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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) BRANCHES FROM ESTONIA

Ain Raal, Rostislav Komarov, Anne Orav, Karmen Kapp, Andriy Grytsyk, Oleh Koshovyi

*For the treatment of the urinary system and kidney diseases, common juniper (*Juniperus communis* L.) fruits are widely used. This raw material is included in the European Pharmacopoeia (Ph.Eur.) and is one of the most popular kinds of official medicinal plant material with diuretic and uroantiseptic activity. However, the main biomass of bushes consists of green branches, which also contain a significant amount of essential oil that can be used in pharmaceutical practice. The branches become waste during bush cutting. So, it is advisable to investigate the chemical composition of essential oil isolated from common juniper branches from different regions of Estonia to prove the possibility of using this essential oil and branches in pharmaceutical practice.*

Aim. *Therefore, the aim of the research was to determine the chemical composition of essential oil from common juniper (*J. communis* L.) branches from Estonia.*

Materials and Methods. *The branches of juniper shrubs were collected in the summer months from 27 different habitats in Estonia. The essential oil was isolated from fresh juniper branches by the modified distillation method described in the European Pharmacopoeia monograph of *Juniperi pseudo-fructus*. GC/MS analysis was carried out using an Agilent 5975 Series MSDMSD, Agilent7890A GC (Agilent Technologies, Inc.) with two detectors (MSMS and FID) on a fused silica capillary column (30 m×0.25 mm) with a bonded stationary phase: poly(5 %-diphenyl-95 %-dimethyl)siloxane (DB-5). The carrier gas was helium with a split ratio of 1:30, and the flow rate of 1.3 mL/min was applied. The temperature program was from 50°–240 °C at 2 °C/min and the injector temperature was 300 °C. The MS detector was operated in the EIEI mode of 70 eV and at a scan rate of 2 scans/s with a mass acquisition range of 20–400 u.*

Research results. *The average amount of juniper essential oil in branches extracted during distillation using the Ph. Eur. method was 0.23±0.04 ml. 103 substances were identified in 27 different samples of juniper branches and quantified by the GC/MS method. The dominant components of Estonian common juniper essential oil are α -pinene (37.5–69.3 %), pinene, sabinene, β -myrcene and β -phellandrene. The juniper essential oils from Estonian raw materials were compared with Serbian, Iran, Portuguese, French and Greek ones. It was established that the common juniper growing in Estonia belongs to the α -pinene chemotype.*

Conclusions. *Common juniper growing in Estonia belongs to the α -pinene chemotype. 103 substances were identified, and their assay was established in 27 different samples of juniper branches. The dominant components of Estonian common juniper essential oil are α -pinene (37.5–69.3 %), so it could be used as a source of α -pinene for the pharmaceutical industry.*

As the essential oils of common juniper branches didn't meet all the requirements of the European Pharmacopoeia for juniper berries oil, so separate regulatory documentation must be developed for the essential oil from the branches

Keywords: *juniper, branches, essential oil, GC/MS analysis, Estonia*

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1. Introduction

Diseases of the urinary system and kidneys occupy a leading place among illnesses all over the world [1]. For their treatment, common juniper (*Juniperus communis* L.) fruits are widely used. This raw material is included in the European Pharmacopoeia (Ph. Eur.) [2] and is one of the most popular kinds of official medicinal plant material with diuretic and uroantiseptic activity [3]. However, the main biomass of bushes consists of green branches, which also contain a significant amount of essential oil that can be used in pharmaceutical practice.

The branches become waste during bush cutting. So, it is advisable to investigate the chemical composition of common juniper branches from different regions of Estonia to prove the possibility of using this essential oil and branches in pharmaceutical practice.

The genus *Juniperus* includes roughly 68 species and 36 varieties and belongs to the *Cupressaceae* family [4, 5]. The plant *Juniperus communis* L. is a shrub or small evergreen tree; a perennial and long-lived coniferous, woody pioneer and colonizing plant, adapted to low nutrient availability in soil and having one the widest distribution ranges

among the different plant species [4, 6]. Its population is spread globally, being the only *Juniperus* species found in both hemispheres, with reports of this plant in Arctic regions of Asia and North America. In Europe, the largest population is found in some parts of the Alps, Scandinavia, Poland, northwest European lowlands, and Mediterranean mountain regions [4, 7]. The wide geographical distribution is the principal reason for the remarkable variation in the morphological characteristics and secondary metabolites' chemical composition [5]. Therefore, it is advisable to conduct comparative studies of raw materials and products of their processing from different regions of the world.

J. communis has been used traditionally in folk medicine for renal suppression, acute and chronic cystitis, catarrh of the bladder, albuminuria, leucorrhea, and amenorrhea [8]. Indeed, this plant presents carminative, diuretic, emmenagogue, digestive, anti-inflammatory [9, 10], antifungal [11, 12], antibacterial [11, 13], analgesic [14], hepatoprotective [15], antidiabetic and anti-hyperlipidemic [16], antioxidant [17], antihypercholesterolemic [18], and anticataleptic effects, and the ability to act as a neuroprotective agent against Parkinson's disease [4, 9].

Relatively to their composition, *J. communis* plant parts are mostly composed of sugars, resins, organic acids, alkaloids, terpenic acids, leucoanthocyanins and flavonoids, gums, lignins and wax. Their essential oils are rich in hydrocarbons of monoterpenes (α -pinene, β -pinene, sabinene and myrcene), diterpenes and sesquiterpenes [4, 19]. All of them contribute to the health-promoting properties shown by this plant [4, 20, 21].

J. communis essential oils from different parts present many volatile organic compounds in their composition, particularly the presence of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes [4, 22]. As well as other phytochemicals, their levels also depend on genotype, origin, cultivation methods, meteorological conditions, and extraction techniques. Even so, among the species, monoterpenes such as α -pinene, β -pinene, and β -myrcene are the most commonly found, followed by some sesquiterpenes compounds, namely germacrene D [4, 23]. There are many studies about the chemical composition of *J. communis* essential oils from Serbia [24], France [25], Portugal [26, 27], Greece [28], Iran [29, 30], Romania [17], USA [28, 31], Algeria [32], Croatia [33], Lithuania [34], Slovakia and Bulgaria [35], Italy [3], Sweden, Switzerland [31] and previously in Estonia [37]. The study regarding the essential oil originating from Estonia included sample collection from only one growing place.

The aim of the research was to determine the chemical composition of essential oil of common juniper (*J. communis* L.) branches from different natural growth sites in Estonia and to determine the chemotype of the local juniper.

2. Planning (methodology) of the research

In Fig. 1, a graphical representation of the research planning process is shown.

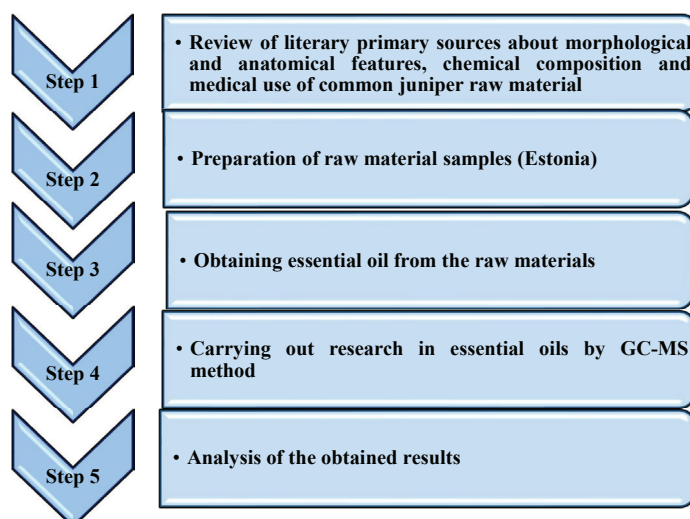


Fig. 1. Planning of the research

3. Materials and methods

The branches of juniper shrubs were collected in the summer months from 27 different possible habitats in Estonia. Most samples were collected in the south and the north of Estonia (Table 1).

The tops of branches (length 20 cm) without berries were cut off from the juniper shrub and were stored fresh in well-closed bags in a freezer at $-18\text{ }^{\circ}\text{C}$. The voucher specimen is deposited in the herbarium of the Institute of Pharmacy, University of Tartu (1 Nooruse Str, Tartu, Estonia). Directly before the distillation of essential oil, the plant material was cut using scissors to fragments about 1 cm, containing needles and woody parts. The essential oils were distilled not later than four months after collecting the plant material.

Isolation of essential oil. The essential oil was isolated from fresh juniper branches by the modified distillation method described in the Ph. Eur. monograph of *Juniperi galbulus* [2, 38] using 30 g of materials, a 500 mL round-bottomed flask, 300 ml water as the distillation liquid and 0.5 mL of xylene in the graduated tube was added to take up the essential oil. The distillation time was 1.5 h at a rate of 3 – 4 ml/min. To improve consecutive chromatographic analyses, hexane was used instead of xylene. The percentage yields obtained were measured by v/w. Only one batch from each sample was analyzed, as it is stated by Ph. Eur. The oils were kept before analysis in well-closed containers at room temperature in the absence of light.

GC/MS analysis. [39]. The full GC-MS analysis of samples 1–17 was carried out using an Agilent 5975 Series MSDMSD, Agilent7890A GC (Agilent Technologies, Inc.) with two detectors (MSMS and FID) on a fused silica capillary column (30 m \times 0.25 mm) with a bonded stationary phase: poly(5 %-diphenyl-95 %-dimethyl)siloxane (DB-5) [40–42]. The film thickness of the stationary phase was 0.25 μm . The carrier gas was helium with a split ratio of 1:30, and the flow rate of 1.3 mL/min was applied. The temperature program was from $50\text{ }^{\circ}\text{C}$ – $240\text{ }^{\circ}\text{C}$ at $2\text{ }^{\circ}\text{C}/\text{min}$, and the injector temperature was $300\text{ }^{\circ}\text{C}$ [43–45]. The MS detector was operated in the EIEI mode of

70 eV and at a scan rate of 2 scans/s with a mass acquisition range of 20–400 u. The method has also been used for detailed analysis of the composition of essential oils of several other plants [46–51]. Determination of the quantitative content of essential oil components was settled in percentages by the method of internal normalization [2].

The GCGC analysis of α -pinene and sabinene in essential oils (samples 18–27) was performed using Agilent GCGC 7890a chromatograph with software Agilent Open Lab CDS Chem Station and with FID on two fused silica capillary columns with stationary phases DB-5 and HP-Innowax (both 30m \times 0.25mm, Agilent). Carrier gas hydrogen with a split ratio 1:150 and a flow rate of 30 mL/min was applied. The temperature program was from 50–250 °C at 2.92 °C/min, and the injector temperature was 250 °C. The identification of the oil components was accomplished by comparing their retention indices (RIRI) on two columns with the RIRI values [53, 54]. Determination of the quantitative content of essential oil components was settled in percentages by the method of internal normalization [2].

Statistical Analysis. Statistical properties of random variables with n-dimensional normal distribution are given by their correlation matrices, which can be calculated from the original matrices. Statistical assessment of all data are reported as mean \pm SEMSEM and were analyzed using STATISTICA 6 software [2].

4. Research results

4.1. Yield and content of essential oil

The essential oil was isolated from fresh juniper branches in amounts of 0.03–0.56 % by the modified distillation method described in the monograph *Juniper galbulus* Ph. Eur. 10 [2, 54] (Table 2). 103 substances were identified, and their assay was established in the 17 samples by the GC/MS method. The contents of the components of the essential oil of juniper branches from the concentration of 0.1 % and more are given in Table 2. The data of the contents of all 103 components can be requested from the corresponding author if interested. Additionally, the content of α -pinene and sabinene in samples 18–27 is shown in Table 3.

5. Discussion of the results

According to Ph. Eur. 10 [2], the content of essential oil in the dried ripe cone berry (*Juniperi galbulus*) must be at least 1 %. None of the 17 samples analyzed met this criterion; the yield was 0.03–0.57 %. Three samples (12–14) of juniper branches contained less than 0.1 % essential oil. The average yield of juniper essential oil extracted during distillation using the Ph. Eur. method was 0.23 \pm 0.04 ml. The average moisture content in juniper branches is 46.95 \pm 3.99 %. The highest essential oil content was in sample 3 (Ida-Virumaa, Iisaku parish, Kauksi village), and its value was 0.57 %.

Table 1

The studied juniper samples from different habitats in Estonia

Sample No.	Administrative locations	Geographical coordinates
1	Ida-Viru county, Narva-Jõesuu	N 59°27'17" E 28°02'51"
2	Jõgeva county, Saare parish, Ruskavere village	N 58°43'47" E 26°50'38"
3	Ida-Viru county, Iisaku parish, Kauksi village	N 58°59'27" E 27°13'50"
4	Valga county, Puka parish, Purtsi village	N 58°07'30" E 26°07'21"
5	Harju county, Kuusalu parish, Kursi village	N 59°27'17" E 25°30'35"
6	Lääne-Viru county, Viru-Nigula parish, Võrkla village	N 59°26'41" E 26°40'41"
7	Põlva county, Rāpina	N 58°06'27" E 27°29'20"
8	Tartu county, Võnnu parish, Hammaste village	N 58°18'14" E 27°00'06"
9	Võru county, Võru	N 57°49'02" E 27°01'29"
10	Põlva county, Ahja hamlet	N 58°12'25" E 27°04'30"
11	Harju county, Kuusalu parish, Pudisoo village	N 59°31'12" E 25°33'28"
12	Tartu county, Tartu	N 58°21'51" E 26°41'06"
13	Valga county, Valga	N 57°47'39" E 26°03'30"
14	Viljandi county, Kõpu parish, Puna village	N 58°20'09" E 25°19'15"
15	Ida-Viru county, Toila parish, Konju village	N 59°22'42" E 27°32'40"
16	Harju county, Tallinn	N 59°24'11" E 24°36'22"
17	Valga county, Tõrva	N 58°00'10" E 25°56'36"
18	Harju county, Kuusalu parish, Viinistu village, Mohni island	N 59° 41'02" E 25° 47'39"
19	Harju county, Jõelähtme parish, Rebala village	N 59° 28'06" E 25° 05'14"
20	Ida-Viru county, Toila parish, Voka village	N 59° 23'23" E 27° 33'48"
21	Ida-Viru county, Iisaku parish, Kauksi village	N 58° 59'20" E 27° 13'37"
22	Lääne-Viru county, Vihula parish, Toolse village	N 59° 31'45" E 26° 27'50"
23	Saare county, Saaremaa island, Salme parish, Tehumardi village	N 58° 10'38" E 22° 15'18"
24	Valga county, Puka parish, Purtsi village	N 58° 05'33" E 26° 04'43"
25	Pärnu county, Häädemeeste parish, Ikla village	N 57° 53'32" E 24° 22'15"
26	Pärnu county, Audru parish, Valgeranna village	N 58° 23'31" E 24° 23'50"
27	Lääne county, Lihula parish, Saastna village	N 58° 44'01" E 23° 33'17"

The lowest essential oil content was in sample 14 (Viljandimaa, Kõpu parish, Puna village) – 0.03 %. This is actually 33 times less compared to the Ph. Eur. requirements for juniper fruits [2]. Thus, a 19-fold increase was observed in the yield of the essential oil in juniper branches changed depending on where the juniper grows. Such a difference is quite large because, in our previous works [42–50], we have found 5...16-fold variations between the minimum and maximum yield of essential oil collected from nature or obtained as commercial samples from different retail pharmacies of various countries. On the other hand, the annual dynamics of the yield of essential oil in the same juniper brunch was 0.05–0.70 % [37].

Table 2

The yield of essential oil and dominant substances of the juniper essential oils from Estonia*

Essential oil	RI DB-5	Content, %																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	-	The yield of essential oil in junipers from Estonia, %																
		0.56± ±0.02	0.29± ±0.01	0.57± ±0.03	0.35± ±0.02	0.32± ±0.01	0.26± ±0.02	0.20± ±0.01	0.10± ±0.02	0.34± ±0.01	0.06± ±0.01	0.16± ±0.01	0.07± ±0.01	0.06± ±0.01	0.03± ±0.01	0.18± ±0.01	0.13± ±0.01	0.23± ±0.02
		The dominant substances of the juniper essential oils from Estonia, %																
Compound		37.5± ±0.3	58.7± ±0.4	51.8± ±0.5	62.0± ±0.5	56.2± ±0.6	69.3± ±0.7	64.8± ±0.7	63.3± ±0.9	66.4± ±0.7	42.6± ±0.5	50.8± ±0.3	50.5± ±0.3	39.0± ±0.4	45.8± ±0.4	38.8± ±0.4	45.8± ±0.5	61.9± ±0.8
α-Pinene	933	14.2± ±0.2	0.5± ±0.1	10.0± ±0.1	0.6± ±0.1	4.9± ±0.2	1.1± ±0.1	0.7± ±0.1	0.6± ±0.1	0.7± ±0.1	0.6± ±0.1	0.5± ±0.1	2.4± ±0.1	11.5± ±0.3	7.6± ±0.3	0.7± ±0.1	2.0± ±0.1	0.7± ±0.1
Sabinene	972	2.0± ±0.2	2.3± ±0.2	1.6± ±0.1	2.4± ±0.1	2.4± ±0.1	2.0± ±0.1	2.7± ±0.2	2.8± ±0.1	1.7± ±0.1	1.8± ±0.1	2.1± ±0.3	2.4± ±0.2	2.1± ±0.1	2.4± ±0.2	2.0± ±0.1	1.6± ±0.1	2.1± ±0.2
β-Pinene	975	4.0± ±0.4	3.4± ±0.3	3.7± ±0.1	3.1± ±0.2	3.7± ±0.4	3.1± ±0.1	3.6± ±0.3	3.3± ±0.1	3.5± ±0.2	3.7± ±0.4	3.7± ±0.4	4.4± ±0.6	3.7± ±0.3	3.7± ±0.2	3.4± ±0.1	3.4± ±0.3	3.4± ±0.2
β-Myrcene	990	2.1± ±0.2	1.4± ±0.1	1.9± ±0.1	1.7± ±0.1	2.0± ±0.2	1.7± ±0.1	2.4± ±0.3	1.3± ±0.1	1.9± ±0.2	2.8± ±0.3	3.6± ±0.4	2.9± ±0.1	1.2± ±0.1	1.4± ±0.2	3.7± ±0.3	2.9± ±0.2	1.8± ±0.1
α-Phellandrene	1003	3.2± ±0.3	0.3± ±0.1	4.6± ±0.2	1.7± ±0.1	3.6± ±0.3	0.5± ±0.1	1.7± ±0.1	8.4± ±0.7	0.2± ±0.0	0.1± ±0.0	0.1± ±0.0	3.6± ±0.2	3.5± ±0.3	4.1± ±0.5	10.7± ±0.8	2.7± ±0.2	0.9± ±0.1
Δ-3-Carene	1009	8.9± ±0.2	13.3± ±0.6	6.1± ±0.1	7.6± ±0.3	8.1± ±0.1	6.7± ±0.4	10.5± ±0.2	5.9± ±0.3	7.6± ±0.4	15.3± ±0.8	16.8± ±0.7	14.0± ±0.5	10.8± ±0.8	10.1± ±0.7	13.5± ±0.1	17.4± ±0.8	8.8± ±0.5
β-Phellandrene + limonene	1027	1.8± ±0.2	0.9± ±0.1	1.8± ±0.2	1.1± ±0.1	1.4± ±0.3	1.1± ±0.1	1.0± ±0.1	1.3± ±0.2	0.8± ±0.1	1.4± ±0.1	1.1± ±0.1	1.3± ±0.2	0.9± ±0.1	1.0± ±0.1	2.0± ±0.3	1.4± ±0.1	1.1± ±0.1
Terpinolene	1086	2.4± ±0.3	0.4± ±0.2	1.6± ±0.2	0.2± ±0.1	0.8± ±0.1	0.2± ±0.1	0.1± ±0.1	0.2± ±0.1	0.2± ±0.1	0.5± ±0.1	0.4± ±0.1	0.3± ±0.1	1.1± ±0.1	0.8± ±0.1	0.1± ±0.1	0.5± ±0.1	0.3± ±0.1
Terpinen-4-ol	1169	0.8± ±0.1	0.6± ±0.1	0.6± ±0.2	0.5± ±0.1	0.6± ±0.1	0.9± ±0.1	0.7± ±0.1	0.8± ±0.1	0.8± ±0.1	0.6± ±0.1	0.7± ±0.1	1.4± ±0.2	6.0± ±0.3	4.1± ±0.3	0.4± ±0.2	0.5± ±0.1	0.7± ±0.1
Bornyl acetate	1280	1.6± ±0.1	0.6± ±0.2	1.6± ±0.2	0.8± ±0.1	0.8± ±0.1	0.1± ±0.1	1.7± ±0.1	0.9± ±0.1	1.4± ±0.1	-	-	1.8± ±0.2	0.4± ±0.2	0.5± ±0.2	1.5± ±0.2	0.9± ±0.1	0.2± ±0.1
α-Terpinyl acetate	1344	0.8± ±0.1	0.3± ±0.1	0.3± ±0.1	1.1± ±0.1	0.6± ±0.1	0.4± ±0.1	0.3± ±0.1	0.5± ±0.1	0.6± ±0.2	1.0± ±0.2	0.3± ±0.1	0.6± ±0.1	0.4± ±0.1	0.5± ±0.1	1.8± ±0.1	0.9± ±0.1	0.6± ±0.1
β-Elementene	1388	0.4± ±0.2	2.0± ±0.2	0.1± ±0.1	1.3± ±0.2	0.8± ±0.1	0.2± ±0.1	1.3± ±0.2	0.3± ±0.1	1.1± ±0.1	0.7± ±0.1	0.1± ±0.1	0.4± ±0.1	1.2± ±0.1	1.4± ±0.1	0.3± ±0.1	0.3± ±0.1	0.3± ±0.1
(E)-β-Caryophyllene	1414	0.1± ±0.1	0.8± ±0.2	0.7± ±0.1	1.5± ±0.2	1.3± ±0.2	1.0± ±0.1	0.8± ±0.1	1.0± ±0.1	1.3± ±0.2	2.1± ±0.3	0.7± ±0.1	0.6± ±0.1	1.1± ±0.1	1.3± ±0.2	2.1± ±0.3	2.1± ±0.1	1.6± ±0.1
Germacrene D	1476	0.7± ±0.1	0.6± ±0.1	0.4± ±0.1	1.0± ±0.1	0.5± ±0.1	0.4± ±0.1	0.2± ±0.1	0.4± ±0.1	0.4± ±0.1	0.6± ±0.1	0.4± ±0.1	0.7± ±0.1	0.5± ±0.1	0.6± ±0.1	1.4± ±0.2	0.9± ±0.1	0.7± ±0.1
δ-Cadinene	1517	0.5± ±0.1	0.4± ±0.1	0.1± ±0.1	0.6± ±0.1	0.3± ±0.1	0.5± ±0.1	0.3± ±0.1	0.6± ±0.1	0.3± ±0.1	1.8± ±0.2	0.7± ±0.1	0.1± ±0.1	0.4± ±0.1	0.4± ±0.1	-	0.2± ±0.1	-
Germacrene B	1550	1.5± ±0.2	0.6± ±0.1	0.1± ±0.1	1.9± ±0.1	0.8± ±0.1	1.4± ±0.1	0.8± ±0.1	1.3± ±0.2	1.0± ±0.1	2.0± ±0.3	1.1± ±0.1	0.1± ±0.1	0.8± ±0.1	2.2± ±0.3	2.2± ±0.3	1.0± ±0.1	0.6± ±0.1
Spathulenol	1569																	

Note: * The samples are described in Tab. 1; tr – traces (<0.05 %); - not determined; bold – content >5%

According to Ph. Eur. 10 [2], essential oil from juniper fruits (*Juniper aetheroleum*) must contain α -pinene 20–50 %, a maximum 20 % of sabinene, β -pinene 1–12 %, β -myrcene 1–35 %, maximum 1 % of α -phellandrene, limonene 2–12 %, terpinene-4-ol 0.5–10 %, bornyl acetate maximum 2 % and maximum 7 % of β -caryophyllene.

The dominant substances in the juniper branches were α -pinene, sabinene, β -myrcene and β -phellandrene. The main component of the essential oil was α -pinene 37.5–69.3 %, the amount of which is higher compared to Ph. Eur. requirements.

Table 3
Content of α -pinene and sabinene (%) in juniper oils from Estonia (samples 18–27*)

Sample No	Content (%) in essential oils	
	α -pinene	Sabinene
18	33.7±0.7	0.3±0.0
19	32.6±0.8	1.0±0.1
20	33.1±0.3	1.4±0.2
21	32.6±0.5	1.3±0.1
22	27.5±0.4	1.0±0.1
23	20.3±0.6	0.6±0.0
24	34.3±0.7	1.0±0.1
25	31.9±0.9	0.02
26	53.5±0.6	2.4±0.12
27	34.0±0.5	1.3±0.1

Note: * The samples are described in Table 1

Consequently, it is possible to state that the common juniper growing in Estonia belongs to the α -pinene chemotype.

Also, below are the concentrations of other main components in essential oils: α -pinene 37.5–69.3 %, sabinene 0.5–14.2 %, β -pinene 1.6–2.8 %, β -myrcene 3.1–4.4 %, α -phellandrene 1.2–3.7 %, limonene and β -phellandrene 5.9–16.8 %, terpinene-4-ol 0.1–2.4 %, bornyl acetate 0.4–4.1 % and β -caryophyllene 0.1–2.0 % (Table 2).

After processing the data, it became clear that not a single sample of the essential oil from branches met all Ph. Eur. requirements. The majority of samples (67 % or 11 essential oils out of 17) contain α -pinene in a concentration greater than 50 %. The amounts of sabinene, α -pinene, β -myrcene and β -caryophyllene in the essential oils were within limits required by Ph. Eur. The content of α -phellandrene is elevated, and no essential oil with an α -phellandrene content of less than 1 % has been analyzed. Samples 13 and 14 contain more bornyl acetate than specified. Also, the content of terpinene-4-ol in most samples (67 % or 11 essential oils out of 17) was a bit lower than needed by the pharmacopoeia. The peaks of limonene and β -phellandrene were not separated, and these substances were determined together. The total concentration of β -phellandrene and limonene was relatively high (8.8–17.4 %). In this way, the essential oil obtained from juniper branches does not meet the requirements of Ph. Eur. monography "*Juniper aetheroleum*" distilled from the juniper fruits, so separate regu-

latory documentation must be developed for the essential oil from the branches.

In the essential oil samples from Serbia and Iran, sabinene content prevailed. The samples from Serbia also showed a high content of Δ -3-carene [24], and the samples from Iran were high in terpinene-4-ol and limonene [24]. In French samples, limonene prevailed (49.3 %), α -pinene content was at the level of 22 % [25]. α -Pinene chemotypes were studied in Portugal, where significant myrcene (16.5 %) and limonene (6.25 %) were observed [26]. Other research showed a high content Δ -3-carene (5–7 %) in the samples from Portugal [27]. In Greece, there were also α -pinene chemotypes with a significant amount of sabinene (6–13.3 %) and myrcene (11.1–18.1 %) [28]. In this study, some samples from Estonia showed a high content of Δ -3-carene (8.4 %, 10.7 %) and β -phellandrene with limonene (6.1–17.4 %).

Therefore, pinene is the main component of essential oil in juniper that grows in many different countries [30–35, 52]. The oils of different juniper varieties were also dominated by α -pinene (16.1–59.3 %) [31]. In our first study of 12 samples of juniper branches of Estonian origin, the oil yields ranged between 0.05–0.70 % within a year, and the essential oils showed the highest content of α -pinene (40.4–62.0 %) [37].

6. Conclusions

Common juniper growing in Estonia belongs to the α -pinene chemotype. The average amount of juniper essential oil in branches extracted during distillation using the Ph. Eur. method is 0.23±0.04 ml. 103 substances were identified, and their assay was established. The dominant components of Estonian common juniper essential oil are α -pinene (37.5–69.3 %), pinene, sabinene, β -myrcene and β -phellandrene.

No sample of the essential oil of common juniper branches met all the requirements of the European Pharmacopoeia for juniper berries oil, so separate regulatory documentation must be developed for the essential oil from the branches.

The prospects for further research. The obtained results could be used for the development of an analytical method for quality control of the essential oil from juniper branches. Further phytochemical and pharmacological studies of the juniper branches and their essential oil will show the prospect of creating new pharmaceuticals.

Conflicts of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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