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Biologically active substances of plants of the *Cupressaceae family of the genus Thuya* and the genus *Juniperus* for phyto- and aromatherapy

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Abstract. Biologically active substances of plants of the *Cupressaceae* family, especially the genera *Thuja* and *Juniperus*, are used in various sectors of the national economy. The quality of essential oils is determined by a set of chemical compounds and their concentrations. In this case, the quality is determined by the ratio between the macro- and micro-components of essential oils. More than 40 biologically active compounds in essential oils have been determined by gas chromatography, which may also depend on the growing region of these plants.

1 Introduction

In recent years, various directions of using plants of the *Cupressaceae* family, especially the genera *Thuja* and *Juniperus*, have been developing more and more actively. In various regions of our country and in many foreign countries, such plants are used in green construction, landscaping of open and closed urban areas [1]. Such plants are factories for the synthesis of biologically active substances that are contained in all their parts. Compounds that are part of plant producers, called essential oils (EO), play a special physiological role. For medicinal purposes, and sometimes vice versa, EO have been widely used since ancient times, and somewhat later their individual components [2-4]**.** Plants of the genus *Thuja* and *Juniperus* contain 0.5-0.9% essential oil, 1.5-2.5% reducing sugars, 1.5-1.8% free acids, about 1.5% tannins. At the same time, the main components of the essential oil are thujone and isotujone (63-73%), fenchone (7-10%), sabinene (4-6%), pinene (1-3%) [5,6]. Myrcene and camphene may be present as impurities in thuja essential oils, the content of which is less than 1%. The difference in the component composition, usually, in the ratio between macro- and micro-components depends on many factors [7]. The main of these factors is the agrochemical composition of the soil, which is widely represented and varies in various soils and urban soils, parks, squares, etc. In addition, it is necessary to consider the regional differences, the agro-climatic conditions of different growing regions of the *Cupressaceae* family plants. In this regard, the component composition and concentration of each of the chemical compounds makes it possible to assess, considering the entire spectrum of compounds, the region of origin of this plant and the direction of use of the selected essential

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oils. It can be perfumery, food, pharmaceutical industry, household chemicals. Research on the use of essential oil with immunostimulating and antivirus properties in homeopathy and phytotherapy is actively developing.

The purpose of this study was to isolate the essential oil and evaluate its chemical composition from samples of Western Thuja (*Thuja occidentalis* L.) and folded Thuja (*Thuja plicata Don ex. D. Don).* These plants are used not only in decorative gardening, but by releasing a large number of volatile compounds, mainly essential oil, they disinfect the environment and help to improve mood, relieve stress, have a tonic and strengthening effect on the central nervous and immune systems, which determines their success with phytotherapists [8].

Another object of research in this work is a plant of the cypress family, which belong to the genus juniper *(Juniperus).* This genus contains a large number of different chemotypes. Of this number, more than 70 species grow in the temperate climate zone in the northern hemisphere, such as: common juniper (*J. communis* L.), Cossack juniper (*J. sabina* L.), etc. The multicomponent composition of juniper essential oil also has pharmacological activity, a wide range of effects on the human body, and is used in phytotherapy. The increasing interest and scope of the use of essential oils in medicine requires constant assessment of the chemical composition of various samples of essential oils obtained from plants in different regions, assessment of the stability of the component composition to obtain the required pharmacological effect [9].

2 Materials and methods

The object of the study is the samples of essential oil, which were obtained from needles and young shoots by steam distillation. The collection of samples was carried out in 2020-2022 on the territory of the botanical gardens of VILAR, NBG, as well as from plants growing in natural conditions in the Forest experimental cottage of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Belarusian Agricultural Academy. Essential oils were extracted by steam distillation and analyzed by chromato-mass spectrometry on the Clarus 600C analytical complex of Perkin Elmer (GC: Elite Wax capillary column - 60 m x 0.32 mm x 0.5 µl; the carrier gas was helium - 1 ml/min, sample volume – 0.5 µl, flow division 1/50; temperature range of the column thermostat: 60°C – 5 minutes, 3^0/min to 195°C, isotherm 15 minutes; t^0 of the injector – 230^0C; detectors PI (230^0C) and MS (simultaneously); MS mode: ionization energy - 70 eV, t^0 of the interface – 210ºC, tº of the source – 180ºC). The content of the essential oil components was calculated from the peak areas of the chromatogram of the detector without using correction or calibration coefficients for three repetitions. The identification of the components of essential oils was carried out using the NIST-05 library of mass spectral data and the library of retention indices, which we previously calculated using the method of ellipsoidal distribution of *n*-alkanes in the mode of arbitrary programming of the analysis temperature for terpene derivatives [10,11].

3 Results and discussion

The results of the chemical analysis of the essential oil of thuja and juniper are given below. More than 100 components of the mono-sesquiterpene series have been identified in the essential oil of various thuja samples, including those contained in the oil in an amount of less than 0.01%. In most species of thuja, the biosynthesis of essential oil proceeds along the path of 4-6-cyclization of limonene to form thuienic (sabinenic) structures with subsequent oxidation to ketones: α- and β-thuions (Figure 1). Table 1 shows experimental data on chemical components in oil, the content of which is more than 0.1%. The total content of these substances in the analyzed samples of thuja oils is in the range of 65-90%. In all samples, α-thujone is the predominant isomer. The maximum content of α-thujone – more than 85% - was observed in samples from Belarus and the Black Sea coast. The minimum amount of α-thujone is observed in samples from Poland and from the Moscow region. In an essential oil with a lower thujone content, another bicyclic ketone, fenchone, was identified in a sufficiently large amount (up to 10%).

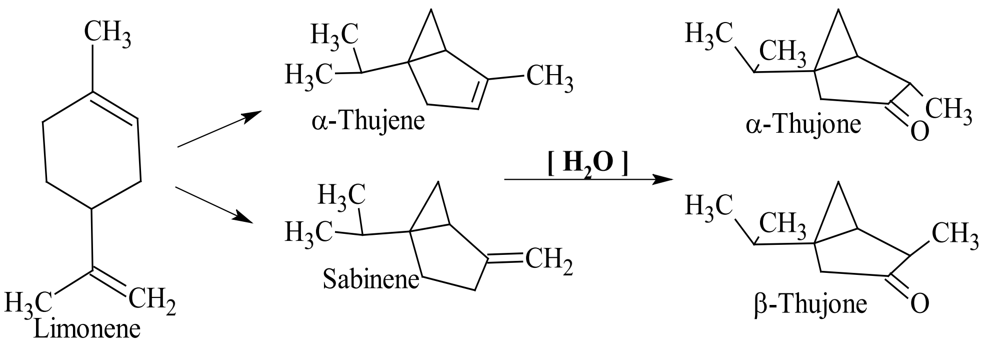

Fig. 1. Biosynthesis of thujone (sabinane) bicyclic structure and their oxidation to ketones.

Table 1. The content of the main components in EO ***Thuja plicata Don ex. D. Don,*** (mass. %).

	Canada 1	Canada 2	Belarus	Poland	Abkhazia	Moscow VILAR	The Republic of Abkhazia	The southern coast of Crimea	Cone - shaped	Spherical
α-Pinene	2.00	1.47	0.08	1.26	0.25	0.16	0.1-0.2	0.06	0.37	0.64
Camphene	0.40	-	0.03	0.15	0.10	0.11	-	-	0.27	0.61
Sabinen	6.40	4.16	0.38	6.00	3.05	1.51	1.95-3.5	1.67	0.13	0.14
β-Myrcene	-	-	0.11	1.76	-	2.70	0.33-0.6	0.17	0.38	0.31
Limonene	0.50	0.80	0.07	1.46	0.42	0.30	0.27-0.4	0.17	0.52	0.64
γ-Terpinen	0.10	1.17	0.03	1.28	0.39	0.16	0.09-0.2	0.37	2.04	2.01
Fenchone	-	-	-	7.06	3.63	10.67	-	-	10.43	15.3
α-Thujone	76.0	73.16	85.64	62.12	78.09	61.67	85.2-86.7	82.58	65.1	56.53
β-Thujone	7.50	8.25	5.37	7.06	7.61	10.82	5.45-5.52	5.66	11.12	9.55
Terpinen-4-ol	1.70	3.17	0.17	4.66	1.83	1.52	1.21-1.3	3.15	1.5	1.45
γ-Terpineol	-	0.86	-	0.20	-	0.56	0.02-0.03	0.09	0.13	0.18
Rimuen	-	0.30	0.52	1.06	-	1.04	0.08-0.15	0.28	0.47	0.67
Bieren	-	0.27	0.25	0.65	-	1.87	0.06-0.1	0.3	0.75	1.42

The data obtained by us correlate with the literature data and the ratio of components in the essential oil of plants of the genus *Juniperus* varies widely. It is noted that monoterpene hydrocarbons prevail most often in the essential oil of most species: α-pinene and sabinene, sometimes D-limonene (*J. jaliscana*) – about 24%. The species *J. ashei, J. pinchotii,* and *J. saltillensis*, for example, are characterized by a high content of camphor, and *J. monticola* –

bornyl acetate. Juniper essential oils are characterized by a large set and variety of sesquiterpene alcohols, but their total amount is usually insignificant. The essential oils of some species contain elemol in concentrations from 0.1 to 8.5% and α-cadinol – from trace amounts to 6%.

We analyzed the essential oil isolated from the green mass of several plant samples growing in different soil and climatic conditions. The figure shows a characteristic GLPC-MS chromatogram of juniper essential oil from the Moscow region, Sergiev Posad district (Fig.2). The oil is mainly composed of limonene. Nevertheless, it can be stated that the essential oil contains in low concentrations a large number of compounds, mainly a number of mono- and sesquiterpenes, their alcohols, acid esters, ketones, oxides and other products. Due to their low concentration, and often the close nature of the decay of a molecular ion during an electron impact (almost identical Mass-spectra), their reliable identification is not always possible.

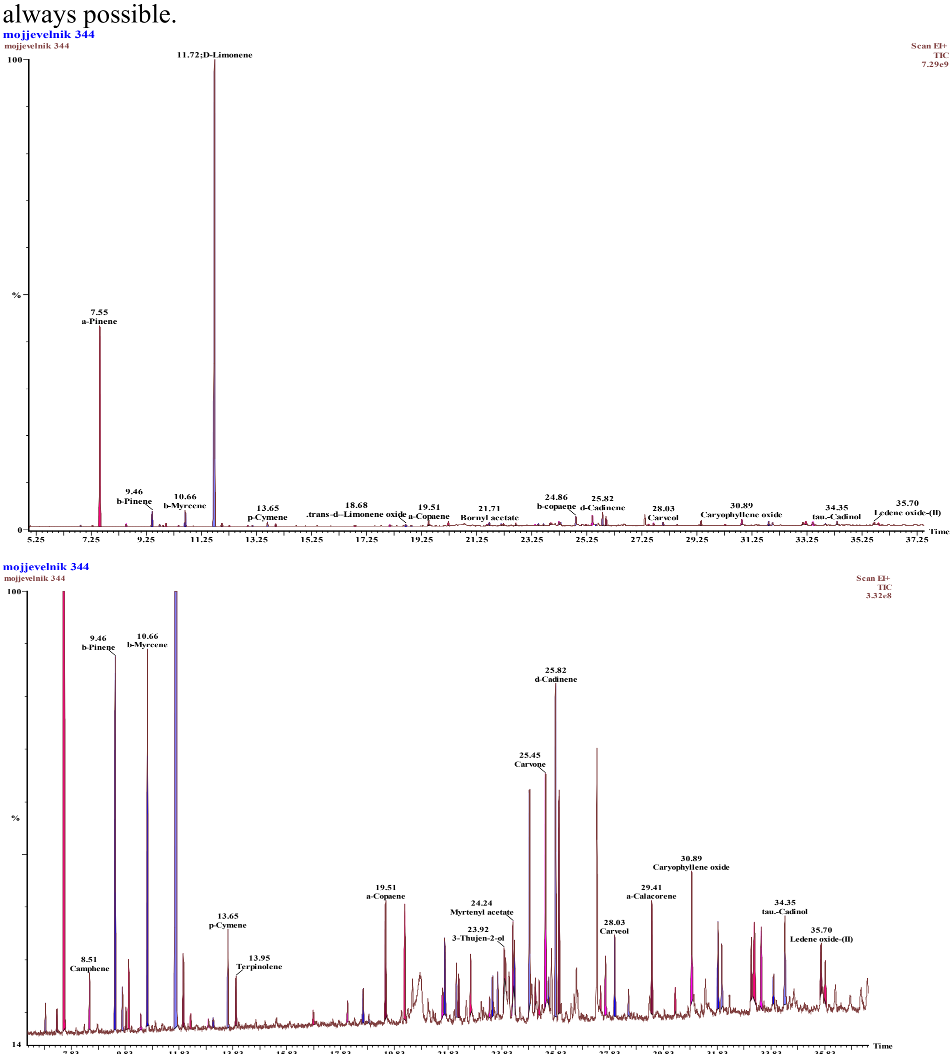

Fig. 2. Chromatogram of Juniperus essential oil with a high content of limonene (according to the total ion current of the maximum peak and greatly increased).

Table 2 shows data on the composition of the components of EO *Juniperus virginiana* L., the content of which in essential oil is more than 1%, and Table 3 shows the comparative ratio of the main components in the studied juniper samples.

Table 2. Composition of *Juniperus virginiana* L. essential oil.

Essential oil Component	Content, %
α-Pinen	0.093 ± 0.008
Sabinen	0.182 ± 0.014
Δ^3-Karen	0.099 ± 0.007
β-Myrcene	0.185 ± 0.016
d-Limonene	25.36 ± 0.19
β-Fellandren	0.262 ± 0.015
p-Cymol	0.078 ± 0.006
α-Terpinolene	0.071 ± 0.005
3-(1-Methyl-2-propenyl)cyclooctadiene-1.5	0.124 ± 0.011
Nonanal	0.108 ± 0.009
Octen-1-ol-3	0.517 ± 0.024
Limonene oxide	0.119 ± 0.012
Linalool	0.086 ± 0.008
1,5-Diethenyl-3-methyl-2-methylencyclohexane	0.467 ± 0.021
Bornyl Acetate	0.093 ± 0.006
Farnesen	0.136 ± 0.009
Terpinen-4-ol	0.177 ± 0.014
trans-p-Mentadiene-2,8-ol-3	0.112 ± 0.010
Estragole	0.094 ± 0.007
Methyl ester of geranium acid	0.297 ± 0.022
Sesquiterpene	0.165 ± 0.013
α-Muurolen	0.314 ± 0.021
Carvon	0.359 ± 0.028
δ-Kadinen	1.46 ± 0.09
γ-Kadinen	1.39 ± 0.07
Citronellol	0.017 ± 0.004
α-Kubeben	0.089 ± 0.007
3,5-Dimethoxytoluene	1.31 ± 0.08
Safrol	5.67 ± 0.29
cis-Bisabolene epoxy	0.156 ± 0.014
Bisabolol	0.679 ± 0.036
Caryophyllene oxide	1.04 ± 0.07

Methylevgenol	2.98 ± 0.19
Hedicariol	3.06 ± 0.18
Nerolidol	0.019 ± 0.005
Germacrene-D-ol-4	5.13 ± 0.26
Camilol	2.03 ± 0.15
Elemol	29.88 ± 0.45
β-Endesmol	2.61 ± 0.17
Gyiaol	0.428 ± 0.054
t-Cadinol	2.12 ± 0.18
γ-Cubenol	0.575 ± 0.031
ε-Eudesmol	1.98 ± 0.14
α-Cadinol	7.82 ± 0.42
Miyristicin	0.239 ± 0.012

According to the Table 2, d-Limonene and Elemol are present in essential oil from macro components with a content of more than 25%. In total, 48 chemical compounds that determine the quality of the essential oil and characterize the region of origin have been identified.

Table 3. The content of the main components in the essential oil of samples of the genus *Juniperus.*

Chemical compound	*Sabina* L.			*Communis* L.			*Virginiana* L.
	Kazakhstan	VILAR	The Blue Arrow	VILAR 3	Belarus	Karelia	Pyatigorsk
α-Pinene	1.05 ± 0.07	1.08 ± 0.08	1.39 ± 0.11	25.4 ± 0.51	49.0 ± 0.82	69.3 ± 1.02	0.09 ± 0.02
Sabinen	41.5 ± 0.88	35.3 ± 0.56	56.7 ± 0.72	13.9 ± 0.33	14.3 ± 0.29	0.07 ± 0.02	0.18 ± 0.03
β-Myrcene	2.92 ± 0.15	3.08 ± 0.28	1.10 ± 0.07	4.31 ± 0.33	4.53 ± 0.37	0.61 ± 0.08	0.18 ± 0.3
β-Pinene	0.10 ± 0.02	-	0.13 ± 0.02	2.06 ± 0.21	2.50 ± 0.18	13.2 ± 0.44	0.05 ± 0.01
β-Fellandren	-	0,44 ± 0.09	1.27 ± 0.09	8.45 ± 0.51	5.42 ± 0.46	0.06 ± 0.01	0.26 ± 0.04
d-Limonene	1.56 ± 0.21	1.50 ± 0.19	1.41 ± 0.12	3.64 ± 0.26	2.05 ± 0.20	8.57 ± 0.49	25.4 ± 0.9
Germacrene D-4-ol	-	2.05 ± 0.14	-	1.08 ± 0.09	-	-	5.13 ± 0.42
4-Thujanol	3.67 ± 0.28	0.05 ± 0.01	5.16 ± 0.33	1.63 ± 0.18	0.56 ± 0.08	0.16 ± 0.03	1.58 ± 0.23
β-Caryophyllen	< 0.03	1.23 ± 0.14	0.31 ± 0.02	0.60 ± 0.08	1.04 ± 0.06	0.41 ± 0.07	< 0.01
trans-Pinocarvil acetate	-	33.5 ± 0.73	-	-	–	-	–

Elemol	-	–	-	-	-	-	29.9 ± 0.8

The results of Table 3 characterize juniper essential oils, moreover the content of chemical compounds varies by different samples and regions of growth. These indicators also characterize the quality of juniper essential oil, the region of origin and the direction of use in the relevant industry.

4 Conclusions

The essential oil of thuja and juniper is characterized by a diverse content of biologically active compounds that determine the use of the oil in various sectors of the national economy. The components of the essential oil of the Cupressaceae family are stable in qualitative composition, but vary in quantitative ratio. Due to the variety of biologically active substances contained in thuja and juniper plants, which have a beneficial effect on human psycho-emotional health, further research of this group of plants is relevant. A promising direction may also be the management of biosynthesis through the use of root or non-root treatments with special chemicals of natural or synthetic origin. This will make it possible to increase the raw material base for the use of essential oils in the medicinal, perfumery, pharmaceutical, and chemical industries.

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