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Supercritical fluid extraction, Hydrodistillation and Soxhlet extraction of the aerial part of winter savory. Comparative evaluation of the extraction method on the chemical composition

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Abstract

Supercritical fluid extraction of the volatile oil of the aerial part of the winter savory was carried out in different conditions of pressure, temperature, mean particle size and CO_2 flow rate and the results were compared with those obtaining by hydrodistillation with a Clevenger-type apparatus. The non-volatile components were extracted by SFE at higher pressures and by soxhlet extraction with different solvent mixtures. Concerning the volatile components, although the extraction yields of SFE and HD are quite similar, SFE extracts are richer in thymoquinone, a component with interest to the pharmaceutical industry due to is anti-cancer, antioxidant and anti-inflammatory properties.

Keywords: supercritical fluid extraction, hydrodistillation, soxhlet extraction, antioxidants

1. Introduction

Winter savory (*Satureja montana* L.) is an aromatic herb frequently used in food industry and in traditional medicine due to its antimicrobial activities (antibacterial and antifungal), as well as the antioxidant, digestive, laxative, expectorant and sedative properties [1].

Hydrodistillation (HD) and solvent extraction (SE) are the common methods to isolate volatile and non-volatile components from aromatic plants, although these two methods present some problems, namely thermal degradation and solvent contamination, respectively. On the other hand, supercritical fluid extraction (SFE) prevents these limitations, since the process is carried out at a temperature lower than that of HD and the extract is solvent free by decompression. Additionally, the manipulation of parameters as temperature and pressure results in different selectivity and, consequently, in the extraction of different components. This behaviour can be useful when a particular component is desired [2, 3].

In the present work, SFE of the volatile oil was carried out with a flow apparatus using a two stage fractional separation technique, which is described elsewhere [4]. Different conditions of pressure (90 and 100 bar), temperature (40 and 50ºC), mean particle size (0.4, 0.6, 0.8 mm) and CO_2 flow rate (0.8, 1.1, 1.3 kg/h) were studied to understand the influence of these parameters on the composition and yield of the volatile oil extraction. HD was performed on a Clevenger-type apparatus, for 4 hours, using the same mean particle diameters, as for the SFE, in order to compare both methods.

After the extraction of the volatile and the essential oils, the same plant material was subject to the extraction of the non-volatile fraction by SFE and SE (pentane and acetone), respectively.

2. Results and discussion

The volatile and the essential oils obtained by the different extraction procedures were analysed by GC (HP 5890 serie II-GC equipped with a DB-5 column) and GC-MS (Perkin Elmer Autosystem XL GC equipped with DB-1 column interfaced with Perkin Elmer Turbomass mass spectrometer (software version 4.1)). The main compounds are *p*-Cymene (6-18%), γ-Terpinene (2-9%), Thymol (8-11%), Carvacrol (41-64%) and β-Bisabolene (2-4%) (Tables 1-3).

Table 1: Yield and composition of the volatile oil extracted at different conditions of pressure (90 and 100 bar) and temperature (40 and 50ºC).

Components		**90bar**	**100bar**	
	RI	**40ºC**	**40ºC**	**50ºC**
p-Cymene	1117	10.14	8.89	17.77
γ-Terpinene	1158	4.26	3.12	5.95
Thymoquinone	1371	2.92	2.89	2.45
Thymol	1419	10.94	11.34	9.11
Carvacrol	1431	52.65	52.83	41.72
β-Bisabolene	1636	2.45	2.21	3.45
Yield % (w/w)		1.38	1.75	0.85

RI - Retention index relative to C_9-C_{16} *n*-alkanes on the DB-5 column.

Table 2: Yield and composition of the essential and the volatile oils extracted using different particles sizes (0.4, 0.6 and 0.8 mm). SFE was performed at 90 bar and 40ºC.

Components		**0.4mm**		**0.6mm**		**0.8mm**	
	RI	**HD**	**SFE**	**HD**	**SFE**	**HD**	**SFE**
p-Cymene	1117	6.87	6.04	12.75	10.14	11.93	10.92
γ-Terpinene	1158	6.42	2.34	8.85	4.26	9.40	4.95
Thymoquinone	1371	0.18	2.95	0.21	2.92	0.17	3.07
Thymol	1419	9.74	9.42	10.99	10.94	8.61	9.82
Carvacrol	1431	62.04	64.04	52.18	52.65	53.76	53.57
β-Bisabolene	1636	2.65	3.01	1.98	2.45	2.15	2.63
Yield % (w/w)		1.60	1.64	1.47	1.38	1.11	1.16

RI - Retention index relative to C_9-C_{16} *n*-alkanes on the DB-5 column.

Table 3: Yield and composition of the volatile oil extracted at different CO_2 flow rates (0.8, 1.1 and 1.3kg/h) using a pressure of 90 bar, a temperature of 40ºC and a mean particle size of 0.6 mm.

Components	**RI**	**0.8kg/h**	**1.1kg/h**	**1.3kg/h**
p-Cymene	1117	7.15	10.14	9.37
γ-Terpinene	1158	3.93	4.26	5.52
Thymoquinone	1371	2.47	2.92	1.64
Thymol	1419	6.04	10.94	7.66
Carvacrol	1431	64.52	52.65	59.10
β-Bisabolene	1636	2.79	2.45	2.63
Yield % (w/w)		1.45	1.38	1.37

RI - Retention index relative to C_9-C_{16} *n*-alkanes on the DB-5 column.

The major difference between these two techniques (SFE and HD) is the relative percentage of thymoquinone, an oxygen-containing monoterpene. This compound is 10 times more concentrated in the SFE extract than in the HD one (Table 2), which is an advantage to the pharmaceutical industry, due to its anti-cancer, antioxidant and anti-inflammatory properties, as well as the neuroprotective effect against forebrain ischemia and alzheimer disease [5, 6, 7].

The non-volatile fraction was isolated after deodorization by SFE at 250 bar and 40ºC and by SE with pentane or acetone for 5 hours. The correspondent extraction yields are presented in table 4.

Table 4: Extraction yield of the non-volatile components (S1- first separator; S2- second separator).

Method	Yield % (w/w)
SFE, S1	0.16
SFE, S2	0.81
SE (pentane)	4.88
SE (acetone)	7.03

At the end of 5 hours, the solvent extraction was more efficient than the supercritical fluid extraction, indicating that a higher pressure was needed.

3. Conclusions

Different methodologies were used to study the volatile and the non-volatile fractions of the aerial part of winter savory. Concerning the volatile components, although the extraction yields of SFE and HD are quite similar, SFE extracts are richer in thymoquinone content. This may be an advantage, as its biological activity has been proved and a synergistic effect may exist between thymol, carvacrol and thymoquinone.
However, for the non-volatile fraction, SE extraction presents higher yields than SFE. Future studies with these extracts will be carried out in order to achieve the best compromise between yield and biological activity. The antioxidant activity will be also studied using DPPH method.

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