Extraction of *Monarda didyma* and *M. fistulosa*, herbs rich in thymoquinone

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ABSTRACT

Volatile oil rich in thymoquinone (TQ) was extracted from aerial parts of scarlet beebalm (*Monarda didyma*) and wild bergamot (*Monarda fistulosa*) with supercritical CO_2. In the investigated range of extraction pressure and temperature, the optimum conditions were determined as 12 MPa and 40 °C for both good yield (0.6 mg TQ/g *M. didyma* dry weight and 1.6 mg TQ/g *M. fistulosa* dry weight) and high TQ concentration in extract (4.8 and 7.7 % w/w, resp.). The difference in the extraction kinetics of volatile oil and cuticular waxes was discussed. The superitical fluid extraction was compared with two alternative separation processes. While TQ degradation occurred under the conditions of hydrodistillation, Soxhlet extraction with hexane ensured good TQ yields (0.7 and 1.1 mg/g, resp.) but TQ concentration in the hexane extracts was lower (2.6 and 1.9 %).

INTRODUCTION

Thymochinon (Fig. 1) is oxygenated monoterpene, a component of essential oils. It presents important biological activities such as anticancer, antioxidant and anti-inflammatory properties, as well as the neuroprotective effect against forebrain ischemia and Alzheimer disease [1,2] and exhibits potent growth-inhibiting activity against gram-positive bacteria [3].

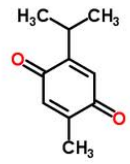

Figure 1: Chemical structure of thymoquinone.

Thymoquinone was identified and isolated from black seed (*Nigella sativa*) fifty years ago. Since then, it was isolated from other plants with therapeutic properties, like *Eupatorium ayapana*, several Origanum species, thyme, *Calocedrus decurrens* and *Nepeta distans* [1]. Kubatova et al. [4] observed that the yield of thymoquinone from garden savory was by extraction with subcritical water 20 times higher than by hydrodistillation. As high yields of thymoquinone were also found by extraction with supercritical CO_2, thymoquinone must be lost during hydrodistillation. When the savory residue after hydrodistillation was extracted with acetone, ca. 50% of the thymoquinone (compared to the amount extracted by subcritical water) was recovered. Thus, both poor extraction and degradation are responsible for low thymoquinone recoveries using hydrodistillation. The loss of thymoquinone during hydrodistillation was observed, to various extents, also for other plants when the yields of hydrodistillation, supercritical fluid extraction (SFE) with CO_2, and other extraction methods were compared (Table 1).

Table 1: Yield of thymoquinone (mg/g dry plant) / concentration of thymoquinone in extract (%)

Plant	Hydrodistillation	SFE	P(MPa)/ T (°C)[a]	Other extraction methods	Ref.
Garden savory *(Satureja hortensis)*	0.02/-	0.29/-	40/50	0.51/-[b]	[4]
Winter savory *(Satureja montana)*	0.03/0.2	0.48/3.0	9/40	-	[2]
Thyme (*Thymus vulgaris*)	0	0.78/6.2	9/40	-	[5]
Black seed (*Nigella sativa*)	0.05/3.0	-	-	(1.2-2.4)/-[c]	[6]
Black seed (*Nigella sativa*)	0.39/21.8	-	-	0.49/24.6[d]	[7]
Black seed (*Nigella sativa*)	0.02/0.5	0.76/0.2	25/40	0.47/0.1[c] 0.17/4.3[e]	[3]
Black seed (*Nigella sativa*)	-	(0.7-2.4)/ (77-86)	9/40	-	[8]

[a]Pressure and temperature of SFE
[b]Extraction with subcritical water
[c]Soxhlet extraction with hexane
[d]Microwave distillation
[e]Steam distillation

Where the SFE conditions indicated in Table 1 are 9 MPa and 40 °C, two separators were used, the non-volatile part of extract was separated from the volatile oil and the percentage of thymoquinone is expressed in relation to the volatile fraction. Alternatively, the concentration of thymoquinone could be increased to tens of percent offline, by extract distillation [3,6].

The aim of the present work was to extract with supercritical CO_2thymoquinone from two species of genus Monardais in the family Lamiaceae, namely aromatic herbs *Monarda didyma* and *Monarda fistulosa*.

MATERIALS AND METHODS

Dry aerial part of herb was milled before the extraction and the particles larger than 0.6 mm were removed on a sieve.

SFE
Dry herb (20 g) was placed in the extractor of 30 mm i.d. and 150 mL volume between two layers of glass beads and extracted with approximately 300 g CO2 (170 L, measured at ambient conditions). The extract was separated from CO_2 by pressure reduction in a micrometer valve to ambient conditions. Fractions of extract were collected separately in cooled vials after 20, 40, 70, 120, and 170 L had passed through the extractor. The CO_2 flow rate was at 0.9-1.2 g/min. The extraction pressure and temperature were adjusted as listed in Table 2. The extraction equipment and procedure were described in detail elsewhere [9].

Table 2: Extraction conditions

Pressure (MPa)	Temperature (°C)	CO_2 density (kg/m^3)[a]
9	40	485.50
12	40	717.76
12	50	584.71
12	60	434.43
30	40	909.89

[a]Estimated using NIST Database [10]

Hydrodistillation
Dried plant (30 g) was immersed in 300 mL of distilled water and distilled for 3 h in Clevenger apparatus. The oil was collected in a side arm of the equipment and its amount was measured gravimetrically.

Soxhlet extraction
The herb (10 g) was extracted with 250 mL *n*-hexane in Soxhlet apparatus for 5 h. The solvent was removed from the extract using a rotary vacuum evaporator.

GC analysis
The composition of volatile compounds in the isolates was determined using GC/MS and GC/FID. The identification was based on the comparison of mass spectra and retention indexes with published results. *n*-Hexadecane was used as internal standard. The details of used analytical methods are given elsewhere [9].

RESULTS

Extraction yield

The effect of SFE conditions on total extraction yield was as expected: the yield increased with increasing density of the solvent and with increasing temperature. The yield of thymoquinone and other volatile substances, however, reached maximum at 12 MPa and 40 °C and it did not increase when the extraction pressure was adjusted at 30 MPa. Thus, the results of other extraction techniques were compared with the results of SFE at 12 MPa and 40 °C.

The yield of individual compounds visible in GC chromatogram, the sum of the yields of volatile compounds and percentage of thymoquinone in this sum, the yield of non-volatile compounds visible in the chromatogram, the total yield of extract, determined gravimetrically, and the percentage of thymoquinone in the extract are listed in Table 3. The yield of CO_2 extract was calculated as the sum of yields of the five collected fractions.

It is evident from the table that the most volatile components (with the shortest retention times) contained in essential oil as a product of hydrodistillation were either partially or completely lost from the other extracts. They could escape dissolved in gaseous CO_2 in the case of SFE, and from the hexane extracts they were most probably evaporated together with the solvent. On the other hand, the oleoresins extracted with CO_2 and with hexane contained large portions of non-volatile substances which could be designated waxes. When the data on thymoquinone content in the extract are mutually compared, it should be taken into account whether the percentage was calculated from areas of peaks in chromatogram or whether it is related to the total mass of extract because the difference could be in order of magnitude. Thymoquinone yield by hydrodistillation was negligible in the case of *M. didyma* and decreased for *M. fistulosa*. Thus, the difficulty of thymoquinone hydrodistillation directly from herbs was confirmed.

Table 3: **Extraction yields (mg/g dry plant)**

Compound	Ret. time (min)	*Monarda didyma*			*Monarda fistulosa*		
		HD[a]	SFE[b]	SE[c]	HD[a]	SFE[b]	SE[c]
α-Thujene	5.42				0.12	0.02	
Sabinene	6.71				0.57	0.11	
1-Octen-3-ol	6.86	0.36	0.17	0.16	0.40	0.14	0.08
3-Octanol	7.39				0.10	0.03	
α-Terpinene	8.09	0.24	0.09		0.28	0.03	
p-Cymene	8.39	1.52	0.65	0.33	2.34	0.47	0.30
Limonene	8.77				0.07		
γ-Terpinene	9.56	0.96	0.34	0.24	0.08		
cis-Sabinene hydrate	10.01					0.04	
Carvacrol methyl ether	16.92		0.09			0.02	
Thymoquinone	17.52	0.002	0.60	0.68	0.72	1.68	1.07
N.I.[d]	18.67					0.14	
Thymol	19.25	1.37	0.98	1.08			
Carvacrol	19.57	0.13		0.10		1.42	0.66
E-Caryophyllene	24.52					0.04	
Germacrene D	27.06	0.19	0.14	0.14	0.30	0.18	0.09
Sum		**4.78**	**3.06**	**2.74**	**4.99**	**4.32**	**2.20**
Thymoquinone in volatile oil (%)		**0.04**	**19.52**	**24.99**	**14.41**	**38.87**	**48.67**
N.I.[a]	49.54		0.05			0.46	
Phthalate	60.73					0.05	
Squalene	67.60		0.09				
Nonacosane	69.73					0.1	
Hentriacontane	74.09		0.08				
Aliphatic hydrocarbon	77.46		0.15				
Aliphatic hydrocarbon	78.31		0.09				
Total yield		**7.16**	**12.49**	**26.24**	**9.99**	**21.81**	**56.36**
Thymoquinone in extract (%)		**0.03**	**4.77**	**2.61**	**7.20**	**7.70**	**1.90**

[a]Hydrodistillation
[b]Extraction with CO_2 at 12 MPa and 40 °C
[c]Soxhlet extraction with hexane
[d]unidentified component

Extraction kinetics

Kinetic data from selected experimental runs are plotted in Figures 2, 3. Within the accuracy of experimental data, the volatile oil could be regarded as one pseudo-component and the remaining mass of extract as the other pseudo-component, “wax”. The minimum feed-to-solvent flow ratio, 20/1.2 = 16.7 min, which is directly proportional to residence time, was large enough to ensure equilibrium at the extractor outlet. Thus, the slopes of extraction yield plotted versus the solvent-to-feed ratio indicate the apparent solubility in the solvent as long as the internal mass transfer resistance does not affect the extraction rate.

The essential oil of Lamiaceae herbs is stored on the surface in glandular trichomes which are

partially destroyed by milling, partially by exposure to supercritical CO_2, and only the walls of remaining intact trichomes represent a substantial mass transfer resistance [11]. At the solvent-to-feed ratio near q = 3, the washing of compounds initially dissolved in CO_2 is finished and the extraction of volatile oil slows down sharply probably because the remaining volatile oil is closed in intact trichomes. It is evident from Figures 2, 3 that the low density of CO_2 at 12 MPa and 60 °C was not sufficient to open most of the trichomes. This holds also for the conditions 9 MPa and 40 °C (not shown here). The extraction of non-volatile substances, located on the surface, continues when the volatile oil from open trichomes is exhausted. Thus, the content of volatile oil in the extract is controlled by the solvent-to feed ratio.

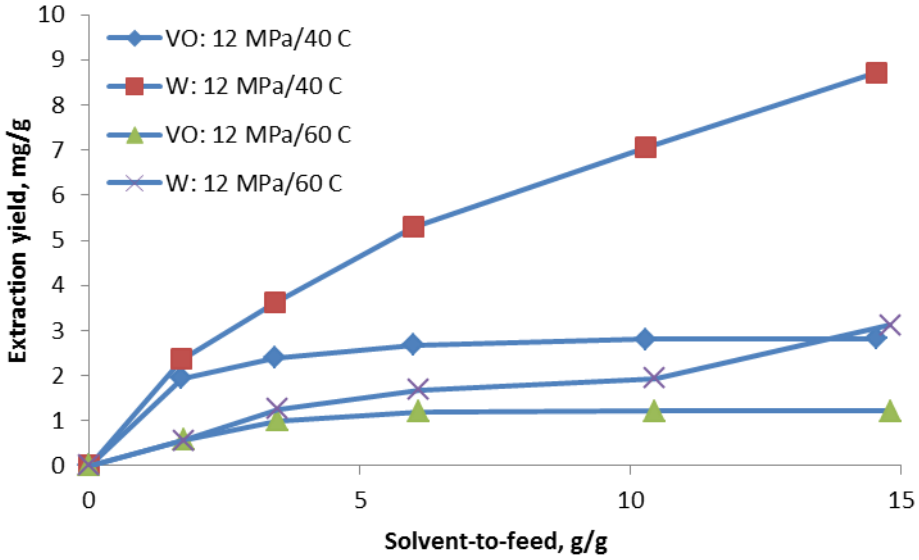

Figure 2: *Monarda didyma:* differences in the extraction kinetics of volatile oil (VO) and non-volatile fraction (W, "waxes").

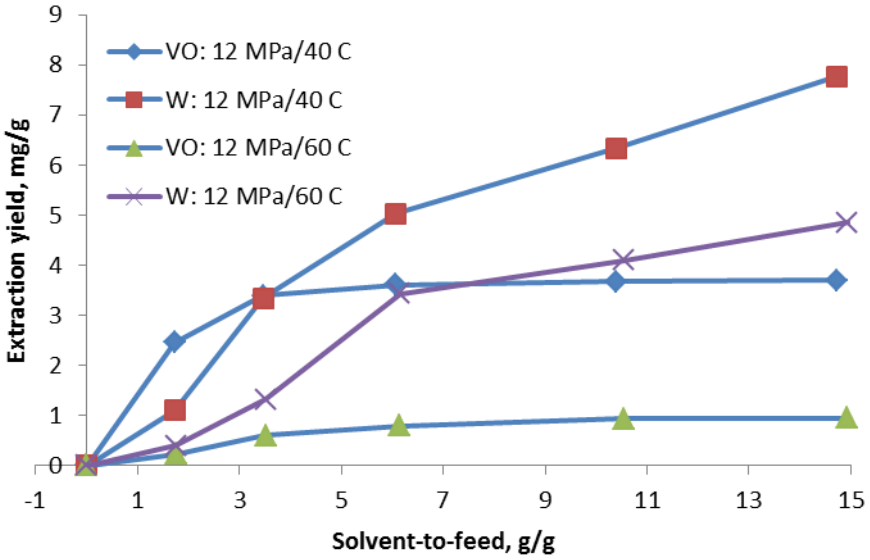

Figure 2: *Monarda fistulosa:* differences in the extraction kinetics of volatile oil (VO) and non-volatile fraction (W, "waxes").

Time fractionation

The changes of extract composition in the course of extraction, so called time fractionation, can be exploited to control the composition of extract fractions. In the present case, the extraction termination at the moment when the initially dissolved volatile oil is washed from the extractor ensures high yield of volatile oil and its high percentage in the extract. The second sampling was conducted slightly later but still the combined experimental data from the first and second sampling indicate good extraction results for the optimum conditions 12 MPa and 40 °C. For M. didyma, 0.48 mg/g and its concentration in extract is 8.0 %, and for M. fistulosa the the corresponding data are 1.42 mg/g and 21.1 % in the extract.

CONCLUSION

Both *Monarda didyma* and *Monarda fistulosa* were confirmed to be a good source of natural thymoquinone. The thymoquinon yields under the optimum conditions for supercritical fluid extraction, 12 MPa and 40 C, were 0.6 mg/g for *M. didyma* and 1.6 mg/g for *M. fistulosa*, which is comparable with the yields of Soxhlet extraction with hexane. The concentration of thymoquinone in CO_2 extracts was 2-4 times higher than in the hexane extracts and could be increased by earlier extraction termination to 8 % (*M. didyma*) and 21 % (*M. fistulosa*) at the expense of slightly lower recovery.

Acknowledgements. The authors thank Viktorija Baskatova for her assistance with experiments. The financial support of the Technology Agency of the Czech Republic (project TA01010578) is acknowledged.

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