Annual Variation of Yield and Composition of the Essential Oil of Common Juniper (*Juniperus communis* L.) Branches from Estonia

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Abstract

The yield and composition of the essential oil of common juniper (Juniperus communis L.) branches from Estonia were analyzed. The yields and composition of the oil isolated by hydrodistillation from dried juniper branches, gathered from one single juniper plant every month in 2006 and in 2007 (12 samples), were compared. The oil yields ranged between 0.05 – 0.70%. A total sixty seven compounds were identified, representing over 96% of the total oil yield. The essential oil from Estonian juniper branches showed a high content of α-pinene (40.4 – 62.0%); the other predominant constituents were limonene (4.2 – 10.0%), α-cadinol (1.9 – 6.3%), δ-cadinene (2.1 – 4.8%), γ-muurolene and germacrene D (1.6 – 4.4%), β-myrcene (2.6 – 3.1%), α- and β-selinene (0.9 – 3.1%), germacren D-4-ol (0.8 – 3.0%) and ?-pinene (1.4 – 2.2%). The yield of essential oil and amounts of mono- and sesquiterpenes depend on the month of collecting the plant material. The best time for harvesting juniper branches seems to be from January to April. The yield and composition of essential oils in branches are rather similar, but did not completely correspond to the standards of European Pharmacopoeia stated to juniper berries.

Key words: *Juniperus communis*; common juniper; branches; essential oil; α-pinene; harvesting time; European *Pharmacopoeia*

Introduction

Trees are valued for many reasons. They are also used as natural sources of plant drugs and medicines in conventional, traditional and alternative medicine (Conway 2001). There are around 50 Juniperus species, many of which are used in medicine. The common juniper (*Juniperus communis* L.) is an evergreen shrub or small tree of Northern temperate region (Conway 2001). Commercial juniper berry oil is rarely a true distillate from berries and may be a by-product from gin or brandy manufacture. It exhibited antimicrobial activity against a range of organism (Bradley 2006). Juniper is stated to possess diuretic, antiseptic, carminative, stomachic, and antirheumatic properties. It has been used for cystitis, flatulence, colic, rheumatic pains in joints and muscles (Ebadi 2007). Pharmacological actions of juniper are primarily associated with the essential oil components. The oil is documented to possess diuretic, gastrointestinal antiseptic and irritant properties (Barnes et al. 2002).

In addition to berries also branches, needles and wood of juniper were and are traditionally in use in Estonian folk medicine (Viires 2000). Juniper needles contain 0.2 - 1.0% of volatile oil. Oil yield depends on the degree of ripeness, seasonal variations, environmental conditions (temperature, sunlight, photoperiod), age of plant latitude and altitude of growing site, a role in selective browsing damage by local herbivores and other factors (Latrasse 1991, Markó et al. 2008, Martz et al. 2009).

The average yield of the essential oil varied from 0.47 to 0.75% in dried needles with young juniper branches and 0.10 - 0.28% in dried branches according to the month of collection. The most significant changes in the content of the oil were found in spring-summer period of vegetation (Kowal and Krupinska 1970).

In the last years and before a number of publications reported the composition of juniper needles or branches oil. Essential oils are traditionally isolated by hydrodistillation (Gelsomini et al. 1988, Chatzopoulou and Katsiotis 1993, Koukos and Papadopoulou 1997, Ochocka et al. 1997, Adams 1998; 2000, Mastelic et al. 2000, Karlsen and Svendsen 2002, 2003; Angioni et al. 2003, Shahmir et al. 2003, Pourmortazavi et al. 2004, Marongiu et al. 2005, Butkiene et al. 2005a, 2006a,b 2007, Gonny et al. 2006, Looman and Svend-

sen 2006, Markó et al. 2008, Milojević et al. 2008, Butkiene et al. 2009, Ottavioli et al. 2009). The composition of needles essential oil varies considerably, consisting mainly of monoterpenes and sesquiterpene hydrocarbons (α-pinene, sabinene, β-myrcene, limonene, β-phellandrene, caryophyllene, muurolenes, germacrene D and B, humulene). The main oxygen-containing monoterpene identified in juniper needles oils were terpinen-4-ol (Chatzopoulou and Katsiotis 1993, Mastelic et al. 2000;, Karlsen and Svendsen 2002, Angioni et al. 2003, Shahmir et al. 2003, Butkiene et al. 2005a,b, 2006a,b), terpinyl acetate (Angioni et al. 2003, Marongiu et al. 2005, Gonny et al. 2006), and oxygen-containing sesquiterpenes were germacrene D-4-ol and α-cadinol (Butkiene et al. 2005a,b, 2006a,b). The best time for harvesting juniper branches is still not clear.

There is no monograph of juniper needles or branches in European Pharmacopoeia (EP) (*European Pharmacopoeia* 2008), but the monograph of Juniper (*Juniperi pseudo-fructus*) is defined as the ripe cone berry of *Juniperus communis* L., which contains a minimum of 10 ml/kg of essential oil. By the monographs of juniper oil (*Juniperi aetheroleum*) the essential oil obtained by steam distillation from the ripe, non-fermented berry cones of *Juniperus communis* L. and the percentages of the components are within the following ranges: α-pinene 20 – 50%, sabinene less than 20%, β-pinene 1 – 12%, β-myrcene 1 – 35%, α-phellandrene less than 1%, limonene 2 – 12%, terpinen-4-ol 0.5 – 10%, bornyl acetate less than 2%, and β-caryophyllene less than 7%. The quality of essential oils of juniper berries and branches have not been compared before.

The demand for dry juniper needles as a raw material for the food, pharmaceutical, and cosmetic industries has increased rapidly in recent years (Martz et al. 2009).

The aim of this paper was to investigate the yield and chemical composition of the essential oil of common juniper branches in Estonia, and to determine the influence of harvesting time to the oil yield and composition of the biologically active constituents.

Materials and methods

Plant material

The branches of the one juniper shrub (height about 2.5 m and width about 2 m) growing in northern Estonia (Harjumaa, Humala village, Figure 1) were collected every month from May 2006 to April 2007. The tops of branches (length 10 cm) without berries were cut off from the juniper and dried at room temperature in a well ventilated room. The branches collected were all at the same level and in the same development stage. All collected drug samples were preserved in well-closed bumper bags at room temperature in the absence of light. The voucher specimen is deposited in herbarium of the Department of Pharmacy our University (1 Nooruse Str, Tartu).

Directly before 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 month after collecting the plant material, kept in well-closed containers at room temperature in the absence of light.

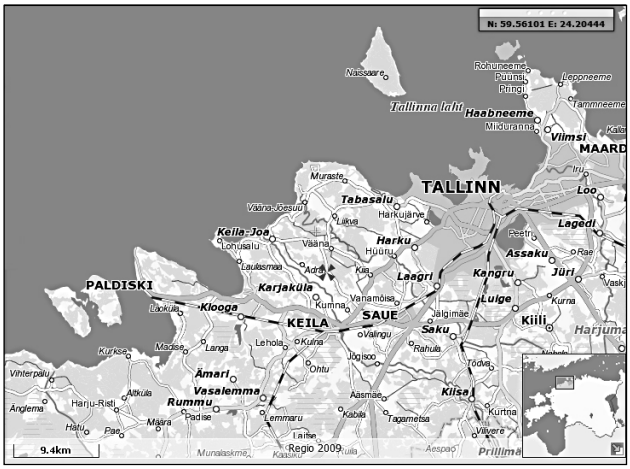

Figure 1. Map of the investigation site
* Humala village

Isolation of essential oil

The essential oil was isolated from dried juniper branches by the distillation method described in the EP monograph of Juniperi pseudo-fructus (*European Pharmacopoeia* 2008) using 20g of drug, a 500 mL round-bottomed flask, 200 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 analyze, 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 EP.

GC/FID analysis

The essential oils from juniper branches were analyzed using a Chrom-5 chromatograph with FID on two fused silica capillary columns with two stationary phases: poly(5%-diphenyl-95%-dimethylsiloxane) (SPBTM-5, 30m ×0.25 mm, Supelco, Switzerland) and polar polyethylene glycol (SW-10, 30m ×0.25 mm, Supelco, Switzerland). Film thickness of both stationary phases was 0.25 μm. Helium with the split ratio 1:150

and the flow rate 30– 35 cm/s, was applied as the carrier gas. The temperature programme was from 50 - 250°C at 2°C/min, the injector temperature was 250°C. A Spectra-Physics SP4100 integrator was used for data processing.

The identification of the oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data bank and with literature data (Davies 1990, Zenkevich 1996, 1997, 1999). RI indices (RI) are relative to n-alkanes. The results obtained were confirmed by GC/MS.

The percentage composition of the oils was calculated in peak areas using normalization method without using correction factors. The relative standard deviation of percentages of oil components of three repeated GC analyses of a single oil sample did not exceed 5%.

GC/MS analysis

GC/MS analysis was carried out using GCMS-QP2010 (Shimadzu, Japan) on fused silica capillary column (30 m x 0.32 mm) with bonded stationary phase: poly (5%-diphenyl-95%-dimethyl)siloxane) (ZB-5, Zebron). The film thickness of stationary phase was 0.25mm. The carrier gas was helium with the split ratio of 1:17 and flow rate 1.8 mL/min was applied. The temperature program was 2 min at 60°C and then from 60 to 280°C at 12°C/min, and the injector temperature was 280°. The MS detector was operated in the EI mode 70 eV at a scan rate 2cans/s with an acquisition mass range of 40 – 500 u.

Results

The pale yellow essential oil of *J. communis* branches with a characteristic odor was obtained by hydrodistillation in a yield 0.05 – 0.70%, based on the dry weigh of the sample (Tables 1 and 2). The yield of the essential oil changed gradually during the year. The most significant changes were observed during spring-summer period of vegetation. The minimum yield of essential oil (0.05%) was found in the sample collected in October, and the maximum (0.7%) in March. A fourteen-fold increase was observed between the minimum and maximum relative concentration of essential oil, the average content was 0.3%. The minimum limit of 0.1% stated by EP was exceeded in all samples excluding the branches collected in October.

Retention indexes (RI) on two capillary columns of different polarity, concentration range and main concentration values of the oil components are reported in Table 1. Sixty seven compounds were identified in twelve essential oil samples comprising 96.8 – 99.0% of the total oils.

Table 1. Composition of the essential oil of juniper branches

Compound	RI		Concentration	Mean, %
	SPB-5	SW-10	range, %	n=12
n-hexanal	800	1076	tr – 0.3	0.11
(E)-2-hexenal	845	1223	tr – 0.4	0.08
tricyclene	914	1009	tr - 0.2	0.13
α-thujene	922	1020	tr - 0.1	0.02
α-pinene	929	1026	40.4 – 62.0	**56.10**
α-fenchene	939	1056	tr - 0.2	0.05
camphene	940	1061	0.3 – 0.7	0.51
sabinene	966	1121	0.3 – 0.7	0.42
β-pinene	969	1105	1.4 – 2.2	**1.92**
β-myrcene	988	1162	2.6 – 3.1	**2.82**
2-carene	998	1130	0.1 – 0.2	0.14
α-phellandrene	1000	1163	0.1 – 0.5	0.19
Δ-3-carene	1006	1145	tr - 0.7	0.24
α-terpinene*	1012	1176	tr - 0.1	0.04
p-cymene	1019	1272	tr - 0.1	0.05
limonene	1024	1200	4.2 – 10.0	**7.40**
β-phellandrene*	1024	1208	0.5 – 2.0	**1.00**
3-methylbutyl butanoate	1051	1269	0.2 – 0.6	0.45
γ-terpinene	1053	1244	tr - 0.1	0.06
trans-4-pentenyl butanoate	1061	1346	tr - 0.1	0.04
terpinolene	1081	1283	0.5 – 0.9	0.75
linalool	1100	1557	tr - 0.1	0.06
n-nonanal	1104	1390	0.1 – 0.7	0.41
3-methylbutyl isovaleriate	1114	1374	0.1 – 0.5	0.36
3-methyl-3-butenyl isovaleriate	1118	1422	tr - 0.1	0.05
campholenal	1118	1508	tr - 0.2	0.06
3-methyl-2-butenyl valeriate	1135	1450	tr - 0.4	0.09
camphene hydrate	1147	1570	tr - 0.5	0.13
p-mentha-1,5-dien-8-ol	1162	1665	tr- 0.2	0.03
terpinen-4-ol	1169	1603	0.2 – 0.7	0.35
α-terpineol	1184	1707	0.2 – 0.8	0.44
myrtenal*	1200	1728	0 – 0.2	0.03
β-citronellol	1235	1794	0 – 0.4	0.05
3-methylbutyl hexanoate	1250	1461	0 – 0.2	0.07
methyl citronellate	1259	1482	0.1 – 0.6	0.27
bornyl acetate	1278	1576	0.5 – 1.2	0.73
2-undecanone	1290	1590	0.1 – 0.3	0.14
myrtenyl acetate	1318	1687	tr - 0.2	0.10
α-terpinyl acetate	1343	1680	tr - 0.7	0.22
trans-myrtanyl acetate	1372	1771	0.1 – 0.2	0.15
β-elemene	1383	1587	0.4 – 1.0	0.67
(E)-β-caryophyllene	1409	1586	0.7 – 1.8	**1.14**
α-humulene	1440	1658	0.6 – 1.5	**0.93**
(E)-β-farnesene	1452	1667	tr - 0.2	0.09
γ-muurolene	1467	1698	1.6 – 4.4	**2.94**
germacrene D	1469	1700		
α-amorphene	1473	1718	0.1 – 0.4	0.26
cadina-3,9-diene	1477	1712	0.1 – 0.2	0.16
α-muurolene	1482	1723	0.4 – 1.3	0.77
β-selinene	1490	1748	0.9 – 3.1	**1.89**
α-selinene	1492	1702		
γ-cadinene*	1500	1750	0.4 – 0.9	0.61
δ-cadinene	1513	1752	2.1 – 4.8	**3.00**
α-farnesene	1528	1739	0.1 – 0.2	0.12
elemol	1540	2078	0 – 0.1	0.03
germacrene B	1542	1815	0.5 – 1.7	**1.08**
spathulenol	1560	2118	0 – 0.3	0.15
caryophyllene oxide	1562	1965	0 – 0.1	0.02
germacrene D-4-ol	1565	2045	0.8 – 3.0	**1.70**
globulol	1582	2100	tr- 0.9	0.23
•-cedrol	1597	2055	tr - 0.2	0.10
cis-ledol	1615	2100	tr- 0.4	0.18
•-cadinol	1630	2165	0.5 – 2.0	0.83
• –cadinol	1634	2182	0.6 – 2.1	**1.10**
T-muurolol	1638	2195	0.2 – 0.8	0.40
• –cadinol	1647	2228	1.9 – 6.3	**3.08**
(Z,Z)-• -farnesol	1688	2317	0.3 – 0.8	0.51
Total, %			96.8 – 99.0	98.25
Oil yield, %			0.05 – 0.70	**0.30**

tr – traces (<0.05%),
Identification confirmed by GC/MS except for *
Boldface designates the principal components

Table 2. Concentration of the main components of the essential oil of juniper branches, %

Compound	Time of sample collection											
	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
•-pinene	**50.7**	**62.0**	**60.6**	**64.8**	**45.4**	**56.2**	**40.4**	**54.6**	**55.9**	**59.4**	**62.1**	**61.7**
•-pinene	1.7	2.2	2.2	2.1	1.8	1.6	1.6	1.4	2.2	2.2	2.2	1.9
•-myrcene	2.6	2.6	2.7	3.0	2.9	3.0	2.6	2.9	3.0	3.1	2.9	2.6
limonene	**10.0**	**6.7**	**4.2**	**5.7**	**6.6**	**6.8**	**6.6**	**8.1**	**7.9**	**7.6**	**8.0**	**9.6**
•-phellandrene	0.8	0.6	0.5	0.6	1.4	1.9	1.0	0.8	0.7	2.0	0.7	0.9
(E)-•-caryophyllene	1.1	1.3	1.2	0.8	1.6	1.0	1.8	1.4	1.3	0.8	0.7	0.7
•-humulene	0.9	1.0	1.0	0.7	1.3	0.8	1.5	1.0	1.1	0.7	0.6	0.6
•-muurolene germacrene D	3.0	3.1	3.6	2.3	4.3	3.5	4.4	3.0	2.7	2.1	1.6	1.7
•-muurolene	0.9	0.7	0.9	0.6	0.9	0.7	1.3	0.9	0.7	0.6	0.4	0.6
•-selinene	1.9	1.6	2.7	1.5	2.8	1.6	3.1	2.2	1.8	1.4	1.2	0.9
•-selinene												
•-cadinene	**2.7**	**2.1**	**2.9**	**2.4**	**4.8**	**3.1**	**4.8**	**3.4**	**2.9**	**2.4**	**2.4**	**2.1**
germacrene B	0.8	1.4	1.3	0.9	1.7	1.3	1.5	1.1	1.2	0.7	0.5	0.5
germacrene D-4-ol	2.0	0.8	1.4	0.8	3.0	1.3	3.0	1.8	2.1	1.5	1.2	1.5
• –cadinol	1.0	0.6	1.3	0.7	1.6	1.0	2.1	1.2	1.0	0.9	1.0	0.8
• –cadinol	**2.8**	**1.9**	**3.3**	**2.3**	**4.8**	**2.6**	**6.3**	**3.2**	**2.9**	**2.6**	**2.3**	**2.0**
Total, %	**82.9**	**88.6**	**89.8**	**89.2**	**84.8**	**86.4**	**82.0**	**87.0**	**87.4**	**88.0**	**87.8**	**88.1**
Oil yield, %	**0.35**	**0.25**	**0.70**	**0.35**	**0.15**	**0.35**	**0.20**	**0.45**	**0.10**	**0.05**	**0.50**	**0.15**

tr – traces (<0.05%)
Boldface designates the principal components

The essential oil from Estonian juniper branches showed a high content of α-pinene (40.4 – 62.0%); the other predominant constituents were limonene (4.2 – 10.0%), α-cadinol (1.9 – 6.3%), δ-cadinene (2.1 – 4.8%), γ-muurolene and germacrene D (1.6 – 4.4%), β-myrcene (2.6 – 3.1%), α- and β-selinene (0.9 – 3.1%), germacrene D-4-ol (0.8 – 3.0%) and β-pinene (1.4 – 2.2%) (Table 1).

Monoterpene hydrocarbons content in studied juniper samples varied from 54.6% to 79.0% (Table 3). The highest relative concentrations of these compounds were found in samples collected in winter (November, December) and in April (78.6-79.0%). The ratios of the amounts of mono- and sesquiterpene hydrocarbons were the smallest in May and July (~3:1) and the highest in November and December (>8:1).

Discussion and conclusions

A fourteen-fold increase was observed in the yield of the essential oil in juniper branches changed during the year (Tables 1 and 2). As shown in the results of studies (Bucko and Salamon 2007), major factors causing differences in the yield of essential oil may be the time between harvest and distillation, the variety of the origin of plants, various harvesting methods, size of green parts, drying, etc. In our previous investigations we have found significant difference between the minimum and maximum yield of essential oil also in other popular medicinal plants: eleven-fold times in *Achillea millefolium* (Orav et al. 2006) and *Salvia officinalis* (Raal et al. 2007), fourteen-fold in *Thymus serpyllum* (Paaver et al. 2008), five-fold in *Pimpinella anisum* (Orav et al. 2008), sixteen-fold in *Levisticum officinale* (Raal et al. 2008a) six-fold in *Valeriana officinalis* (Raal et al. 2008b) and ten-fold in Chamomilla recutita (Orav et al. 2010), collected or obtained as commercial samples from different retail pharmacies of various countries.

By the maximum yield of essential oil, the best time for harvesting juniper branches seems to be from Jan-

Table 3. Component groups concentration of the essential oil of juniper branches, %

Compound group	Time of sample collection											
	Jan	Feb	March	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Monoterpene hydrocarbons	68.0	76.4	72.2	**78.7**	61.0	72.9	54.6	70.7	72.1	77.5	**78.6**	**79.0**
Sesquiterpene hydrocarbons	13.4	12.8	15.8	10.8	**19.8**	13.6	**21.0**	14.8	13.7	10.2	9.2	8.8
Oxygen-containing monoterpenes	**4.5**	1.8	1.4	1.9	3.1	2.8	**4.2**	2.6	2.4	2.6	1.9	2.2
Oxygen-containing sesquiterpenes	8.9	5.1	8.1	6.0	**12.1**	6.8	**15.3**	8.4	8.2	7.0	6.4	7.0
Other compounds	**3.0**	1.9	1.2	1.6	1.6	1.8	1.7	1.8	2.0	1.4	2.0	1.4
In Total	97.8	98.0	98.7	99.0	97.6	97.9	96.8	98.3	98.4	98.7	98.1	98.4

uary to April. J. R. Ochocka et al. (1997) studied to what extent the season of harvesting influences the composition of the juniper essential oil. They found increasing yield of limonene (1.5 – 6.7%) and decreasing contents of β-myrcene (9.5 – 4.0%) and β-phellandrene (19.1 - 4.9%) in needles samples between autumn and spring. Analyses of essential oils from Estonian juniper branches showed increasing yield of β-phellandrene and decreasing yield of limonene in summer (Table 2).

The juniper chemotype, growing and analyzed in Estonia is rich in α-pinene and seems to be similar to samples from Lithuania (Butkiene et al. 2005a, 2006a,b), Norway (Karlsen and Svendsen 1968, 2002, 2003), Bulgaria (Yankov et al. 1969), Sweden (Adams 2000) or Italy (Marongiu et al. 2005). But in the composition of needle oil of juniper from India the major component was sabinene (27.5- 48.8%), the yield of α-pinene (4.0 - 6.2%) was conspicuously low (Pande and Mathela 2000, Singh et al. 2005). There was found three chemical races – the α-pinene type, the sabinene type and one with intermediate contents of these terpenes among both *J. communis* and *J. nana* in Poland (Filipowicz et al. 2006). In the oil of Estonian juniper the sabinene was a minor component (0.3 – 0.7%). On the other hand, the amount of α-pinene decreased and the amount of myrcene increased with increasing hydrodistillation rate (Milojević et al. 2008). As it was found in the results of investigations (Butkiene et al. 2009), the needles and berries of the same juniperus growing wild in Lithuania produced essential oils of different chemotypes: the juniperus with needles producing sabinene-chemotype oils were found only in three localities from the 34 investigated habitats, beside the plants with needles biosynthesizing α-pinene-chemotype essential oils.

In the 109 samples of essential oil isolated from the needles of *J. communis* ssp. *alpina* growing in wild in Corsica, was the main component limonene (up to 53.9%) (Ottavioli et al. 2009).

α-Pinene and β-pinene have exhibited a spasmogenic activity towards smooth muscle, with no effect on cardiac muscle (Barnes et al. 2002). β-Phellandrene (4.1 – 11.4%) was the second major constituent in needle oils from Lithuania (Butkiene et al. 2005a). In Estonian oils, yield of β-phellandrene was only 0.5 – 2.0%. The second main compound in Estonian oil was limonene (4.2 – 10.0%), as in needle oils from Sweden (Adams 2000) and Italy (Marongiu et al. 2005).

The limits of all other compounds excluding α-pinene and terpinen-4-ole in the braches' oils corresponded to the standards stated by EP to juniper berries (*European Pharmacopoeia* 2008). By the EP the yield of terpinen-4-ol should be between 0.5 and 10%, but in the juniper sample analyzed, the amount of terpinen-4-ol was under EP standard (0.2 – 0.7%). As much the mentioned essential oil component is reported to increase the glomerular filtration rate, the diuretic activity of juniper has been attributed just to terpenen-4-ol. However, on the other hand, it is also stated to be an irritant to kidneys (Barnes et al. 2002).

The yield of α-pinene in analyzed oils usually did not correspond to the EP standard (20 – 50%) for berries; only in samples collected in May and July the amount of α-pinene was lower than 50%. As shown (Butkiene et al. 2007), the essential oils of the needle, unripe and ripe berry of juniper of Lithuanian origin were of α-pinene chemotype (42.4 – 67.4%), but the amounts of α-pinene in the wood oils (15.9-31.0%) were lower than those in the oils mentioned above. The most abundant compounds in one wood oil were nootkatone (18.4%), α-pinene (15.9%) and α-cadinol (3.8%) and in the other oil α-pinene (31.0%), p-mentha-1,5-dien-8-ol (4.5%) and δ-3-carene (4.0%). In the juniper growing in Norway, the main ingredients of the needles were α-pinene and β-phellandrene, and in the branches, α-pinene and limonene (Karlsen and Svendsen 1968). So the yield of α-pinene in the oils of juniper berries, needles, branches and wood can be rather different.

The sums of mono- and sesquiterpenoids were close (89.7 - 95.5%) in all five essential oils distilled from ripe berries of juniper growing wild in northeast Lithuania (Butkiene et al. 2005b). In the oils of unripe and ripe berries from five plants of juniper growing wild in Vilnius district, the monoterpene hydrocarbons (61.4 – 80.8%) prevailed (Butkiene et al. 2004). But different quantities of monoterpenoids (24.6 and 60.1%) and sesquiterpenoids (63.4 and 23.2%) were found in the two essential oils of the juniper wood (Butkiene et al. 2007). Martz et al. recently described the terpenoid composition of juniper needles collected in Finland (n = 125) and demonstrated that the content of these compounds clearly increased with latitude and altitude with, however, a stronger latitudinal effect - a higher content of monoterpenoids (Martz et al. 2009).

In our investigation, it was shown that the yield of essential oil and ratios of the amounts of mono- and sesquiterpenes depended on the month of collecting juniper branches. The best time for harvesting juniper branches seems to be from January to April. The yield and composition of essential oils of branches were rather similar, but did not completely correspond to the standards of EP stated to juniper berries.

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References

Adams, R.P. 1998. The leaf essential oils and chemotaxonomy of Juniperus sect. Juniperus. *Biochemical Systematics and Ecology* 26: 637 – 645.

Adams, R.P. 2000. Systematics of Juniperus section Juniperus based on leaf essential oils and random amplified polymorphic DNAs (RAPDs). *Biochemical Systematics and Ecology* 28: 515 – 528.

Angioni, A. Barra, A., Russo, M.T., Coroneo, V. Dessi, S. and Cabras, P. 2003. Chemical composition of the essential oil of Juniperus from ripe and unripe berries and leaves and their antimicrobial activity. *Journal of Agricultural and Food Chemistry* 51: 3073 – 3078.

Barnes, J., Anderson, L.A. and Phillipson, J.D. 2002. Pharmaceutical Press, London, Chicago, p. 317 – 319.

Bradley, P. 2006. British herbal compendium: A handbook of scientific information on widely used plant drugs. British Herbal Medicine Association, Bournemouth, p. 237 – 241.

Bucko, D. and Salamon, I. 2007. The essential oil quality of chamomile, Matricaria recutita L., after its large-scale distillation. *Acta Horticulturae* 749: 269 – 273.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2004. Chemical composition of unripe and ripe berry essential oils of *Juniperus communis* L. growing wild in Vilnius district. *Chemija* 15: 57 – 63.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2005a. α-Pinene chemotype of leaf (needle) essential oils of *Juniperus communis* L. growing wild in Vilnius district. *Chemija* 16: 53 – 60.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2005b. Volatile compounds of ripe berries (black) of *Juniperus communis* L. growing wild in north-east Lithuania. *Journal of Essential Oil Bearing Plants* 8: 140 – 147.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2006a. Differences in the essential oils of the leaves (needles), unripe and ripe berries of *Juniperus communis* L. growing wild in Vilnius district (Lithuania). *Journal of Essential Oil Research* 18: 489 – 494.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2006b. Leaf (needle) essential oils of *Juniperus communis* L. growing wild in eastern Lithuania. *Journal of Essential Oil Bearing Plants* 9: 144 – 151.

Butkiene, R., Nivinskiene, O. Mockute, D. and Miliute, A. 2007. Variety of the essential oils composition of wood, needles (leaves), unripe and ripe berries of *Juniperus communis* var. communis growing wild in Druskininkai district. *Chemija* 18: 35 – 40.

Butkiene, R., Nivinskiene, O. and Mockute, D. 2009. Two chemotypes of essential oils produced by the same *Juniperus communis* L. growing wild in Lithuania. *Chemija* 20: 195 – 201.

Chatzopoulou, P.S. and Katsiotis, S.T. 1993. Chemical investigation of the leaf oil of *Juniperus communis* L. *Journal of Essential Oil Research* 5: 603 – 607.

Conway, P. 2001. Tree medicine: A comprehensive guide to the healing power of over 170 trees. Piatkus, London, p. 1; 204 – 206.

Davies, N.W. 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *Journal of Chromatography* 503: 1 – 25.

Ebadi, M. 2007. Pharmacodynamic basis of herbal medicine. 2nd Ed. Taylor & Francis, Boca Raton, p. 97.

European Pharmacopoeia. 2008. 6th Ed. Council of Europe, Strasbourg, p. 2206 – 2208.

Filipowicz, N., Piotrowski, A., Ochocka, J.R. and Asztemborska, M. 2006. The phytochemical and genetic survey of common and dwarf juniper (*Juniperus communis* and Juniperus nana) identifies chemical races and close taxonomic identity of the species. *Planta Medica* 72: 850 – 853.

Gelsomini, N., Vidrich, V., Fusi, P. and Michelozzi, M. 1988. Capillary gas chromatography of the terpenic fractions of *Juniperus communis* L. black, green berry and leaf extracts. *Journal of High Resolution Chromatography & Chromatography Communications* 11: 218 – 220.

Gonny, M., Cavaleiro, C., Salgueiro, L. and Casanova, J. 2006. Analysis of *Juniperus communis* subsp. alpina needle, berry, wood and root oils by combination of GC, GC/MS and 13C-NMR. *Flavour and Fragrance Journal* 21: 99 – 106.

Karlsen, J. and Svendsen, A.B. 1968. Terpenes and related compounds. VI. Gas-liquid chromatography of the monoterpene hydrocarbons of the volatile oil of separated needles and of branches from *Juniperus communis*. *Meddelelser fra Norsk Farmaceutisk Selskap* 30(1): 1 – 5.

Karlsen, J. and Svendsen, A.B. 2002. The influence of the isolation method on the composition of the essential oil of leaves and twigs of *Juniperus communis* L. var. saxatilis Pall. growing in Norway. *Scientia Pharmaceutica* 70: 87 – 92.

Karlsen, J. and Svendsen, A.B. 2003. The content of terpinen-4-ol in the essential oil of separate leaves and in hydrodestilled oil of leaves and terminal twigs of *Juniperus communis* L. var. *saxatilis* Pall. *Scientia Pharmaceutica* 71: 35 – 37.

Koukos, P.K. and Papadopoulou, K.I. 1997. Essential oil of *Juniperus communis* L. grown in northern Greece: variation of fruit oil yield and composition. *Journal of Essential Oil Research* 9(1): 35 – 39.

Kowal, T. and Krupinska, A. 1970. Essential oils from leaves with young terminal branches and older branches (without leaves) of *Juniperus communis*. *Annales Pharmaceutici* (Poznan) 8: 83 – 91.

Latrasse, A. 1991. Fruits III. In: Maarse, H. (Ed.). Volatile compounds in foods and beverages. Marcel Dekker, New York, p. 329 – 387.

Looman, A. and Svendsen, A.B. 2006. The needle essential oil of norvegian mountain juniper, *Juniperus communis* L. var. Saxatilis Pall. *Flavour and Fragrance Journal* 7, 2006, 23 – 25.

Markó, G., Gyuricza, V., Bernáth, J. and Altbacker, V. 2008. Essential oil yield and composition reflect browsing damage of junipers. *Journal of Chemical Ecology* 34: 1545 – 1552.

Marongiu, B., Porcedda, S., Piras, A., Sanna, G., Murreddu, M. and Loddo, R. 2005. Extraction of *Juniperus communis* L. ssp. nana Willd. Essential oil by supercritical carbon dioxide. *Flavour and Fragrance Journal* 21: 148 – 154.

Martz, F., Peltola, R., Fontanay, S., Duval, R.E., Riitta, J.T. and Stark, S. 2009. Effect of latitude and altitude on the terpenoid and soluble phenolic composition of juniper (*Juniperus communis*) needles and evaluation of their antibacterial activity in the boreal zone. *Journal of Agricultural and Food Chemistry* 57: 9575 – 9584.

Mastelic, J., Miloš, M., Kuštrak, D. and Radonic, A. 2000. Essential oil and glycosidically bound volatile compounds from the needles of common juniper (*Juniperus communis* L.). *Croatia Chemica Acta* 73: 585 – 593.

Milojević, S.Z., Stojanović, T.D., Palić, R., Lazić, M.L. and Veljković, V.B. 2008. Kinetics of distillation of essen-

tial oil from comminuted ripe juniper (*Juniperus communis*) berries. *Biochemical Engineering Journal* 39: 547 – 553.

Ochocka, J.R., Asztemborska, M., Zook, D.R., Sybilska, D., Perez, G. and Ossinini, L. 1997. Enantiomers of monoterpenic hydrocarbons in essential oils from *Juniperus communis*. *Phytochemistry* 44: 869 – 873.

Orav, A., Arak, E. and Raal, A. 2006. Phytochemical analysis of the essential oil of *Achillea millefolium* L. from various European countries. *Natural Product Research* 20(12): 1082 –1088.

Orav, A., Raal, A. and Arak, E. 2008. Essential oil composition of *Pimpinella anisum* L. fruits from various European countries. *Natural Product Research* 22(3): 227 – 232.

Orav, A., Raal, A. and Arak, E. 2010. Content and composition of the essential oil of *Chamomilla recutita* (L.) Rauschert from some European countries. *Natural Product Research* 24(1): 48 – 55.

Ottavioli, J., Gonny, M., Casanova, J. and Bighelli, A. 2009. Chemical variability of the needle oil of *Juniperus communis* ssp. alpina from Corsica. *Chemistry & Biodiversity* 6: 2192 – 2199.

Paaver, U., Orav, A., Arak, E., Mäeorg, U. and Raal, A. 2008. Phytochemical analysis of the essential oil of *Thymus serpyllum* L. growing wild in Estonia. *Natural Product Research* 22(2): 108 – 115.

Pande, C. and Mathela, C.S. 2000. Chemical composition of the leaf oil of *Juniperus communis* L. from the Kumaon region. *Journal of Essential Oil Bearing Plants* 3: 135 – 137.

Pourmortazavi, S.M., Baghaee, P. and Mirhosseini, M.A. 2004. Extraction of volatile compounds from *Juniperus communis* L. leaves with supercritical fluid carbon dioxide: Comparison with hydrodistillation. *Flavour and Fragrance Journal* 19: 417 – 420.

Raal, A., Arak, E., Orav, A., Kailas, T. and Müürisepp, M. 2008a. Composition of the essential oil of Levisticum officinale W.D.J. Koch from some European countries. *Journal of Essential Oil Research* 20(4): 318 – 322.

Raal, A., Arak, E., Orav, A., Kailas, T. and Müürisepp, M. 2008b. Variations in the composition of the essential oil of commercial Valeriana officinalis L. roots from different countries. *Journal of Essential Oil Research* 20(6): 524 – 529.

Raal, A., Orav, A. and Arak, E. 2007. Composition of the essential oil of Salvia officinalis L. from various European countries. *Natural Product Research* 21(5): 406 – 411.

Shahmir, F., Ahmadi, L., Mirza, M. and Korori, S.A.A. 2003. Secretory elements of needles and berries of *Juniperus communis* L. ssp. communis and its volatile constituents. *Flavour and Fragrance Journal* 18: 425 – 428.

Singh, G., Singh, O.P., Maurya, S., Marimuthu, P., de Lampasona, M.P. and Catalan, C. 2005. Composition of needle oil of *Juniperus communis* L. from north India. *Indian Perfumer* 49(2): 163 – 167.

Zenkevich, I.G. 1996; 1997; 1999. Analytical parameters of components of essential oils for their GC and GC-MS identification. Mono- and sesquiterpenes. Oxygen containing derivatives of mono- and sesquiterpenes hydrocarbons. Acetates of terpenic alcohols. Rastitel'nye Resursy 32: 48 – 58; 33: 16 – 28; 35: 30 – 37.

Viires, A. 2000. Puud ja inimesed [The trees and people]. Ilmamaa, Tartu, p. 98 – 103, (in Estonian).

Yankov, L.K., Tsutsulova, A.M., Stoyanova-Ivanova, B. and Nikolov, K. 1969. Composition of essential oils from concretes of the needles of some coniferous trees, grown in Bulgaria. Rivista Italiana Essenze, Profumi, Piante Officinali, Aromi, Saponi, Cosmetici, Aerosol. 51(11), 571 – 578.

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СОДЕРЖАНИЕ И СОСТАВ ЭФИРНОГО МАСЛА ВЕТВЕЙ МОЖЖЕВЕЛЬНИКА ОБЫКНОВЕННОГО (*JUNIPERUS COMMUNIS* L.) В ЭСТОНИИ

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Резюме

Были проанализированы содержание и состав эфирного масла кустов можжевельника обыкновенного (*Juniperus communis* L.). Были сравнены состав и количество эфирного масла, изолированные из ветвей можжевельника ежемесячно в 2006-2007 (12 образцов). Амплитуда количества добытого масла составила от 0.05 до 0.7%. В общей сложности было идентифицировано 77 соединений, что отвечает более 96%-ам масел. Эфирное масло, полученное из эстонского можжевельного куста, показало высокое содержание α–пинена (40.4-62.0%), преобладающий второстепенный компонент был лимонен (4.2-10.0%), α-кадинол (1.9 – 6.3%), β-кадинен (2.1-4.8%), γ-муролен и гермакрен Д (1.6-4.4%), мирцен (2.6-3.1%), α- и β-селинен (0.9-3.1%), гермакрен-Д-4-ол (0.8-3.0%) и β-пинен (1.4-2.2%). Содержание эфирного масла и количество моно- и сесквитерпенов зависит от месяца сбора растительного материала. Лучшее время для сбора урожая можжевельного куста с января по апрель. Содержание и состав эфирных масел не полностью соответствует стандартам Европейской Фармакопеи учреждённым для можжевельных ягод..

Key words: *Juniperus communis*; можжевельник; ветки; эфирное масло; α-пинен; время для сбора; Европейская Фармакопея