

Comparative Study of the Volatile Constituents from *Juniperus communis* Berries Obtained under Different Methods of Extraction

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Abstract

The volatile constituents obtained from *Juniperus communis* berries by the application of different methods, Hydro distillation (HD), Super Critical Fluid Extraction (SFE) and Headspace (HSE) static technique were examined as regards their qualitative and quantitative composition. Significant differences were noticed among the percentages of the main components of the extracts: the monoterpene hydrocarbons, the oxygenated ones and the sesquiterpenoids. Monoterpene hydrocarbons predominated using all three methods. The highest yield - as far it concerns the main constituents (89.9%) was obtained using the headspace technique, following by 60.6% in the distilled oil and 56.2% in the supercritical CO₂ extract. α -Pinene was the most abundant monoterpene hydrocarbon, ranging from 51.3% to 36.4%, in the headspace and SFE-aroma products, respectively. The percentages of the sesquiterpene constituents varied also among the extracts. The SFE extraction was more effective giving 22.7% versus 21.9% in the hydro distilled oil, while the equilibrium headspace technique gave only 4.2%.

INTRODUCTION

Juniperus oil is an important natural product, used widely in many pharmaceutical and technical preparations, cosmetic products and as a flavouring agent in beverages (Wichtl, 1984, Lamparsky and Klimes, 1985). Hydro distillation and solvent extraction are the oldest methods used for the isolation of essential oils from aromatic and medicinal plants and are the procedures most used today. Nevertheless, other more sophisticated techniques for the effective isolation of aromatic components, or the determination of the aromatic profile, such as simultaneous distillation-extraction, the headspace technique and supercritical fluid extraction have been introduced (Likens and Nickerson, 1964; Chialva et al., 1983; Moyler and Heath, 1986). A closer examination of these methods and the parameters involved, verify that they influence directly and some times drastically the yield of the obtained scent and consequently its quality, and probably none of them reveal the true profile of the "aroma" of a plant material (Lamparsky, 1985; Chatzopoulou and Katsiotis, 1995; Arino et al., 1999).

Because of the great industrial interest of juniper berries oil, our aim was to study its volatile constituents applying different techniques such as the classical hydro distillation, supercritical fluid extraction and the static headspace. Thus any improvement in technology and the method used for obtaining the volatiles could be evaluated.

MATERIALS AND METHODS

Plant Material

The berries were collected from wild growing Juniper shrubs on the Olympus mountain at an altitude of 1300 m. The plant material (just berries) prior to the execution of the extraction method, was placed in liquid N₂ for 30 min, and then was comminuted in

a closed type mill with a degree of comminution of 1mm.

Experimental Procedures

1 Hydro Distillation (HD) Hydro distillation was performed using the European Pharmacopoeia suggested apparatus (Clevenger type) (3 times). 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 (Katsiotis, 1988). The lighter than water, slightly yellow and limpid oil was dried over anhydrous Na₂SO₄ and stored in sealed containers under refrigeration (-20°C).

2. Headspace Extraction (HSE) The plant material (comminuted juniper berries) was placed into the headspace vials with an ethanolic solution of isoborneol - as external standard- for the quantitative analysis. The static headspace technique was performed using a Headspace Sampler Perkin Elmer HS 40, connected with a GC Hewlett Packard 5890. The samples were equilibrated in the system at 120° C for 40 min. The conditions in the headspace system were: carrier gas: N₂ 1.5 bar, auxiliary gas: air 3.3 bar, time of pressing: 10 sec, time of injection: 5 sec, temperature into the transfer line: 150°C.

3. Supercritical - CO₂ Extraction (SFE) The berries were extracted with super critical carbon dioxide in an extraction pilot plant system produced by the SITEC Co – Switzerland. A 50 g sample of the plant material was packed as tightly as possible in a stainless steel cylinder (0.6L), which was subsequently brought into the extraction vessel, that was also kept at the selected extraction temperature. The SFE extraction was carried out for 1 hour, under a pressure of 125 bars and a temperature of 40°C.

Chromatographic Analysis

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. Oven temperature: 45-220°C (3.5°C/min); carrier gas: nitrogen, 140Kpa; injection temperature: 220°C; detector temperature: 300°C. In addition a GC-MS was also performed, using a CP-Wax 52 CB column, and a gas chromatograph Packard 438 A interfaced with a Finnigan MAT Ion Trap Detector. 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 wild growing juniper berries' essential oil contained at least 101 constituents of which at least, 77 of them were identified and only 22 constituents recorded yields higher than 0.5% (Chatzopoulou and Katsiotis, 1993). The majority of the volatile components were monoterpenic hydrocarbons following by the sesquiterpenic group. The compounds present in the headspace and supercritical CO₂ extracts were similar to the hydro distillate.

In Table 1 are listed the main (>0.5%) volatile constituents of Juniper berries obtained by HSE, SFE and HD. As can be seen, there are mainly quantitative differences between the samples, as well as some qualitative ones. The monoterpene hydrocarbons were extensively isolated by HSE (89.9 %) following by 60.6 % in the distilled oil and 56.2 % in the supercritical extract (Fig. 1). α -Pinene was the predominant component in all three extracts, decreasing from 51.3 % in the HSE, through 40.3 % in the HD, to 36.4 % in the SFE extract. Sabinene, myrcene, β -pinene and limonene were determined as the most abundant monoterpenic hydrocarbons in all three extracts, while α -thujene, α -terpinene, p-cymene and γ -terpinene were detected in greater than 0.5%, only in the HSE. Limonene and terpinolene were the two exceptions, isolated in higher percentages from the SFE and the HD, respectively.

Sesquiterpenoids represented a considerable portion of the volatile extracts, mainly in those obtained from the SFE and the HD (Fig. 1). Germacrene-D was the major sesquiterpene, attaining its highest yield in the SFE (13.6 %). Other sesquiterpenes

detected in significant amounts were β -caryophyllene, α -humulene, δ -cadinene, caryophyllene oxide and T-cadinol. The percentage yield of the sesquiterpenoids was only 4.2 % in the HSE, while the SFE extract had the highest one (22.7 %).

A minor amount of oxygenated monoterpenes, represented particularly by terpinen-4-ol, was detected in the HSE (0.7 %) and HD (0.5 %).

All three different applied methods proved to be suitable for the extraction of the volatiles from juniper berries, even though revealing a different "aroma" pattern. The HSE seemed to be more efficient for the isolation of the low boiling point monoterpene hydrocarbons as concluded also by Wilson et al. (1992) and Buchbauer et al. (1994). The SFE extracted to a greater extent the higher boiling point volatile constituents, while the co-extraction of cuticular waxes and fats should not be excluded (Reverchon, 1995). This method should be considered as the most appropriate for the obtaining of sensitive substances; as in our case, the maximum yield of the compound most labile to heat and acids, germacrene-D, was achieved by the SFE method (13.6 %). Caryophyllene oxide, also, was detected in the SFE-extract in greater amount (3.2 %) in comparison to the hydro distillate {due probably to the hydrolytic conditions occurring during the hydro distillation (Quirin et al., 1988)}.

Finally, we may conclude that the trend of the constituents during the extraction could be explained by their volatility and the action mode of the above mentioned methods. By the HSE were obtained the more volatile components, while by the SFE those of higher molecular weights and those dissolving to a greater extent in the supercritical CO₂.

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Tables

Table 1. The percentage yields (mean values) of the main volatile constituents of Juniper berries obtained by Headspace (HSE), Supercritical CO₂ extraction (SFE) and Hydro distillation (HD)

Constituents	HSE	SFE	HD	Constituents	HSE	SFE	HD
A-Thujene	2.16	(*)	(*)	Bornylacetate	(*)	(*)	0.51
A-Pinene	51.30	36.43	40.30	α -Cubebene	(*)	(*)	0.88
Sabinene	11.09	3.97	3.84	β -Caryophyllene	1.83	2.28	2.58
B-Pinene	3.12	2.42	2.75	α -Humulene	0.96	1.79	2.13
Myrcene	16.7	10.17	10.63	Germacrene D	1.42	13.63	10.36
A-Terpinene	0.66	(*)	(*)	α -Muurolene	(*)	(*)	0.97
p-Cymene	0.75	(*)	(*)	δ -Cadinene	(*)	(*)	1.92
Limonene	2.37	3.20	1.90	Caryophyllene oxide	(*)	3.22	0.44
Γ -Terpinene	1.09	(*)	(*)	Humulene oxide	(*)	(*)	0.83
Terpinolene	0.64	(*)	0.77	T-Cadinol	(*)	0.52	0.97
Terpinen-4-ol	0.73	(*)	0.51	α -Cadinol	(*)	(*)	0.81

(*) <0.5%

Figures

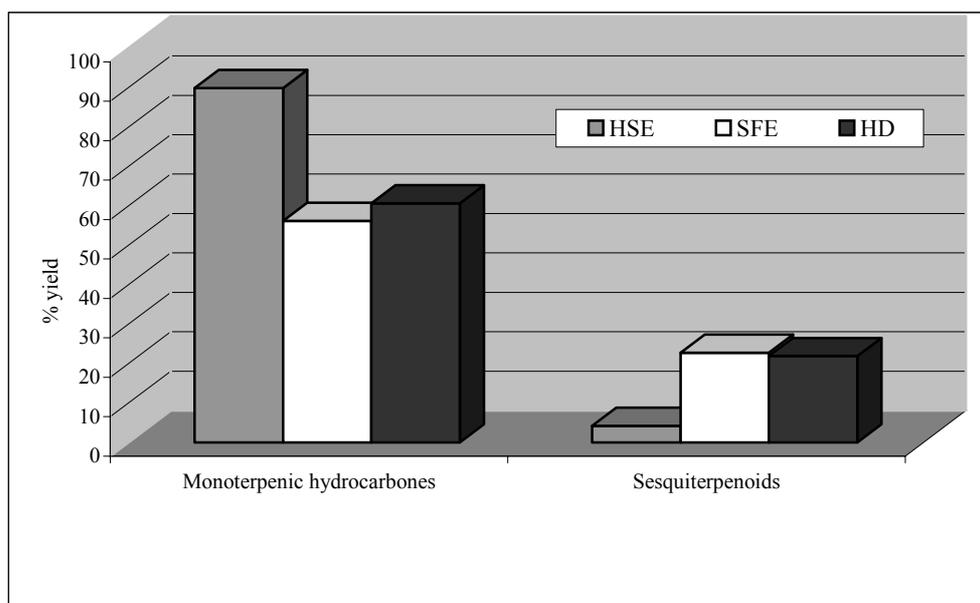


Fig. 1. The percentage yield of the monoterpenic hydrocarbons and the sesquiterpenoids obtained by HSE, SFE and HD.