Variation of Secondary Metabolites (Essential Oils) in Various Plant Organs of Juniperus communis L. Wild Growing in Lithuania

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Abstract

Variation of some secondary metabolites of Juniperus communis among populations as well as at individual plant level was studied. Chemical composition and yield of the essential oils from branches, leaves (needles), sprouts, debarked wood and bark of common juniper collected in Eastern Lithuania was detailed. The oils of ten samples were obtained by hydrodistillation and analyzed by GC and GC-MS. A remarkable variation could be observed in the oil composition and yield depending on plant organs. The yield of the oils varied from 0.06% (v/w, on a dry weight basis) in wood to 0.46% – in the needles: α-Pinene was found to be the first major compound in the first-year sprout (43.5-57.1%), needle (31.0-44.7%) and shoot (25.4-46.3%) oils. Essential oil composition of bark and debarked wood differed drastically from oils of the other organs. Bark oil was characterized by predominance of longifolene (25.3-28.8%) and longiborneol (10.8-11.6%), while wood oil contained appreciable quantities of 1-epi-cubenol (up to 10.5%) and δ-cadinene (δ=13.4%).

Data of J. communis (of Lithuanian origin) essential oils obtained from debarked wood and bark was presented for the first time. Altogether there were identified 160 components (one of them tentatively, 25 ones in quantity more than 3%), which made up 74.3-98.0% of total oil content. The most of the investigated material was characterized by monoterpen hydrocarbon fraction (31.3-72.8%) in the essential oils, with exception for wood and bark, where the main fraction was oxygenated sesquiterpenes. Compounds with pinane and cadinane skeletons predominated in the J. communis essential oils. Additionally, a detailed survey of the previous published data on essential oils from J. communis of Lithuanian origin was presented in the paper.

Key words: Juniperus communis var. communis, secondary metabolites, essential oils, α-pinene, longifolene, longiborneol, 1-epi-cubenol, δ-cadinene.

Introduction

Juniperus (consisting of approximately 70 species and 40 varieties) is the second most diverse genus of the conifers. The genus is divided into three sections, and one of them is Juniperus (syn: sect. Oxycedrus Spach), containing 12 species. Juniperus communis (Cupressaceae Rich. Ex Bartl.), a highly variable taxon that is distributed around the Northern hemisphere (including and Baltic Sea region) has the largest distribution of any juniper species (Adams 2004). The clustering data of RAPD molecular markers showed that communis complex formed three groups: (I) J. communis var. communis and J. communis var. saxatilis, (II) J. communis var. sibirica (syn. J. sibirica) and (III) J. communis var. oblonga (syn. J. oblonga) (Adams 2004).

J. communis (known as common juniper) is a plant bearing volatile secondary metabolites, i.e. essential oils. Many constituents present in juniper essential oils are bioactive. The oils are used widely for medicinal purposes, in aromatherapy, in cosmetics, chemistry and food industry as well. Juniper oils (mainly from berries) possess antiseptic, diuretic, antihemathic, deputative, antispasmodic, stimulating, stomachic, astringent, carminative, vulnerary, antiinflammatory, tonic, antibacterial and antifungicial properties (Angioni et al. 2003, Barnes et al. 2002, Cabral et al. 2012, Filipowicz et al. 2003, Martz et al. 2009, Ragažiškinienė et al. 2005).

Chemical composition of the oils has a huge range of variability (both quantitative and qualitative) depending on plant origin and individual plant level as well. Detailed variation in juniper oils between different wild and cultivated populations from Lithuania and various plant organs was presented in Table 1.

1989, 1990, 2003, 2006, 2008, Mastelic et al. 2000, Damjanovic et al. 2003, Filipowicz et al. 2006). Juniper berry oils of sabinea chemotype have been found only in high mountains (Shamir et al. 2003, Lawrence 2003). Some juniper needle essential oils from Scandinavian countries, Poland, Iran, Portugal and Italy were of sabinea chemotype beside mostly identified a-pinene profile (Angioni et al. 2003, Cabral et al. 2012, Filipowicz et al. 2006, Lawrence 2003, 2006, Shamir et al. 2003). Germacrene D-ol and germacrene D were the principal constituents in the oils of needles, collected at various latitudes and altitudes in Finland (Martz et al. 2009), while a wide variability for different monoterpoids and sesquiterpenoids was observed in the berries of junipers grown in this country (Kallio and Junger-Mannermaker, 1998).

The essential oils of dried juniper needles investigated in Estonia were characterized by predominance of α-pinene, sabine, β-caryophyllene, α-humulene and germacrene D, while fresh needle contained higher amounts of (E)-2-hexenal and the berry oil was rich in α-pinene (in α-pinene, β-mycene and germacrene D (Oray et al. 2010). Raal et al. (2009) have determined that branch oils of Estonian origin are rich in α-pinene (≥62.0%), limonene (≤10.0%) and α-cadinol (≥6.3%). Another monoterpone, limonene, was determined as a major constituent in needle and berry oils of juniper from Corsica (Gonny et al. 2006, Ottavoli et al. 2009). Monoterpincarboxylic hydrocarbons composition has been studied in needles, berries and wood oils of junipers from various locations in Poland (Ochoka et al. 1997). Major fraction in the J. communis wood oils from Sweden and South-Western France was of sesquiterpenoids with predominant constituent thujaosene (Adams 2004, Lawrence 2003), while the wood oils from Corsica and Sardinia islands were comprised mainly by monoterpens or (oxygenated derivatives), such as limonene, α-terpinyl acetate, β-phellandrene, α-terpinene, α-pinene and p-cymene (Gonny et al. 2006, Marongiu et al. 2006).

The aim of the present study was to evaluate variation in volatile secondary metabolites (essential oils) among populations and in various plant organs (shoots, needles, sprouts, debarked wood and bark) of Juniperus communis var. communis growing in Lithuania.

Table 1. Variability in essential oil composition of Juniperus communis L. growing wild in Lithuania (data published during the last decade)

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Time of hydrodistillation (%)</th>
<th>Plants origin</th>
<th>Major components (or over 5.0%) essential oils (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh ripe (black) berries</td>
<td>In ripe berries: 1.2; in ripe berries: 0.9</td>
<td>Vīna district, five localities</td>
<td>α-Pinene (35,7-64,9), myrcene (4,5-16,4), terpin(4-1-7,1), terpin(4-10)</td>
<td>Butkienė et al. 2004</td>
</tr>
<tr>
<td>Fresh needles</td>
<td>3 h; 0.37</td>
<td>Vīna district, six localities</td>
<td>α-Pinene (35-38,9-59,5), terpin(4-1-1,14), terpen(4-1-3,3)</td>
<td>Butkienė et al. 2005a</td>
</tr>
<tr>
<td>Fresh ripe berries (black)</td>
<td>3 h; 0.0</td>
<td>North-Eastern Lithuania, five habitats</td>
<td>α-Pinene (21-0,4-6,4), terpin(4-10)</td>
<td>Butkienė et al. 2005b</td>
</tr>
<tr>
<td>Fresh bags with unripe (green) and ripe (black) berries, fresh leaves</td>
<td>3 h; unripe berries: 0,12; ripe berries: 0.03, needles: 0,37</td>
<td>Druskinkelai district</td>
<td>α-Pinene (27-7-64,9), myrcene (4,5-16,4), terpen(4-1-6,1), α-cadinol (≤2)</td>
<td>Butkienė et al. 2007</td>
</tr>
<tr>
<td>Unripe and ripe berries of light-green and blue-green berries</td>
<td>2 h</td>
<td>Druskinkelai district</td>
<td>Plants cultivated in the field collection of the Institute of Botany (Vilnius), Viesbūnų, Sakalnių, Minkūnų, Trakai, Anykščiai</td>
<td>Butkienė et al. 2009</td>
</tr>
<tr>
<td>Leaves and unripe cones</td>
<td>2 h</td>
<td>Eleven natural habitats (samples 110)</td>
<td>α-Pinene (17-7-64,9), terpin(4-1-6,9-9), terpin(4-1-9,2)</td>
<td>Luziene et al. 2010</td>
</tr>
<tr>
<td>Leaves and ripe cones</td>
<td>2 h, unripe cones: 0.9-0.14, ripe cones: 0.44-0.51</td>
<td>Eleven natural habitats (samples 110)</td>
<td>α-Pinene (17-7-64,9), terpin(4-1-6,9-9), α-pinene (27-5-64,7-4), myrcene (4,3-12,1), ε-pinene (4-1-13,5)</td>
<td>Luziene and Labukas, 2011</td>
</tr>
<tr>
<td>Leafy twigs with unripe cones</td>
<td>2 h, unripe cones: 0.1-0.5 in leaves</td>
<td>Eleven natural habitats (samples 110)</td>
<td>α-Pinene (17-7-64,9), terpin(4-1-6,9-9), α-pinene (27-5-64,7-4), myrcene (4,3-12,1), ε-pinene (4-1-13,5)</td>
<td>Luziene and Labukas, 2011</td>
</tr>
<tr>
<td>First-year cones, current-year shoots</td>
<td>2 h, leaves: 0.1-0.3, cones: 0.3-4.2</td>
<td>Eleven natural habitats (samples 110)</td>
<td>α-Pinene (17-7-64,9), terpin(4-1-6,9-9), α-pinene (27-5-64,7-4), myrcene (4,3-12,1), ε-pinene (4-1-13,5)</td>
<td>Luziene and Labukas, 2011</td>
</tr>
</tbody>
</table>

*Essential oil yield was expressed in v/w dry weight basis

Material and Methods

Plant material. The aerial parts (10-50 g): sprouts (one-year old), leaves (needles), branches, debarked wood and bark of Juniperus communis var. communis of two individual plants from two wild populations in Eastern Lithuania (Svėdasai, Anykščiai district (in mixed forest) and Vievis, Elektrėnai (in marshy forest)) were gathered at full flowering stage, in June (2008). Plant material of separated organs was dried at room temperature (20-25°C). Voucher specimens with numbers 68931 (Vievis) and 68343 (Svėdasai) were deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania. The essential oils from separated aerial parts were prepared by hydrodistillation (2 h) in a Cleveenger-type apparatus according to the European Pharmacopoeia and a mixture of hexane and diethyl ether (1:1) was used as a collecting solvent. Light yellow or colorless pure oils were obtained from 50 g of dry material. Yield ranged from 0.06 to 0.46% (v/w) on a dry weight basis.

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Analyses of the essential oils were carried out by GC and GC/MS. The quantitative analysis was performed on a capillary column HP-FFAP (30m × 0.25mm × 0.25mm) using chromatograph HP 5890II equipped with FID. The GC oven temperature was programmed as follows: from 60°C (isothermal for 3 min) increased to 160°C at a rate of 5°C/min (isothermal for 1 min) and to 250°C at a rate of 10°C/min and the final temperature was kept for 3 min. The temperatures of the injector and the detector were 250°C. The flow rate of carrier gas (helium) was 1 ml/min. At least 2 repetitions (n=2) per analysis were performed.

Analyses by GC/MS were carried out by HP 5890 gas chromatograph equipped with HP 5971 mass selective detector and HP 7673 split/splitless injector on a capillary column DB-5 (50m × 0.32mm × 0.25 mm). The chromatographic conditions were the same as in GC (FID) analysis. Mass spectra in electron mode were generated at 70 eV, 0.97 scans/second, mass range 35-650 m/z.

The percentage composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on comparison of retention indexes on both columns, co-injection of some terpenes references and C15-C30 n-alkane series; and mass spectra with corresponding data in the literature (Adams, 2007) and computer mass spectra libraries (Wiley and NBS 54K).

**Results**

The yield of *J. communis* essential oils varied at individual plant level (Table 2). The lowest one was determined in wood (0.06%) and the highest was found in the needles (0.46%). It should be mentioned, that yield of the volatile oils is a very important factor during industrial production of juniper essential oils.

**Table 2.** Essential oil yields in different plant organs of *J. communis*, % (v/v, expressed on a dry weight)

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>Essential oil yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-year old sprouts</td>
<td>~0.2</td>
</tr>
<tr>
<td>Leaves (needles)</td>
<td>0.34-0.46</td>
</tr>
<tr>
<td>Branches (without needles)</td>
<td>~0.16</td>
</tr>
<tr>
<td>Bark from stem</td>
<td>~0.975</td>
</tr>
<tr>
<td>Debarked wood (of ~5 cm diameter, without bark)</td>
<td>~0.06</td>
</tr>
</tbody>
</table>

Main chemical composition (over 3.0%, at least in one sample) of *J. communis* essential oils of various plants organs (sprouts (one-year old), leaves (needles), shoots (branches), debarked wood and bark) was presented in Table 3. One hundred and sixty compounds were identified (one of them tentatively, 25 in quantity more than 3%) and they made up 74.3-98.0% of total oil content.

Compounds with pinane and cadinane skeletons predominated in the *J. communis* essential oils. Most of the investigated material was characterized by monoterpene hydrocarbons fraction (31.3-72.8%) in the essential oils, with exception for wood and bark, where the main fraction was sesquiterpenes or oxygenated sesquiterpenes (39.3-45.7%).

Content of a monoterpene, α-pinene, varied drastically according to the plant organs. It was found to be as a principal constituent in shoot, needles and sprout essential oils (25.4-46.3%, 31.0-44.7% and 43.5-57.1%, respectively), while quantity of this compound was only up to several percents in the wood oil. Quantities of another two monoterpene, δ-3-carene and limonene differentiated not only in various plant organs, but also according to the collecting sites. The highest quantity of δ-3-carene was found in bark oils from Svėdasai (15.1%) and in branches from Vievis (9.1%), while the smallest amount of this compound was in wood and sprout oils from both juniper populations. α-Pinene, δ-3-carene and limonene are common and characteristic constituents for juniper oils of different geographical origins (see Introduction).

The major fraction was found to be sesquiterpenoids in wood and bark oils in the study. Oils of the bark was characterized by appreciable amounts of two sesquiterpenes, longifolene (25.3-28.8%) and longiborneol (10.8-11.6%), while these compounds even have not been detected in the other parts of the plants. To the best of our knowledge, there are no already published data on chemical composition of essential oils from bark of *J. communis*.

The juniper oils from debarked wood contained appreciable quantity of 1-epi-cubenol (up to 10.5%) and δ-cadinene (~13.4%). It should be pointed out that debarked wood composition was found to be different from previously investigated juniper wood (together with bark) oils in our laboratory (Butkiene et al. 2007) and absolutely dissimilar from wood oils investigated in other countries (Adams 2004, Lawrence 2003, Gonny et al. 2006, Marongiu et al. 2006). Wood oils from juniper growing in Druskinkiai district were characterized by major compounds nootkatone (18.5%) and α-pinene (≤31.0%), while contents nootkatone and α-pinene were significantly smaller (~4.9 and 0.5%, respectively) in the debarked wood oils under this study.

**Conclusions**

A remarkable variability (both in chemical composition and yield, also depending on plant organs and collecting sites) of volatile secondary metabolites of juniper (*Juniperus communis* L.) of Lithuanian origin was evaluated. The lowest essential oil yield was de-
### Table 3. Essential oil composition (≥0.0 %, at least in one oil sample) of *Juniperus communis* growing in Easter Lithuania

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>Anykčiūlai d., Svėdasai, %</th>
<th>Elektrėnai d., Vievis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>sprout needle shoot bark</td>
<td>debarked wood</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>939</td>
<td>43.5 31.0 46.3 10.1 0.6</td>
<td>57.1 44.7 25.4 4.2 3.1</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>979</td>
<td>4.0 2.0 11.1 0.4 tr.</td>
<td>3.7 2.3 1.1 0.4 0.2</td>
</tr>
<tr>
<td>Myrcene</td>
<td>990</td>
<td>4.1 2.3 0.3 -</td>
<td>3.7 3.4 0.9 0.2 -</td>
</tr>
<tr>
<td>δ-3-Carene</td>
<td>1011</td>
<td>1.8 6.9 3.5 15.1 0.2</td>
<td>1.0 2.0 9.1 4.8 1.1</td>
</tr>
<tr>
<td>Limonene</td>
<td>1029</td>
<td>1.4 1.9 5.5 1.8 0.2</td>
<td>5.5 9.8 1.0 1.0 0.4</td>
</tr>
<tr>
<td>Longifolene (Juniperene)</td>
<td>1407</td>
<td>- - 25.3 -</td>
<td>- - - - 28.8 0.1</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1419</td>
<td>2.2 tr. 0.6 0.8 3.9</td>
<td>0.4 0.6 5.7 0.5 1.5</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1454</td>
<td>0.5 3.2 0.9 0.9 4.1</td>
<td>0.7 0.6 3.9 0.8 1.9</td>
</tr>
<tr>
<td>cis-Cadin-1(6),4-diene</td>
<td>1463</td>
<td>0.2 0.2 tr. 0.1 3.0</td>
<td>- 0.3 - - 0.2</td>
</tr>
<tr>
<td>γ-Muurolene</td>
<td>1479</td>
<td>1.5 0.5 2.3 -</td>
<td>1.9 0.2 3.0 -</td>
</tr>
<tr>
<td>epi-Cubeol</td>
<td>1494</td>
<td>1.6 0.4 0.2 -</td>
<td>6.4 0.2 0.7 tr. - 3.0</td>
</tr>
<tr>
<td>Cubeol</td>
<td>1515</td>
<td>- - -</td>
<td>- - - - 3.9</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>1523</td>
<td>4.4 5.6 1.9 2.2 13.4</td>
<td>2.8 1.8 5.4 1.6 7.2</td>
</tr>
<tr>
<td>Germacrene D-4-ol</td>
<td>1575</td>
<td>2.0 4.1 0.6 -</td>
<td>1.4 0.8 2.1 -</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>1578</td>
<td>- 0.9 3.3 1.0 -</td>
<td>- 1.8 1.9 0.7 - 7.0</td>
</tr>
<tr>
<td>Unknown (M ~ 284)</td>
<td>1589</td>
<td>- - -</td>
<td>- - - -</td>
</tr>
<tr>
<td>Longiborneol</td>
<td>1598</td>
<td>- - 11.6 -</td>
<td>- - - - 10.8</td>
</tr>
<tr>
<td>1-epi-Cubenol</td>
<td>1628</td>
<td>0.5 0.5 0.5 -</td>
<td>10.0 0.2 0.1 4.4 tr. 10.5</td>
</tr>
<tr>
<td>epi-α-Cadinol</td>
<td>1640</td>
<td>1.6 1.9 1.3 1.4 3.0</td>
<td>0.5 0.6 - - 3.0</td>
</tr>
<tr>
<td>epi-α-Muurolol</td>
<td>1642</td>
<td>3.7 2.3 - - 1.4 -</td>
<td>1.7 0.9 - -</td>
</tr>
<tr>
<td>Cubenol</td>
<td>1647</td>
<td>- - 7.0 -</td>
<td>- - - -</td>
</tr>
<tr>
<td>δ-Cadinol</td>
<td>1654</td>
<td>6.0 6.8 3.3 -</td>
<td>2.5</td>
</tr>
<tr>
<td>Longiborneol acetate</td>
<td>1655</td>
<td>- - -</td>
<td>- - 1.1 -</td>
</tr>
<tr>
<td>14-hydroxy-α-Muurolone</td>
<td>1780</td>
<td>- - -</td>
<td>- - 3.1 -</td>
</tr>
<tr>
<td>Nootkatolone</td>
<td>1805</td>
<td>- - -</td>
<td>- - 4.9 -</td>
</tr>
</tbody>
</table>

### MS (RI 1589) of unknown: M ~ 284 (84), 213 (45), 202 (100), 199 (47), 91 (5), 81 (5), tr. in amounts ≥0.05 %

*Including corresponding compounds with quantity less than 3.0 %:

- hexanal, (2E)-hexenal, n-hexanal, tricycene, α-thujene, (2E)-heptanal, thuj-2,4(10)-diene, α-fenchene, camphene, sabine, verbene, hexanoic acid, 1-octen-3-ol, 2-pentyl furan, n-octanal, 1,3,5-trimethyl benzene, α-, β-phellandrene, (2E,4E)-hepta-
dienal, α-terpinene, β-, p-cymene, 1,8-cineole, (2E)-octen-1-ol, pentyl-isobutanate, γ-terpinene, p-meththa-2,4(8)-diene, α-terpinolene, linalool, n-nonanal, isopentyl-2-methyl butanoate, isopentyl-isovalerate, 3-methyl-3-butenyl-3-methyl butanoate, α-
campholenal, trans-pinocarveol, 3-methyl-2-butenyl-2-methyl butanoate, cis-, trans-verbenol, p-meththa-(1)(7),2-dien-8-ol, cam-
phor, pinocarvote, borneol, 1,3-dimethoxybenzene, p-meththa-1,5-dien-8-ol, trans-4-ol, α-terpinene, m-, p-cymen-8-ol, myrtille, verbenone, (2E,4E)-nonadienal, trans-carvone, 4-methyl isopropyl carvone, citronellol, methyl thymol, (E)-ocimene, isobornyl formate, methyl citronellate, (2E,4Z)-decadienal, bornol acetate, carvacrol, (Z)-methyl cinnamal, α-terpinene-4-ol acetate, α-terpinel acetate, (2E,4E)-decadienal, myrtenol acetate, δ-elemene, α-, β-longipinene, dihydro iso-jasmone, α-cube-
bene, α-ylangene, α-copaene, β-elemene, sativene, trans-myrtanol acetate, β-bourbonene, 2,5-dimethoxy p-cymene, β-copaene, β-
gurjunene, γ-elemene, aromadendrene, alo-alonadendrene, (2Z)-β-farnesene, 6,9-guaiadene, cis-, trans-muurola-3,5-diene, 9-
epi-(E)-caryophyllene, γ-curcumene, β-selinene, cis-, trans-muurola-4(14),5-diene, citronellol butanoate, trans-cadin-1(6),4-
diene, trans-cadin-1,4-diene, γ-gurjene, amophra-4(11)-diene, germacrene D, α-muurolene, δ-amorphene, γ-cadinene, 10-
epi-cubeol, α-cadinene, zonolene, elemol, selina-3,7(11)-diene, germacrene B, (E)-nerolidol, α-, β-calacorene, globulus, virid-
iflorol, caryophyllene oxide, salvia-4(14)-en-1-one, humulene epoxide II, gleenol, β-olopenene, 1,10-di-epi-cubeol, cis-dihy-
dro muurolene, cis-cadinen-10-ol, trans-calamen-10-ol, α-muurolol, cadalene, 14-hydroxy-4(Z)-caryophyllene, guaia-3,10(14)-
dien-11-ol, selin-11-en-4-α-ol, khusinol, germaca-4(15),5,10(14)-triene-1-α-ol, 14-oxy-α-muurolene, eudesma-4(15),7-dien-
1β-ol, cis-14-nor-muurol-5-en-4-one, eudesm-7(11)-en-4-ol, amophera-4,9-dien-14-ol and abietiatriene.

terminated in wood (0.06%) and the highest one was found in the needles (0.46%). The most predominant fraction was of monoterpenic hydrocarbons (31.3-72.8%), containing compounds characteristic for juniper oils), with exception for wood and bark essential oils, where the main fraction was sesquiterpenes or oxygenated sesquiterpenes (39.3-45.7%). Composition of essential oils of debarked wood drastically from the oils of juniper of other world origins. To the best of our knowledge, the composition of juniper bark essential oils was evaluated for the first time under this study.

To know chemical composition it is very important for industrial production of juniper essential oils. Also, utilization of all plant parts of juniper during production process, avoiding the waste, is of huge commercial significance.
References


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