Abstract

The present investigation reports the experimental data a) from the recovery and the composition of the extract under supercritical fluid extraction from Juniperus communis L. "berries" (cones), and b) their comparison with those of the essential oil obtained by hydrodistillation. For the extraction of the juniper oil different values of temperature and pressure were applied; furthermore, the degree of comminution of the plant material was also considered – a) integral "berries" and b) comminuted "berries". The quality of the oil recovered from the "berries" by supercritical carbon dioxide extraction was found to be highly dependent on the applied conditions. The comminution affected greatly the oil recovery and consequently the final composition of the extracts. Significant differences were recorded between the supercritical CO₂ extract and the distilled oil, the latter being more enriched in monoterpenoid hydrocarbons.

Key words
Juniperus communis · Cupressaceae · volatiles · supercritical CO₂ extraction · hydrodistillation · GC/MS analysis

Introduction

Juniperus oil is an important natural product known as a source for compounds to be used directly in fragrance compounding [1]. The oil is used to scent pharmaceutical and technical preparations, as well as in cosmetic products such as soaps, room sprays, disinfectants and other products.

In pharmacy the oil has been used over the centuries for its diuretic and antiseptic properties as rubefacient or in spirits. Furthermore, it is universally known for its use in the perfumery and especially in the alcoholic drinks.

In the last few years a number of papers dealt with the composition of the volatile oil from Juniperus communis "berries" (cones) [2], [3], [4], [5], [6]. All studies involved the analysis of the composition of the distilled oil. A closer examination of the different methods of obtaining the volatile compounds, as far as the various factors or conditions applied, revealed that they influence directly and some times drastically the yield of the obtained essential oil and consequently its quality [7].

The extraction of volatile constituents with dense CO₂ from aromatic/medicinal plants is possible in any case [8], as the solubility of the essential oils in supercritical carbon dioxide is very high, offering the particular advantage that the final product retains the organoleptic characteristics of the starting plant material. However, supercritical CO₂ has high selectivity to other lipophilic compounds located on the surface or the inside parts of the plant tissues such as fatty oils, waxes, etc. The dissolving capacity of supercritical carbon dioxide is associated with its density, increasing at high pressures and temperatures. To produce a super-
critical extract as close as possible to the essential oil, the CO₂ should be used at low solvent power and this takes place in the supercritical region, close to the critical point [9]. On the contrary, the total extract from a natural product is obtained at maximum solvent power of CO₂. The temperature and pressure applied affect the supercritical CO₂ density as far as the dissolving capacity and furthermore the composition of the mixture of volatiles present in the various fractions of the extracts [10], [11].

The present study presents the results concerning the extraction of the volatile compounds from Juniperus “berries” using supercritical fluids (SCF) - carbon dioxide. Moreover, we have attempted a comparison with the results from the classic hydrodistillation method. With the aim to ensure the possibly better quality of the essential oil obtained from Juniper “berries”, its most characteristic compounds were also investigated more closely. Three parameters were involved in the experimental design: the pressure and the temperature of the supercritical CO₂ extraction and the degree of comminution of the berries. The last one, the grinding of the plant material, as also resulted from our previous works [7], [12] remains one of the most important factors influencing drastically the obtained oil.

Materials and Methods

Plant material

The Juniperus communis “berries” (cones) were collected from the mountain Olympus above 1000 m during the summer (voucher specimen is kept in the Department of Aromatic and Medicinal Plants, National Agricultural Research Centre of Macedonia & Thrace, Thessaloniki, Greece). The plant material [5] before the extraction procedure was kept in liquid N₂ for 30 min and then was reduced in size by a cutting mill (closed type) at two different degrees of comminution determined from preliminary studies: a) particles between 2 and 1 mm (mean diameter 1400 µm) and b) less than 1 mm (mean diameter 800 µm). For every degree of comminution all the particles passed the bigger sieve and at least 40% the next smaller one; the mean diameter of the plant material particle was determined by mechanical sieving. In addition, some experiments were conducted with uncommuninated “berries”.

CO₂ extraction

After each reduction in size, the plant material was extracted immediately with supercritical carbon dioxide (of 99.5% purity), in an extraction pilot plant system produced by the SITEC Co, Switzerland. The CO₂ flow rates were adjusted between 0.3 and 0.9 kg/h, though different flow rates had no influence on the extracted oil yield and composition.

A 50 g sample of the plant material was packed as tightly as possible in a stainless steel cylinder (0.6 L), which was subsequently brought into the extraction vessel, that was also kept at the selected extraction temperature.

The values of the parameters applied were as follows: temperature: 40, 50°C; pressure: 90, 125 bars and degree of comminution (particles size): a) < 1 mm b) between 2 mm and 1 mm and c) intact cones. The extraction runs were executed under combination of the above conditions. The CO₂ extractions were carried out for 1 hour, and the density of CO₂ used was in the vicinity of 0.290 g/cm², deemed to be most favorable from preliminary studies.

The pressure and temperature in the separator influence also the quality of the obtained CO₂ extract; therefore relatively low values were applied, such as 55 - 56 bar and 23 - 24°C, resulted as the best from our previous studies [13]. In each experiment, the fraction was collected in liquid carbon dioxide in the separator and after the decompression it was dissolved in pentane and finally subjected to GC analysis.

Hydrodistillation

The distilled essential oil percentage yield determination of the “berries” resulted using the European Pharmacopoeia apparatus (Clevenger type) and after 3 performances in a relative standard error Srel of ± 0.05%. Samples of 20 g of comminuted “berries” with 340 ml of deionised water were distilled for 2 hours at distillation rate of 3 - 3.5 ml/min [14]. The lighter than water, slightly yellow and limpid oil was dried over anhydrous Na₂SO₄ and stored in sealed containers under refrigeration (–20°C).

Gas liquid chromatography

The GC analyses were carried out on a dual gas chromatograph, Hewlett Packard model 5890 Series II, equipped with one injection port and a two-channel system of columns and respective FIDs connected to a Dual Channel Integrator Hewlett Packard 3396 Series II. Two fused silica columns of different polarity were used: a) Durabond DB 1 and b) DB-Wax both of 60 m x 0.25 mm i.d, film thickness 0.25 µm (J & W Scientific Inc., Rancho Cordova, California, USA). Oven temperature: 45 – 220°C (3.5°C/min); carrier gas: nitrogen, 140 Kpa; injection temperature: 220°C; detector temperature: 300°C. Routinely the analyses were carried out by injecting three times 0.5 µl of the extract or the dissolved essential oil in n-pentane (1:20). The percentage composition was computed from the GC peak areas without correction factors. The relative standard deviations of the peaks averaged from ± 0.03% to ± 1.46%.

Gas chromatography and mass spectrometry

A GC-MS was also applied, using a 60 m x 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 (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 results from the supercritical CO₂ extraction showed that the method is efficient for the obtaining the volatile fraction from the Juniper “berries” (cones). The yields obtained under different representative combinations of pressure and temperature, and considering the degree of comminution are recorded in Table 1.

From the results it is assumed that all the parameters involved in the supercritical CO₂ extraction of the Juniper “berries”, the temperature, the pressure and the comminution influenced the ob-
Table 1 The percentage yield of the CO2 extracted oil under different conditions of temperature, pressure and comminution

<table>
<thead>
<tr>
<th>Conditions</th>
<th>% Yields [mean (SDE)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncommminated berries</td>
</tr>
<tr>
<td>Pressure 90 b</td>
<td>0.89 (0.035)</td>
</tr>
<tr>
<td>Temperature 40 °C</td>
<td>4.90 (0.20)</td>
</tr>
<tr>
<td>Pressure 125 b</td>
<td>6.31 (0.17)</td>
</tr>
<tr>
<td>Temperature 50 °C</td>
<td>10.60 (0.25)</td>
</tr>
</tbody>
</table>

*% Yield is calculated by weight of the material charged in the extractor.

Table 2 The main constituents of the supercritical CO2 extracts (obtained under different conditions) and the distilled oil from Juniperus communis cones

<table>
<thead>
<tr>
<th>CO2 extraction</th>
<th>Hydrodistillation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (bar)</td>
<td>90</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>40</td>
</tr>
<tr>
<td>Degree of comminution</td>
<td>2 mm</td>
</tr>
<tr>
<td>Constituents</td>
<td>Composition (% of peak area)</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>35.28</td>
</tr>
<tr>
<td>Sabine</td>
<td>3.54</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>2.33</td>
</tr>
<tr>
<td>Myrcene</td>
<td>9.88</td>
</tr>
<tr>
<td>Limonene</td>
<td>3.00</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>0.26</td>
</tr>
<tr>
<td>α-Terpinol</td>
<td>0.17</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>2.20</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1.72</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>1.12</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>3.12</td>
</tr>
<tr>
<td>T-Cadinol</td>
<td>0.50</td>
</tr>
<tr>
<td>α-Cadinol</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The CO2 extract of the uncomminuted “berries” was also analysed. No qualitative differences were observed among the main volatile constituents compared to the comminuted ones (Table 2). The percentages of the monoterpenes and sesquiterpenes were lower than those from the comminuted cones and no further investigation was conducted on the influence of the pressure and temperature on the composition of the oil because of the very poor extract yield (Table 1). At this point it must be also noticed that there was evidence that the total content of undesirable components such as substances of high molecular weight, fats or waxes located at the outer surface of the “berries”, was co-extracted in a much greater extent from the integral “berries”; this remains a matter for future investigation.
ANOVA, was verified as statistically significant \( (p \leq 0.01) \). Furthermore, the execution of the Duncan's Multiple Range test for the means comparisons, showed that the highest yields of the monoterpene hydrocarbons were obtained at the lowest applied temperature, 40 °C, and particularly from the bigger particle sizes; 56.21% and 54.20% at pressure 125 and 90 bars respectively (Fig. 1). The combinations 125 bars or 90 bars and 40 °C, proved to be the most efficient for both particle sizes. Moreover, the main constituent \( \alpha \)-pinene attained its highest yields 36.43% and 32.17% (from the bigger and smaller particle size batches, respectively) at 125 bar and 40 °C.

As regards the monoterpene sesquiterpenoid group, it was retrieved mostly by the application of the highest temperature (50 °C), and from the smaller particle sizes (Fig. 2). The maximum yields of \( \beta \)-caryophyllene (3.50%) and germacrene-D (16.03%) were obtained under the conditions of 125 bar and 50 °C.

**Comparison of hydrodistillation to \( \text{CO}_2 \) extraction of Juniper berries volatiles**

The supercritical \( \text{CO}_2 \)-extracted volatile part from Juniper “berries” (cones) was furthermore compared with the essential oil obtained by hydrodistillation. The composition of the distilled oil has been investigated and presented previously [5]. More than 100 constituents were detected from which 77 were identified. In Table 2 are presented the main components and their percentages in the essential oil obtained by hydrodistillation.

The main constituents were identical in the oils obtained by hydrodistillation and \( \text{CO}_2 \) extraction although \( \alpha \)-pinene was the predominant constituent in both of them, significant differences were noticed concerning their quantitative compositions. The amounts of numerous monoterpene hydrocarbons were higher in the distilled oil, in accordance also to Moyler et al. [17]. The synthesis of the supercritical-\( \text{CO}_2 \) oil, obtained under pressure 125 bars, temperature 40 °C, and degree of comminution between 2 mm and 1 mm resembled more those from hydrodistillation. The percentage of \( \alpha \)-pinene in the distilled oil was 40.29%, while in the \( \text{CO}_2 \) extracts it varied among 26.95 and 36.43%.

Terpinen-4-ol, the most characteristic main oxygenated compound of Juniper “berries” oil, was registered in minor quantities in both the distilled and in the \( \text{CO}_2 \) extracted oil (0.30% and 0.46%, respectively).

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**Fig. 1** The variation of the monoterpene hydrocarbons in the \( \text{CO}_2 \) extracts obtained under different combinations of pressure, temperature and degree of comminution of the berries.

**Fig. 2** The variation of the monoterpene sesquiterpenes in \( \text{CO}_2 \) extracts obtained under different combinations of pressure, temperature and degree of comminution of the berries.
The group of the sesquiterpene hydrocarbons (\(\beta\)-caryophyllene 3.50\%, humulene 2.92\%, germacrene D 16.03\% and \(\delta\)-cadinene 2.26\%) resulted in higher levels through the CO\(_2\) extractions. Meanwhile the amounts of those obtained by hydrodistillation were 2.59, 2.13, 10.36 and 1.92\%, respectively.

Kerrola et al. [18] recorded in a study on coriander fruits, just slighter differences between the supercritical CO\(_2\) extract and the hydrodistilled amount of the monoterpenoid group. Although Poiana et al. [19] reported great differences between the distilled oil and the CO\(_2\) extract of Citrus medica, the hydrodistillation yielding much higher monoterpenoid hydrocarbons, while the monoterpenoid sesquiterpenes were obtained at higher yields from the supercritical CO\(_2\) extraction. The authors assumed that by means of hydrodistillation the more volatile constituents were largely isolated from a “matrix”, while on the other hand the CO\(_2\) dissolves in a greater extent components with higher molecular weights and boiling points, favoring mostly the constituents of the sesquiterpenoid group.

Moreover, in the present study, a significant difference was observed between the hydrodistillation and the CO\(_2\) extract of the cones, in terms of the percentage yield of caryophyllene oxide.

In the distilled oil, it was determined only in minor quantities (0.44\%), whereas under the specific conditions of the CO\(_2\) extraction it was achieved up to 5.20\%. This is probably due to the hydrolytic conditions arising during the hydrodistillation, which often cause alterations in many substances. Similar findings are also recorded by Reverchon et al. [16] with respect to the amount of linalool and linalyl acetate in the CO\(_2\) extract and the hydrodistilled oil from lavender.

In conclusion, the results above revealed that by modifying properly the operating conditions in a supercritical CO\(_2\) extraction, it is feasible to intervene drastically in the composition of the Juniper oil. Finally, it is assumed that the composition of the CO\(_2\) extracted volatile part resembled more the inherent flavour pattern of the botanical source than the hydrodistillation’s procedure product; the high extraction power of the supercritical CO\(_2\) is shown by its enriched concentration of high boiling components.

Acknowledgements

The authors are grateful to Mrs A. Looman for valuable technical assistance in the GC–MS analysis of the sample.

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