *Nitraria schoberi*, *Ceratoides papposa*, *Leymus racemosus*, *Festuca valesiaca*, and *Agropyron desertorum* are the typical plants for the desert flora of our region. The vegetative cover of the desert is characterized with a high dynamic range that is caused by dramatic changes in conditions of habitat including soil deformation. In general, the flora of the desert totals 160–200 species, the leading families are composites (*Compositae*), pigweeds (*Chenopodiaceae*), and gramineous (*Gramineae*).

The family of wormwood—*Artemisia* L. (*Asteraceae family*)—unites over 400 species mainly widespread in the moderate zone of the northern hemisphere, 174 species grow in the CIS (Commonwealth of Independent States). The wormwood species are often met in steppes, others grow in semi-deserts and deserts, and some are weeds in all zones.

The interest to wormwoods is explained by pharmacological active agents, sesquiterpene lactones that were found in the studied species. As a result of the comprehensive study, some preparations from wormwood are offered for medical application.

The study of the dependence of chemical composition of essential oils on ecological factors shows the allelopathic phenomena in phytocenoses and has an important practical value.

This chapter represents the data on studying the chemical composition of essential oils from four species of wormwood, on the influence of ecological factors on accumulation, the contents and composition of essential oil from plants of the *Artemisia* L. growing in the climatic conditions of the Astrakhan region.

The components in the composition of essential oil are often identical, but there are also differences [2].

Austrian wormwood (*Artemisia austriaca*) is a perennial gray-white root-sucker herbaceous plant [3]. Stems are upright, branching, and leafy. Leaves are twice pinnatisect into small linearly mucronated parts. Flower baskets are broadly ovate, small, drooping, and are collected in paniculate inflorescence. Involucral leaflets are linear and pilary. All flowers and floral calathidia are tubular. The blossoming period is July–August.

Wormwood sand (*Artemisia arenaria*) is a subshrub with a height of 20–100 cm. At the bottom, the stems are the ligneous, and vegetative sprouts are truncated. Leaves are green, slightly succulent, almost naked, dissected into narrow segments, and linearly lanceolate final segments; the lower leaves are macropodus, the others are assidenous. Anthodes are ovoid, assidenous, or on the truncated pedicles, collected in a sprawling whisk. *A. arenaria* grows on sand in steppes and on the sea coasts of the Balkan Peninsula, on the coast of the Azov and Black seas, in western Ciscaucasia, in the Caspian Sea region, and in the Aral Sea region. Thanks to the fast vegetative reproduction, *A. arenaria* fixes friable sand easily forming unproductive pastures–sandy wormwood areas.

Wormwood santonian (*Artemisia santonica*) is a desert-steppe species of the Caspian region. It is a perennial subshrub with a height of 70 cm. Leaves are alternate, twice plumose-dissected, and the lower stem leaves are petiolar, let down, and gray. Flowers are small, 2–3 mm long, telianthus, without flower-cups; corollas are tubular, quinquedentate with oil droplets. Inflorescences are oblong-ovoid, assidenous anthodes collected in narrow contracted panicles. Halophilous-meadow-steppe Black Sea-Kazakhstan species grows on damp saline soils, on salt-marsh edge, in the areas where salted ground waters come out.

Wormwood Lerch (*Artemisia lerchiana*) or white wormwood is a perennial subshrub with a height of 16–50 cm. At the beginning, all the plant is covered with grayish dense fluffy hairs; later, it is partially naked. The bush is built up of perennial ligneous, strongly truncated stems and short leaf-bearing one-year sprouts. Fruit-bearing sprouts are numerous; in the top half, they are branchy. Leaves in unfertile sprouts and in bottom stems are petiolate, 2–3 pinnatisected; middle—sessile, 2 pinnatifid, at the basis with pinnatisected ears; overhead leaves are simple, linear. Inflorescences are assidenous anthodes collected in contracted panicles. Corollas are yellow or pink. Fruit is achene. Blossom period is from August–September. It grows in meadows, pastures, in steppes with strongly saline, black humus, and brown soils. It is the indicator of soil alkalinity and a fodder plant. Because of soil salination, *A. lerchiana* shows properties that are specific to succulents. This fact singles *A. lerchiana* out of mesophytes as the cells of the latter lose water in the conditions of water shortage [4].

Nowadays, when preventing and treating many diseases, pharmaceutical preparations are demanded to possess specificity, maximum efficiency, and lack of by-effects. Multicomponent forms that contain biologically active agents from medicinal vegetative raw materials meet this demand. Studying phytochemical properties of wild-growing plants and preparing medicines on their basis to meet the necessary requirements are of some practical interest.

Lycopus high (*Lycopus exaltatus* L.) and European lycopus (*Lycopus europaeus* L.) are wild-growing perennial grassy plants of *Lamiaceae* family, they grow in a temperate climate of many countries of Europe, in the European part of Russia, Central Asia, Caucasus, and in Western and Eastern Siberia. In the Astrakhan region, large populations of these plant species dominate in the humid meadows of the Delta and the floodplain of the Volga River, and these also can be met as weed plants in vegetable gardens.

The folk-medicine advises to use European lycopus tea as antiinflammatory, restorative, and analgesic agent to treat Basedow's disease, to reduce high blood pressure and tachycardia. The extracts of lycopus high are used for the treatment of paludism, diarrhea, gastric distresses, metrorrhagia, and neurosis.

The scientific interest to vegetative raw materials of lycopus high and European lycopus is growing. Shelukhina *et al.* [5] studied the chemical composition of European lycopus. Through the methods of ^{1}H, ^{13}C NMR, UV-spectroscopy, and HPLC–MS methods, they have isolated phenolic compounds and identified caffeic acid ethyl ester, 3,4-dimethoxybenzaldehyde, 5,3′,4′-trihydroxy-6,7-dimethoxyflavone, apigenin, apigenin-7-glucuronide ethyl ester, luteolin, luteolin-7-glucuronide methyl ester, luteolin-7-glucuronide ethyl ester, caffeic acid, rosmarinic acid, and rosmarinic acid methyl ester. The sum of phenolic compounds in terms of rosmarinic acid and absolutely dry European lycopus extract made 3.5%. After studying a number of researches on the influence of extractions from European lycopus on thyroid body and tissue metabolism of iodine in experimental animals (guinea pigs, etc.), Alefirov *et al.* [6] proved that extracts from grassy European lycopus possess antihypothyroid activity on the experimental model of thyrotoxicosis in rats that led to the normalization of the state and behavior of animals, the thyroid hormones level in blood serum. It was found out that water extraction from grassy lycopus showed the most evident medicinal effect similar to the action of thyrozol. The study of pharmacological activity of vegetative raw materials of European lycopus by Alefirov *et al.* showed antihypothyroid action of extracts from the plant makes possible to use the preparations on the basis of European lycopus in the treatment of Basedow's disease as alternatives in case of intolerance to hormonal antithyroid agents. The study of pharmacological activity of vegetative raw materials of European lycopus by Alefirov *et al.*and contributors showed antihypothyroid action of extracts from the plant that makes possible to use the preparations on the basis of European lycopus in the treatment of Basedow''s disease as alternatives at while the intolerance to hormonal antithyroid agents. Earlier, during the phytochemical researches of aboveground parts of European lycopus and lycopus high [7], at the department of organic, inorganic, and pharmaceutical chemistry of the Astrakhan state university, in water extracts of vegetative raw materials of these plants, when carrying out qualitative reactions, hydrolyzable and condensable tannins were found. The content of tannin in leaves of *L. exaltatus* L. and *L. europaeus* L. made 0.01% and 1.6%, respectively. The evaluation of total flavonoids in equivalent to luteolin-7-glucozide in aqueous–alcoholic (60%) extracts of lycopus high showed in stems was 7.2 mg, in leaves—11.7 mg; the European lycopus extracts showed in stems were 5.3 mg, in leaves—8.5 mg per 100 g of dry raw materials. The sum of flavonoids in equivalent to rutin in stems and leaves made: 6.8 and 12.2 mg per 100 g of dry raw materials respectively for lycopus high; 5.3 and 6.25 mg per 100 g of dry raw materials respectively for European lycopus. In water extraction from grassy lycopus high, the sum of triterpene saponins made 3.8% in equivalent to oleanolic acid.

European lycopus and lycopus high are the plants with similar pleasant aromatic smells that become stronger when grinding both fresh and dried-up raw materials. The smells are kept during a storage time (2 years) that provides the evidence for the content of essential oil in different parts of these plants. According to the present data, the yield of essential oil from raw materials of European lycopus that grows in the conditions of Uzbekistan made 0.2%. In essential oil, limonene, terpinene, linalool acetate, linalool, bornyl acetate, geranyl acetate, nerol, geraniol, *p*-cymene, γ-terpinene, α-pinene, camphene, terpinolene, etc were identified. The study of European lycopus that grows in the northern part of Serbia [8] showed that the yield of essential oil is 0.5%. Moreover, in the composition of the oil, the following components were identified: copaene, geranyl acetate, selinene, cadiene, ledol, hexadienol, borneol, terpineol, decanal, geraniol, furfural, hexanol, benzaldehyde, nonadienal, isocitral, lavandulol, nonalol, etc. While further studying the sample of European lycopus from the northern part of Serbia, antimicrobial activity of essential oil components against *Escherichia coli* and *Klebsiella pneumoniae* was found. The data concerning the composition of essential oil from lycopus high are not submitted in the scientific literature, the pharmacological activity of essential oil from this plant is not studied.

In recent years, the interest to essential oil plants of *Lamiaceae* family to which hyssop belongs has increased significantly. Probably, this plant originally comes from Southwest Asia and Southern Europe. This subshrub is cultivated in Eastern and Central Europe, in France, Italy, the Balkans, Crimea, and in Asia [9, 10].

The essential oil is the main physiologically active component of *Hyssopus officinalis.* The content of oil in leaves makes 0.3–1.5%, in inflorescences 0.9–2.0%, and in stems, only trace amounts are found. Above-land parts of the plant (leaves, inflorescences, and softwood stems) are used as raw materials to receive essential oil which is consumed by food, cosmetic, and pharmaceutical industry. The yields of essential oil from dried and fresh plant raw materials received by steam distillation make 0.15–0.3% and 0.3–0.8%, respectively. In large volumes, this oil is produced in France, Italy, and in the countries of Former Yugoslavia.

The essential oil from *H. officinalis* is a light green or light yellow liquid with a characteristic camphor smell.

It possesses antibacterial, antiviral, antifungal, and expectorative activity [11–13]. The recent researches figured that the essential oil produced from a hyssop shows antiplatelet activity [14]. In addition, spasmolytic activity of essential oil from a hyssop medicinal is revealed [15].

The yield and chemical composition of essential oil from *H. officinalis* depend on many external factors (climatic conditions, soil type, plant origin, time of raw materials preparation, etc.) [16, 17].

According to the literary data, the main components of essential oil from *H. officinalis* are isomeric pinocamphones, β-pinene, pinocarvone, limonene, linalol, β-caryophyllene, germacrene D, tujones, and myrtenol [18–20].

The chemotype of hyssop growing in Turkey differs from the chemotype of hyssop cultivated in Poland by the content of its dominating ingredient—pinocarvone [21, 22]. The essential oil of hyssop medicinal from Spain is characterized by high concentration of 1,8-cyneol (52.89%) [23]. Hyssop medicinal cultivated in France differs by the domination of linalool in its essential oil (49.6%). This oil is also characterized by the low content of monoterpene ketones [24].

The study of the chemical composition of essential oil (yield of 0.34%), received by the method of gas chromatography–chromato-mass-spectrometry, from hyssop leaves picked near Khandiza (former Uzbek SSR), showed that its main components are pinocamphone (71%), β-pinene (8.6%), and 1,8-cyneol (6.4%), but limonene and isopinocamphone were not found [25].

The chemical composition of essential oil from hyssop medicinal, cultivated in the Astrakhan region, has not been studied before. The need to detailed studying the components of essential oil composition is caused by the potential content of toxic compounds. According to the scientific data [26], methyl eugenol, for example, possesses a cancerogenic activity, and unterpene ketones show an obvious epileptogenic activity [27].

In recent years, the interest to fennel gianthisson (*Lophantus anisatum* Benth.) has significantly increased in Russia. It was improved by Ukrainian plant selection breeders [28], then gardeners began to grow it up in their private grounds more often, and it is wildly cultivated by beekeepers as this plant is an excellent honey herb [29].

Fennel gianthisson belongs to Labiate family (Lamiaceae), it is a perennial, winter-hardy plant, and a semi-grassy bush with the height not exceeding a meter. Stems are tetrahedral; leaves are petiolar oval, and seldom have rough edges, 7–10 cm long and 4–5 cm wide. Root is fibrous. Flowers are hermaphrodite with a long stoma. Inflorescences are spiciform, white, or violet, sometimes different colored, up to 20 cm long or more, with anisic smell. The vegetative period lasts till steady frosts. In the first year of crops, seeds ripe at the end of September, and in the next years, it happens 2–3 weeks earlier.

In folk medicine, *L. anisatum* Benth. is applied as antiinflammatory and bactericide agent.

L. anisatum Benth. is believed to increases body resistance and facilitates the adaptation to adverse environment as well as has a sedative effect on the central nervous system.

Water extracts from leaves of this plant are used to treat inflammatory processes in gastrointestinal tract, liver, and urinary tracts diseases, as a medicine for acute respiratory diseases, bronchitis, pneumonia, and bronchial asthma, to remove radionuclides and to reduce cholesterol content in blood. Gel produced from leaves of *L. anisatum* Benth. cures the skin diseases caused by fungi successfully.

From this point of view, it is important to know what substances including biologically active compounds are contained in this plant.

Despite the wide range of pharmacological properties of *L. anisatum* Benth., its chemical composition is nearly unknown.

The present work is purposed to study the chemical composition of essential oil samples from four endemic species of wormwood (*A. lerchiana, A. santonica, A. arenaria,* and *A. austriaca*), two species of *Lycopus*—Bugleweed high (*L. exaltatus* L.) and European bugleweed (*L. europaeus* L.), and also *H. officinalis* and *L. anisatum B*enth., growing in the Astrakhan region, depending on type of above-land parts and vegetation period, as well as quantitative determination of essential oil main components [30–33].

15.2 MATERIALS AND METHODOLOGY

15.2.1 RAW MATERIALS

Above-land parts of *A. santonica*, *A. lerchiana* Web., *A. arenaria*, and *A. austriaca* Jack. were gathered and collected not far from the populated areas of the Astrakhan region (Dzhakuevka, Privolzhe, Kucherganovka, Yaksatovo, Streletskoye, Kamyzyak, and Enotaevka). The raw materials were picked in a blossoming phase in July and analyzed in a dry state.

To receive essential oils samples from two species of bugleweed, the vegetative raw materials were gathered in natural habitats: the vegetative and blossoming stems of lycopus high (*L. exaltatus* L.) were picked in Il'inka village; European bugleweed (*L. europaeus* L.) were gathered from the banks of the Volga river in the Astrakhan surroundings during the period from the end of June to September.

H. officinalis and *L. anisatum* Benth. (above-land parts) were provided by All-Russian Research Institute of the irrigated melon growing (a pharmaceutical kitchen garden, Kamyzyak, Russia). The raw materials were analyzed in fresh and dry states.

The dry raw materials were received according to the rules of picking and drying pharmaceutical herbs [34]. To avoid the destruction of biologically active agents and to remove excessive moisture, all raw materials were dried up right after gathering by the most widespread method—the air drying based on a free access of air to the plant material which is spread out in the darkened place.

The essential oil was extracted out from air-dry raw materials weighing 5 kg that consisted of beated land parts (leaves, stems, and inflorescences) through the method of hydrodistillation at atmospheric pressure in the device made of stainless steel, the distillate was collected throughout 5 h. The oil was dried by waterless sodium sulfate; then, it was separated from the drying agent by decantation. The duration of hydrodistillation process is fixed experimentally on the basis of studying the dynamics of change in essential oil yields in time. The yield of essential oil was estimated in percentage (%) terms of the weight of absolutely dry raw materials. Physical and chemical properties of essential oils were determined by the standard techniques [35].

Qualitative and quantitative compositions of essential oil samples were carried out by the chromatograph with a mass-selective detector Shimadzu QP 2010. To identify components, the mass spectra libraries of NIST 02, 05, 11 were used.

The sample of essential oil was dissolved in benzene to the concentration of 0.1% by volume. The column MDN-1 (methyl silicone, firmly bound) is 30 m, the diameter is 0.25 mm. The chromatography mode is the following: the injector is 180°C; the detector is 200°C; the interface is 210°C; the carrier gas is helium (99.99999%), 1 ml/min at the division of the stream 1:10; the thermostat is 60°C in 1 min, 2°/min to 70°C, 5°/min to 90°C, 10°/min to 180°C, 20°/min. to 280°C, further the isotherm is

1 min. The mode of spectra mass registration is 39–350 *m/z*. To determine linear indices, the samples of essential oil and normal paraffins (nonane, undecane, tridecane, and pentadecane) were dissolved in benzene. *n*-Paraffins were diluted to the concentration of 0.007% by volume, the essential oil is 1:30,000 by volume. The quantitative content of essential oil components was calculated over the areas of gas-chromotographic of peaks without using correcting indices. The qualitative analysis was carried out by the comparison of linear retention indices [36] and full mass-spectra of components with the relevant data of pure compounds.

Linear retention indices (*RI*) were calculated by the following formula:

$$\mathrm{RI}_x = 100n + 100k\left(\frac{t_{Rx} - t_{Rn}}{tR_{(n+k)} - t_{Rn}}\right),$$

where n is the number of carbon atoms of n-paraffin, k is the difference of the number of carbon atoms in two n-paraffins, t_{Rx} is the time of substance retention, t_{Rn} is the retention time of n-paraffin with n carbon atoms, and $tR_{(n+k)}$ is the retention time of n-paraffin with $n + k$ carbon atoms.

15.2.2 THE STUDY OF ANTIFUNGAL ACTIVITY OF *LOPHANTUS ANISATUM* BENTH. ESSENTIAL OIL

Studying of antifungal activity was carried out according to the M27 standard by the method of serial dilution of NCCLS [37, 38] in Saburo solid and liquid medium [38].

In a test tube, a microorganism suspension was added to the preparation serially diluted in dimexidum, and the minimum concentration of substance capable to detain growth of test culture was determined. Microorganisms of *Microsporum canis*, *Trichophyton rubrum*, and *Candida albicans* were used as test cultures.

Test tubes were thermostated at 24 ± 3°C for 7 days (*C. albicans*) and for 30 days (*M. canis, T. rubrum*). To determine the nature of the preparation activity (fungistatic—FS) or (fungicide—FTs), the wort-agar from all the test tubes was plated in the Petri-dish. The cups were placed into the thermostat at 24 ± 3°C for 7 days (*C. albicans*) and for 30 days (*M. canis, T. rubrum*). Econazole was used as a preparation of comparison. The results were statistically processed with the use of Student's *t*-test.

15.3 RESULTS AND DISCUSSION

The samples of essential oil from four species of wormwood were submitted to the determination of color and refraction index, and the results of these indicators study are given in Table 15.1.

TABLE 15.1 The Area of Picking Raw Material, Yield, Appearance, Refraction Index of Essential Oil from Four Species of Wormwood.

Wormwood species	Area of picking raw material	Appearance	Refraction index n_D^{20}	Yield of essential oil, %
Artemisia austriaca	Kucherganovka	The jellylike mass of light yellow color with a strong smell of camphor	–	0.42
	Kamyzyak		–	0.33
	Privolzhye		–	0.30
	Streletskoye		–	0.37
Artemisia santonica	Dzhakuyevka	The oil of yellowish color with a strong smell of camphor	1.5044	0.70
	Kucherganovka		1.4984	0.65
	Streletskoye		1.5031	0.70
	Yaksatovo		1.5033	0.54
Artemisia lerchiana	Kucherganovka	Colorless oil	1.4822	0.24
	Dzhakuyevka		1.4820	0.32
	Privolzhye		1.4821	0.27
	Streletskoye		1.4822	0.26
Artemisia arenaria	Privolzhye	The oil of yellowish-green color	1.5049	0.48
	Dzhakuyevka		1.5170	0.68
	Streletskoye		1.5055	0.64
	Enotayevka		1.5048	0.67

In Table 15.2, the compounds identified in essential oil from four species of wormwood are given as well as their quantitative contents as percentage of the whole oil.

The evidence presented in Table 15.2 suggests that the essential oils from the four studied species of wormwood differ from each other in their chemical compositions considerably. The main components of essential oil from wormwood of *A. lerchiana* are 1,8-cyneol (31.0–32.3%), camphor (48.5–49.4%), isoborneol (5.6–6.5%), and terpine-4-ol (3.4–3.5%).

TABLE 15.2 The Composition of Essential Oil Samples From *Artemisia lerchiana* (Samples No. 1–4), *Artemisia santonica* (Sample Nos. 5–8), *Artemisia arenaria* (Sample Nos. 9–12), *Artemisia austriaca* (Sample Nos. 13–16)

The name of a sample	RI	Sample number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
α-Pinene	931	0.3	0.3	0.2	0.3	–	–	–	–	8.4	7.9	8.2	7.9	0.1	0.2	0.2	0.3
Camphene	946	2.0	1.9	1.8	2.1	0.6	–	–	–	0.5	0.4	0.6	0.5	–	–	–	–
Amyl vinyl carbinol	951	0.3	0.3	0.4	0.2	0.5	0.4	0.2	0.5	–	–	–	–	0.3	0.4	0.2	0.4
Sabinen	972	–	–	–	–	–	–	–	–	1.3	1.1	0.9	1.4	–	–	–	–
β- Pinene	975	0.5	0.4	0.6	0.5	–	–	–	0.4	23.3	24.3	23.1	23.6	–	–	–	–
Oct-1-en-3-ol	978	0.2	0.2	0.3	0.1	–	–	–	–	–	–	–	–	–	–	–	–
Δ^2-Carene	984	0.1		0.1	0.1	–	–	–	–	–	–	–	–	–	–	–	–
β-Myrcene	991	0.1		0.2	0.1	–	–	0.3	–	1.5	1.1	1.3	1.5	–	–	–	–
p-Cymol	1023	0.8	0.6	0.5	0.7	–	0.3	0.2	–	1.4	1.5	1.4	1.4	–	–	–	–
Limonene	1028	–	–	–	–	–	–	–	–	7.3	7.2	7.1	6.9	–	–	–	–
1,8-Cyneol	1033	31.0	32.3	31.5	31.0	15.4	15.0	15.2	14.0	1.3	0.9	1.2	1.4	1.6	1.6	1.8	1.6
Santolina alcohol	1037	–	–	–	–	0.4	0.4	0.3	0.4	–	–	–	–	–	–	–	–
γ-Terpinen	1058	0.4	0.2	0.5	0.5	0.2	–	0.1	0.1	0.3	0.3	0.2	0.5	–	–	0.2	–
M = 152	1078	0.5	0.6	0.5	0.5	–	–	0.2	–	–	–	–	–	–	–	–	–
Camphenilone	1085	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	0.4	0.1
cis- Sabinen hydrate	1094	–	–	–	–	0.6	0.7	0.5	0.7	–	–	–	–	–	–	–	–
Linalool	1100	–	–	–	–	–	0.1	–	–	0.1	–	0.1	0.3	–	–	–	–
M = 154	1103	0.3	0.3	0.1	0.4	0.3	0.4	0.4	0.3	–	–	–	–	1.0	0.7	1.1	0.9
isopentyl-3-methylbutanoate	1105	0.3	0.3	0.2	0.2	0.3	0.4	0.4	0.5	–	–	–	–	1.0	0.9	0.9	0.9

TABLE 15.2 *(Continued)*

The name of a sample	RI	Sample number															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
3-Tujone	1107	–	–	–	–	–	–	–	0.1	–	–	–	–	0.1	0.2	0.3	0.1
α-Tujone	1108	–	–	–	–	–	–	0.2	–	–	0.2	–	0.1	1.5	1.3	1.5	1.6
M = 152	1118	–	–	–	–	–	–	–	–	–	–	–	–	0.8	0.8	0.9	0.7
M = 140	1134	0.5	0.4	0.4	0.4	–	–	–	–	–	–	–	–	–	–	–	–
trans-Pinocarveol	1139	1.0	0.5	0.8	1.0	–	0.1	–	0.2	0.5	0.6	0.5	0.6	0.3	0.3		0.4
Camphor	1146	48.8	49.0	48.5	49.4	60.6	59.8	60.2	59.7	5.4	5.2	5.3	5.4	74.1	73.9	73.8	74.0
Isoborneol	1148	5.6	6.0	6.5	5.7	9.8	9.6	9.5	9.8	1.3	1.0	1.3	1.4	3.4	3.6	3.5	3.1
trans-Verbenol	1150	–	–	–	–	–	0.2	–	0.1	–	–	–	–	0.6	0.5	0.5	0.6
Pinocarvone	1161	0.5	0.4	0.4	0.4	0.8	0.8	0.7	1.0	0.2	0.4	0.1	0.3	0.7	1.0	0.8	0.8
Estragol	1172	0.3	0.1	0.3	0.2		0.3	0.1	0.1	0.2	0.1	0.4	0.4	–	–	–	–
Terpine-4-ol	1177	3.4	3.5	3.4	3.4	4.9	4.7	4.8	5.1	1.0	0.9	1.3	0.9	2.1	1.7	1.9	2.3
Myrtenal	1180	0.6	0.3	0.4	0.5	0.6	0.6	0.6	0.5	–	–	–	–	0.7	0.8	0.6	0.4
α-Terpineol	1189	1.1	1.0	0.9	1.2	2.1	1.9	2.4	3.1	0.4	–	0.6	0.5	0.7	0.6	0.7	0.5
Myrtenol	1196	0.5	0.4	0.5	0.2	0.6	0.6	0.4	0.5	0.3	0.3	0.5	0.4	0.8	0.9	0.8	0.8
Cytronellol	1215	–	–	–	–	–	–	–	0.2	0.2	0.3	0.3	0.2	–	–	–	–
trans-Carveol	1219	0.2	0.1	0.2	0.1	0.4	0.5	0.4	0.4	–	–	–	–	0.2	0.5	0.2	0.4
3Z-Hexenyl-2-methylbutanoate	1233	–	–	–	–	–	–	–	–	–	–	–	–	0.7	0.7	0.6	0.7
trans-Pinocarvyl acetate	1237	–	–	–	–	0.1	0.4	0.4	0.5	–	–	–	–	3.0	2.9	3.0	3.1
Carvone	1243	0.2	0.3	0.1	0.3	0.4	0.6	0.3	0.3	–	–	–	–	0.8	0.6	0.6	0.8
Phelladral	1271	–	–	–	–	0.4	0.5	0.4	0.4	–	–	–	–	0.5	0.5	0.4	0.6

TABLE 15.2 *(Continued)*

The name of a sample	RI	Sample number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mertenyl acetate	1325	–	–	–	–	–	–	–	–	–	0.1	–	–	0.1	0.1	0.3	0.3
Nerol acetate	1346	–	–	–	–	–	–	0.1	–	0.2	0.3	0.2	0.2	–	–	–	–
α-Terpinyl acetate	1350	–	–	–	–	0.1	0.2	0.1	0.1	–	–	–	–	–	–	–	–
Octahydro-1,4,9,9-tetramethyl-1*H*-3a, 7-methanoazulene	1390	–	–	–	–	–	–	–	–	0.2	0.3	0.1	0.2	–	–	–	–
Eugenol methyl ether	1406	0.5	0.6	0.7	0.5	0.4	0.5	0.4	0.2	1.8	2.2	2.4	1.9	0.4	0.5	0.5	0.4
β-Cedrene	1413	–	–	–	–	–	–	0.2	–	0.4	0.3	0.5	0.4	–	–	–	–
β-Farnesene	1456	–	–	–	–	–	–	–	–	0.2	0.2	0.1	0.1	–	–	–	–
Germacrene D	1483	–	–	–	–	–	0.5	0.2	0.2	–	–	–	–	2.9	3.0	3.0	2.7
γ-Elemene	1500	–	–	–	–	–	–	–	0.1	–	0.2	–	0.1	0.2	0.2	0.2	0.3
Nerolidol	1540	–	–	–	–	–	–	0.2	0.1	1.2	1.3	1.4	1.1	–	–	–	–
Spathulenol	1578	–	–	–	–	–	–	–	–	1.9	1.9	1.7	1.8	–	–	–	–
1,7,7-trimethylbicyclo [2.2.1]hept-2-yl acetate	1584	–	–	–	–	0.5	0.5	0.5	0.4	2.8	2.9	2.6	2.7	0.2	0.4	0.1	0.1
M=196	1615	–	–	–	–	–	–	–	–	–	–	–	–	0.5	0.6	0.5	0.7
Bisabolol oxide II	1655	–	–	–	–	–	–	–	–	1.6	1.6	1.7	1.3	–	–	–	–
α-Bisabolol	1688	–	–	–	–	–	–	–	–	34.7	34.9	34.8	34.5	0.5	0.6	0.5	0.4

Note: *M*—unidentified compounds and *RI*—retention index.

At the same time, *A. santonica* essential oil is characterized by higher concentration of camphor (59.7–60.6%), isoborneol (9.5–9.8%), terpine-4-ol (3.9–4.9%), and much smaller contents of 1,8-cyneol (14.0–15.4%). The distinctive characteristic of the chemical composition of *A. arenaria* essential oil is a higher concentration of α-bisabolol (34.5–34.9%), β-pinene (23.1–24.3%), α-pinene (7.9–8.4%), the presence of limonene (6.9–7.3%) unlike other types of wormwood, and the lower contents 1.8-cyneol of (0.9–1.4%) and camphor (5.2–5.4%). In *A. austriaca* essential oil as the main components, there are camphor (73.8–74.1%), isoborneol (3.1–3.6%), *trans*-pinocarvil acetate (3.9–3.1%). Unlike other types of wormwood, in *A. austriaca* essential oil gemacrene D and γ-elemene are identified.

The received samples of essential oil from two species of bugleweed are mobile liquids of yellow color with a characteristic pleasant smell. The essential oil from European bugleweed (*L. europaeus* L.) possesses an evident flower smell with a light aroma of menthol and bergamot. The essential oil of bugleweed high (*L. exaltatus* L.) has a light flower smell. The content of essential oil in vegetative raw materials of *L. exaltatus* L. and *L. europaeus* L. made 0.9% and 0.7%, respectively, during the blossoming period of plants; 0.7% and 0.5% per 100 g of air-dry raw materials, respectively, during the vegetative period. While determining the chemical composition of samples in the essential oil from *L. europaeus* L., the percentage of 31 components was calculated and 12 components were identified. The results are given in Table 15.3.

TABLE 15.3 The Blend Composition of the Essential Oil from *Lycopus europaeus* L. and *Lycopus exaltatus* L.

Component	Content, %	
	***Lycopus europaeus* L.**	***Lycopus exaltatus* L.**
Amyl vinyl carbinol	1.59	–
Benzyl alcohol	1.52	–
Phenylethyl alcohol	2.62	–
trans-Pinocarveol	1.14	–
cis-Verbenol	2.61	–
Myrcenol	0.34	–
4-Terpineol	3.65	0.52
α-Terpineol	9.06	0.48

TABLE 15.3 *(Continued)*

Component	Content, %	
	Lycopus europaeus L.	*Lycopus exaltatus* L.
Benihinal	2.72	–
Carvone	2.13	–
Pelargonic acid	1.08	–
Isoeugenol	3.00	–
Damascenone	0.44	–
Jasmone	0.83	–
Copaene	1.95	–
Isoeugenol methyl ether	9.43	–
Caryophyllene	2.51	–
Geranyl acetone	0.56	–
α-Caryophyllene	0.48	–
β-Farnesene	0.78	–
β-Ionone epoxide	0.39	–
Isocyclocytral	1.39	–
α-Selinene	3.48	–
Viridiflorol	1.32	–
Z-α-*trans*-Bergamotol	0.98	–
Z-α-*trans*-Bisabolene epoxide	0.62	–
Caryophyllene oxide	10.30	–
Ledene oxide	0.60	–
2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate	1.56	–
Ledol	2.24	1.05
Patchulane	0.75	0.56
Paraffines	–	14.11
2,4-Hexadien-1-ol	–	5.05
2-Decenal	–	1.08
2,4-Decadienal	–	51.72
A-Limonene, diepoxide	–	1.61
2-Hepten-1-ol	–	0.35
3,4-Dimethyl-2-hexanone	–	0.35
6,10-Dimethyl-5,9-dodecadien-2-one	–	0.33
Unidentified compounds	27.93	22.79

According to the obtained data, the main components of essential oil from *L. europaeus* L. are terpenoids such as *cis*-verbenol, amyl vinyl carbinol, phenylethyl alcohol, benzyl alcohol, *trans*-pinocarveol, myrcenol, α-terpineol, 4-erpineol, benihinal, carvone, isoeugenol, damascenone, jasmone, isoeugenol methyl ether, geranyl acetone, β-ionone epoxide, and isocyclocytral. In addition, sesquiterpenes are met: α-caryophyllene, caryophyllene, copaene, α-selinene, patchulane, and β-farnesene. Sesquiterpenoids of essential oil from *L. europaeus* L. are viridiflorol, Z-α-*trans*-bisabolene epoxide, Z-α-*trans*-bergamotol, caryophyllene oxide, ledene oxide, and ledol.

Similar to the oil from *L. europaeus* L., the main components of essential oil from *L. exaltatus* L. are terpenoids. They are α-terpineol, 4 terpineol, 2,4-hexadien-1-ol, 2-decenal, 2,4-decadienal, α-limonene diepoxide, 2-hepten-1-ol, 3,4-dimethyl-2-hexanone, 6,10-dimethyl-5,9-dodecadien-2-one. The sesquiterpene of essential oil from *L. exaltatus* L. is patchulane, and the sesquiterpenoid is ledol which is also found in the essential oil from *L. europaeus* L. In the essential oil of from *L. exaltatus* L., there are also paraffins.

On the basis of the total contents of different terpenoids groups in essential oil from *L. europaeus* L., in particular, alcohols (not less than 22.53%), phenols (not less than 12.43%), ketones (not less than 3.96%), aldehydes (not less than 1.39%), etc., it is possible to assume that it shows antiseptic activity (it can stop the growth of bacteria, viruses, fungi, or kill the latter), as well as anesthetizing and antiinflammatory activities, stimulates immune system.

In the essential oil from *L. exaltatus* L., the component structure is less various. However, it has the high concentration of terpene derivatives: aldehydes (not less than 52.8%) and alcohols (not less than 19.16%). So, it is supposed to have much higher antimicrobic, febrifugal, and antiinflammatory activity than the essential oil from *L. europaeus* L. In general, the determination of both pharmacological and potential toxic effects of essential oils from *L. europaeus* L. and *L. exaltatus* L. needs further researches.

The study of the dependence of essential oil yield in terms of vegetation and types of above-land parts of *H. officinalis* showed that the greatest oil yield is received from inflorescences (Table 15.4).

In Table 15.5, the compounds identified in the essential oil from *H. officinalis* (above-land parts in the blossoming phase) are given, as well as their quantitative contents.

TABLE 15.4 The Essential Oil Yield from Different Above-Land Vegetative Parts of *Hyssopus officinalis* and in Different Vegetation Terms.

An above-land vegetative part of *Hyssopus officinalis*	Vegetation terms	Essential oil yield, %*
Leaves	May–the beginning of June	$\frac{0.3}{0.2}$
Stems	May–the beginning of June	$\frac{0.1}{0.1}$
Leaves	Mid-June–the beginning of July (the blossoming period)	$\frac{0.4}{0.3}$
Stems	The blossoming period	$\frac{0.2}{0.1}$
Inflorescences	–	$\frac{0.8}{0.6}$

*The numerator and the denominator show the essential oil yield from fresh and dry vegetative raw materials, respectively.

TABLE 15.5 The Quantitative Composition of the Essential Oil from *Hyssopus officinalis*.

The name of a component	*RI*	Whole oil, %
Sabinen	951	0.19
β-Pinene	954	1.58
Mol. mass = 112*	959	0.22
Eucalyptol	1002	0.34
Dihydrocarveole	1081	0.60
Nopinone	1105	0.48
trans-Pinocarveol	1118	1.41
Verbenol	1124	0.22
Mol. mass = 152*	1131	2.52
Isopinocamphone	1143	63.55
Myrtenal	1161	1.58
α-Terpineol	1166	0.21
Myrtenol	1171	1.39
trans-2-Pinalol	1181	0.72

TABLE 15.5 *(Continued)*

The name of a component	*RI*	Whole oil, %
Pinanediol	1212	9.45
Myrtanal	1292	0.86
α-Bourbonene	1372	0.99
β-Caryophyllene	1404	0.37
Aromadendrene	1443	0.21
Limonen-6-ol, pivalate	1452	0.61
α-Caryophyllene	1457	0.32
Germacrene D	1462	0.95
γ-Elemene	1477	0.18
Mol. mass = 220*	1487	4.64
ε-Muurolene	1494	0.19
Elemol	1524	0.71
Spathulenol	1549	1.08
Caryophyllene, oxide	1553	1.42
Mol. mass = 182*	1577	2.31
τ-Cadinol	1614	0.18
Cubenol	1636	0.33
Mol. mass = 268*	1818	0.18

Note: *RI*—Retention Index.

*Unidentified compound.

In Table 15.6, the content of terpenes, terpenoid, seskviterpen, and seskviterpenoid in the essential oil from *H. officinalis* is given.

The evidence presented in Table 15.2 suggests the main components of essential oil from hyssop medicinal are oxygenated monoterpenes: isopinocamphone (63.55%) and pinanediol (9.45%).

The comparative analysis of the obtained experimental results and literary data on the component composition of essential oils from hyssop growing in other countries (Serbia, Poland) [12, 23] shows a significant difference in the chemical composition of essential oil from hyssop medicinal cultivated in the Astrakhan region. So, the content of β-pinene in the essential oil is much low (1.58%) than in the essential oil from hyssop which is grown up in Poland (6.14%) or in India (18.4%).

TABLE 15.6 The Content of Main Components of Essential Oil.

Components of essential oil	Content, %
Monoterpene hydrocarbons (β-pinene, sabinen)	1.77
Sesquiterpene hydrocarbons (β-caryophyllene, germacrene D, aromadendrene, α-bourbonene, ε-muurolene, γ-elemene, α-caryophyllene)	3.21
Oxygenated monoterpenes (isopinocamphone, nopinone, α-terpineol, *trans*-pinocarveol, myrtanal, pinanediol, myrtenol, limonen-6-ol pivalate, *trans*-2-pinalol, verbenol, dihydrocarveole, eucalyptol, myrtenal)	81.42
Oxygenated sesquiterpenes (cubenol, τ-cadinol, caryophyllene oxide, spathulenol, elemol)	3.72
Unidentified compounds	9.87
Total	100

Studying the dependence of essential oil yield on vegetation terms and a type of above-land parts of *L. anisatum* Benth. showed that the maximum yield is received from inflorescences and leaves of the plant in the blossoming phase (Table 15.7).

TABLE 15.7 The Essential Oil Yield from Different Above-Land Vegetative Parts of *Lophantus anisatum* Benth. and in Different Vegetation Terms

An above-land vegetative part of *Lophantus anisatum* Benth.	Vegetation terms	The essential oil yield, %*
Leaves	May–the beginning of June	0.45 / 0.43
Stems	May–the beginning of June	0.32 / 0.27
Leaves	Mid-June–the beginning of July (blossoming period)	0.50 / 0.48
Stems	Blossoming period	0.32 / 0.30
Inflorescences	–	0.55 / 0.54
Seeds	End-April–the beginning of August	0.25

*The numerator and the denominator show the essential oil yield from fresh and dry vegetative raw materials, respectively.

The samples of essential oil with a characteristic pleasant smell of anise were submitted to the determination of color, specific weight at 20°C, and the index of refraction, and the results of these indicators determination are presented in Table 15.8.

TABLE 15.8 The Index of Refraction and the Specific Weight of Essential Oil Samples from *Lophantus anisatum* Benth.

Above-land parts of the plant	Color	*d*, g/sm^3	n_D^{20}
Leaves in the vegetation phase	Slightly yellowish	0.9360	1.4700
Leaves in the blossoming phase	Slightly yellowish	0.9365	1.4780
Stems in the vegetation phase	Light yellow	0.9370	1.4782
Stems in the blossoming phase	Light yellow	0.9372	1.4782
Inflorescence	Yellowish-green	1.0070	1.5200
Seeds	Yellowish	0.9532	1.4932

In Table 15.9, the compounds identified in the essential oil from *L. anisatum* Benth. as well as their quantitative contents are given.

TABLE 15.9 The Quantitative Composition of the Essential Oil from *Lophantus anisatum* Benth.

The name of a component	Retention index, *RI*	Percentage (%) of the whole oil
Amyl vinyl carbinol	957	0.32
β-Myrcene	990	0.06
D-Limonene	1014	8.14
Linalool	1086	0.07
1-Octenyl acetate	1094	0.50
Methyl chavicol	1172	62.08
Chavicol	1215	0.12
Mol. mass = 162*	1217	1.19
Eugenol	1330	0.09
β-Elemene	1394	0.59
Methyl eugenol	1453	24.01
Caryophyllene	1403	1.28
Germacrene D	1480	0.80

TABLE 15.9 *(Continued)*

The name of a component	Retention index, *RI*	Percentage (%) of the whole oil
δ-Cadinene	1516	0.15
Germacrene D-4-ol	1536	0.12
τ-Muurolol	1564	0.24
α-Cadinol	1637	0.24

*Unidentified compound.

The evidence presented in Table 15.9 suggests the main components of essential oil from *L. anisatum* Benth. are methyl chavicol (62.08%), eugenol methyl ether (24.01%) and D-limonene (8.14%).

The results of studying antifungal effect of the essential oil from *L. ophantus anisatum* Benth. are presented in Table 15.10.

TABLE 15.10 The Fungistatic and Fungicide Activities of *Lophantus anisatum* Benth. Essential Oil.

The studied sample	Concentration, mcg/ml*		
	Microsporum canis	*Trichophyton rubrum*	*Candida albicans*
Lophantus anisatum Benth. essential oil	80 / 100**	80** / 100	100 / 200**
Econazole	40 / 80	40** / 80	40 / 80

*The numerator is a fungistatic activity; the denominator is a fungicide activity.

**The distinctions between repeated patterns are reliable at $p = 0.95$.

The obtained experimental data show that essential oil from *L. anisatum* Benth. has a fungistatic and fungicide activity against the studied test cultures.

15.4 CONCLUSIONS

The obtained data enlarge and supplement the knowledge of the chemical composition of wormwood growing on the Caspian Plain significantly, as well as they allow to expand the raw-material base of essential-oil plants due to the plant species of the *Artemisia* L. family. Some compounds that are not typical for the *Artemisia* L. family such as Amyl vinyl carbinol, octahydro-1,4,9,9-tetramethyl-1H-3a, 7-methanoazulene, and neryl acetate were identified. It is supposed to be the result of the complex of soil climatic conditions and vegetative biocenoses in the Caspian Sea region.

It is found out that the content of essential oil in *L. europaeus* L. and *L. exaltatus* L. is higher when picking raw materials in a blossoming phase. Through the method of chromatography–mass-spectrometry, 31 and 12 components were identified in grassy European bugleweed (*L. europaeus* L.) and in grassy bugleweed high (*L. exaltatus* L.), respectively. European bugleweed and bugleweed high raw materials are of interest for the pharmaceutical, perfumery, and cosmetic industries as well as for aromatherapy.

The qualitative and quantitative chemical composition of *H. officinalis* L. and *L. anisatum* Benth. essential oils cultivated in the Astrakhan region is determined. The main components of *H. officinalis* L. are isopinocamphone and pinanediol. *L. anisatum* Benth. essential oil that contains methyl chavicol and methyl eugenol as the main components. The study of antifungal activity showed that the essential oil from *L. anisatum* Benth. possesses a rather high antifungal activity against *M. canis*, *T. rubrum*, and *C. albicans*.

KEYWORDS

- **wormwood**
- **lycopus**
- **hyssop**
- **giant hyssop**
- **hydrodistillation**
- **chromatography**
- **isopinocamphone**
- **pinanediol**

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