Study of the Essential Oil from *Juniperus communis* “Berries” (Cones) Growing Wild in Greece

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**Abstract**

Capillary GC and GC-MS analysis of the essential oil from the cones of *Juniperus communis* L. grown wild in Greece showed a high content of α-pinene (ca. 27%), sabinene (13%), germacrene-D (10%), and myrcene (9%). The total oil (yield 2.95%) consisted of almost 105 constituents, 77 of which representing 93.1% were identified. As the main component of the oxygenated monoterpenes was found the terpinen-4-ol (1.37%). Seasonal investigation of the essential oils showed mainly quantitative variations among the constituents.

**Keywords**

*Juniperus communis* L., Cupressaceae, berries, cones, Greek mountain wild juniper, essential oil, GC analysis, GC/MS, seasonal variations.

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**Introduction**

The genus *Juniperus* (Cupressaceae) which contains more than 60 species is quite widespread and grows wild all over the northern hemisphere. In the Greek flora six species are present. Among them, *Juniperus communis* L. grows on the mountains of Macedonia, Epirus and Thessalia. It was already known as "χάδος μυκοτά" by Dioscurides or "ἀνθέκυς αὐτος" by Theofrastos.

The oil of *J. communis* female cones (so-called berries) has been used for centuries as a diuretic, based on its terpinen-4-ol content (1). The plant is also used in folk medicine as a carminative, antiseptic, and as a remedy for indigestion. Some antimicrobial properties of the essential oil have also been reported (2, 3).

Juniper cones are widely used in compounding spices, and the oil is used in perfumes, pharmaceutical and cosmetic products. The oil and the extracts are also used for the flavouring of beverages and liqueurs.

Whereas analytical data on volatile components of *Juniperus* needle and cone oils (4–8) as well as of alcoholic extracts (9, 10) have been reported and are scattered in the literature, the Greek cone essential oil has never been studied. So, it seemed of interest to investigate the composition of the oil produced from the cones of this shrubby tree, growing wild, widespread in central and northern Greece.

**Materials and Methods**

**Plant material**

The plant material (female cones) was collected from wild growing *Juniperus communis* shrubby trees in three different periods of the year – February (sample A) when the whole plant was covered by snow, June (sample B), and October (sample C) – from the same marked trees, on the Olympus mountain at an altitude of 1300 m. Two kinds of sample were selected: the black mature cones and green unripe ones.

**Isolation and yield of the essential oil**

The cones were stored in a freezer at −20°C. The percentage oil yielded was determined using the European Pharmacopoeia apparatus (Clevenger-type) and after 3 replications giving an S rel of ± 0.1%. The cones were subjected to hydrodistillation after drying to a moisture content of 25% (11). The plant material was placed in liquid N₂ for 30 min and then comminuted in a closed type mill with a degree of comminution of 1 mm (12). Samples of 20 g of comminuted cones with 340 ml of deionized water were distilled for 3 h at distillation rates of 3–3.5 ml/min (13). The lighter than water, slightly yellow and limpid oil was dried over anhydrous Na₂SO₄ and stored in sealed containers under refrigeration (−20°C). The physicochemical constants of the sample were: ε₂⁰ = 0.860, [α]₂⁰⁻ = −14.8°, and ρ₂⁰ = 1.4791.

**Fractionation of the oil**

A prefractionation of the essential oil by means of silica gel "mini extraction columns" (LSC) was carried out to obtain three fractions to ease the identification of the oil components.

**Liquid-solid chromatography**

The following disposable columns were used for the LSC: 1 ml Bond Elute silica columns (Analytichem International Scientific BV, The Netherlands) and 3 ml Baker 10 SPE (Solid Phase Extraction) silica columns (J. T. Baker Chemicals BV, The Netherlands). Aliquots of 30 µl oil were introduced to the columns after being washed with a small amount of pentane (5 ml). Elution was carried out with 10 ml pentane (hydrocarbons), after-
wards with 8 ml pentane-diethyl ether 97:3 and subsequently with 10 ml diethyl ether (oxygen-containing compounds). The eluates obtained were concentrated under reduced pressure in a rotary evaporator at ca. 0 °C to 1 ml subsequently, 1 μl was used for gas chromatography.

**Gas-liquid chromatography**

The essential oil samples, as well as the fractions obtained by LSC, were analyzed by gas chromatography, using a gas chromatograph Hewlett-Packard 5890 Series II, equipped with one injection port and a two-channel system of columns and respective FIDs connected with a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Three different polarity fused silica columns were used: a) Durabond-DB 1, b) DB-Wax both of 60 m × 0.25 mm i.d., film thickness 0.25 μm (J & W Scientific Inc., Rancho Cordova, California, USA); and c) CP-Sil 19 CB, 25 m × 0.25 mm i.d., film thickness 0.20 μm (Chrompack Nederland, Middelburg, The Netherlands). Oven temperature: 45° – 220°C (3.5 °C/min); carrier gas: nitrogen, 140 Kpa; Injection temperature: 220°C; Detectors temperature: 300°C. The percentage compositions were computed from the GC peak areas without correction factors.

**Gas chromatography and mass spectrometry**

A GC-MS was also applied, using a 60 m × 0.245 mm i.d. fused silica CP-Wax 52 CB column, film thickness 0.25 μm (Chrompack Nederland BV) and a gas chromatograph Packard 438 A interfaced with a Finnigan MAT Ion Trap Detector (software version 3.0; Finnigan Mat, San Jose, California, USA). Oven temperature: 45° – 240°C (3 °C/min); carrier gas: helium (pressure 200 Kpa); splitting ratio 1:40; scan time, 1 s.

**Results and Discussion**

The yield of the essential oil obtained from *J. communis* cones was 2.95 % v/w, one of the highest reported in literature [0.8% for a Norwegian juniper (14), 0.8-1.6% by Guenther referred to the Italian and Hungarian ones (15)]. It was higher than the limits defined by the Analytical Methods Committee [0.5-2.5% (16)].

The total oil consisted of approximately 105 constituents, 77 were identified, representing 93.1 % of the oil. The percentages given are based on the data obtained with the DB-1 and DB-Wax columns after three chromatographic runs (S rel. ± 1.01 – ± 1.2 %).

As can be seen from Table 1 (sample A), 20 of the constituents were monoterpene hydrocarbons related.

<table>
<thead>
<tr>
<th>Table 1 continued.</th>
<th>Percentage composition</th>
<th>Method of Identification</th>
</tr>
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<tbody>
<tr>
<td>Components</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>tricyclicene</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>α-Thuene</td>
<td>1.49</td>
<td>0.30</td>
</tr>
<tr>
<td>α-pinene</td>
<td>27.14</td>
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</tr>
<tr>
<td>α-fenchene</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>β-Fenchene</td>
<td>0.01</td>
<td>0.03</td>
</tr>
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<td>Camphene</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
<td>2,4-pentadiene</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Sabinene</td>
<td>13.21</td>
<td>3.84</td>
</tr>
<tr>
<td>β-pinene</td>
<td>2.15</td>
<td>2.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Qualitative and quantitative analysis of the essential oil from cones of <em>Juniperus communis</em> growing wild in N. Greece.</th>
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<td>Components</td>
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</tbody>
</table>

| Reference: A = February collection, B = June collection, C = October collection. |
| Identification method: 1 = relative retention time, 2 = reference oils, 3 = mass spectra. |
| t = traces < 0.010 %. |
presenting 57.8% of the total essential oil. Major components are α-pinene (27.14%), sabinene (13.2%), myrcene (9.55%), β-pinene (2.14%), and limonene (1.91%). It should be noted that the corresponding amounts of the monoterpene hydrocarbon α-pinene are lower than samples of other origins (70.8%) in a German sample (17), and 34.5 and 35%, respectively, of the α-pinene for Norwegian (14) and Hungarian (16) oils.

Finally, the presence of p-cymene in a rather low percentage showed that no oxidation of α-, γ-terpinine and α-phellandrene had occurred [as has been mentioned for other Juniper essential oils (18, 19)]. Similarly, no autooxidation of terpinolene was observed, because p-dimethylstyrene was not detected (14).

In spite of the large number of oxygenated constituents (24 identified in the oil) the total amount was only 4.25%, with terpinen-4-ol as the main component (1.37%). Bornyl acetate (0.22%), α-terpinol (0.13%), citronellol (0.11%), bornol (0.08%), and 1,8-cineol (0.80%) were found in lesser amounts. Vernin et al. (20) identified a large number of oxygenated components in a sample of juniper cone oil from France, but no percentage data were given. Likewise, in other previous studies on the oil, terpinen-4-ol, which is responsible for the diuretic properties of the cone maturation, a large number of oxygenated components were identified in minor quantities, among the seasonal samples. Furthermore, during the ripening cycle of the cones, similar results, drastic reduction of essential oil yield have also been reported by von Rudloff in studies of certain Pinaceae (23).

In our sample germacrene-D was the largest component (10.28%). This amount of germacrene-D is in fairly good agreement with those previously reported by Melegari et al. (0.09–14.68%) (22), Kallio et al. (22), and Vernin et al. (20).

Apart from sample A (collection of February) the Juniperus cone samples of June (B) and October (C) were also investigated. The results of the analysis, performed in the same way as for the sample (A), are shown in Table 1.

The percentage yields of essential oil were 2.95% for A, 2.70% for B, and 1.35% v/v for C. This fluctuation is likely due to changes during the ripening cycle of the cones. Similar results, drastic reduction of essential oil yield have also been reported by von Rudloff in studies of certain Pinaceae (23).

As seen in Table 1, an increase of the monoterpene hydrocarbons occurred from spring (57.8%) to summer (61.6%) after which a decrease (54.6%) was observed. This finding was in accordance with those found by von Rudloff (23). In addition, the highest level of oxygenated monoterpenes was found in the autumn (4.9%), while the sesquiterpenes maximized at the end of winter (32.1%), decreasing afterwards (24).

Nevertheless, some qualitative variations were observed, for components found in minor percentages, among the seasonal samples. Furthermore, during cone maturation, large quantitative variations were noted for α-pinene, sabinene, β-caryophyllene, α-humulene and germacrene-D.

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References