

An investigation of CO₂ extraction of marigold (*Calendula officinalis* L.)

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Abstract: Essential oil content (0.05 %) of marigold (*Calendula officinalis* L.) was determined using the official steam distillation procedure. High-pressure CO₂ extraction of plant material under supercritical (100, 200 and 300 bar and 40 °C) and subcritical (60, 90 and 120 bar and 15 °C) conditions for 3 h was investigated. It was found that the increase in the pressure promoted an increase in the yield. The essential oil contents obtained from the investigated CO₂ extracts by steam distillation were significantly higher (1.52–2.70 times) and increased with pressure. Major constituents of the oil, identified using GC-MS and GC-FID, were α -cadinol (26.54 %), T-cadinol and T-muurolol (9.80 %), γ -cadinene (2.99 %), hexadecanoic acid (2.95 %), and ledane (2.45 %). In addition, the essential oils of the CO₂ extracts contained δ -cadinene (6.50–19.87 % under supercritical and 16.09–19.41 % under subcritical conditions), which was not found in the essential oil obtained from the plant by steam distillation. The extraction kinetics was investigated at 200 bar and 40 °C. The total extract obtained after 10 h of extraction was 6.54 % and essential oil content in it, referring to plant material, was 0.209 %, which is 4.16 time more than the one determined by the standard steam distillation procedure.

Keywords: marigold, *Calendula officinalis* L., CO₂ extraction, essential oil, GC-MS analysis.

INTRODUCTION

High-pressure solvent extraction (HPSE) is becoming increasingly popular in the chemical, food and pharmaceutical areas. Extraction by means of CO₂, extensively studied in the last decade, is a good technique for the production of flavors and fragrances from plant material.^{1–3} Conventional processes, such as steam distillation, solvent extraction, etc., often require additional steps, such as separating

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the extract, and their selectivity is usually inferior to that of CO₂. Due to the lower temperature and low water content in HPSE, thermal degradation and hydrolysis are avoided. The extract obtained in this manner contains all active compounds unaltered from the plant and exhibits a scent more similar to the starting material.^{4–6}

Marigold (*Calendula officinalis* L.) is a widely cultivated plant in Europe and America for ornamental and medicinal purposes. In folk medicine, the flowers of this plant are used to treat inflammatory conditions of internal organs, gastrointestinal ulcers, diuretic and diaphoretic convulsions.^{7,8} Calendula extracts are also used in diverse preparations, mainly ointments for the treatment of some dermatological conditions, such as ulcers, eczema, burns and hemoroides.^{9,10} Pharmacological studies of conventional marigold extracts (infusions, tincture, fluid extract) show that its most important constituents are saponines, glycosides of sesquiterpenes, flavonoides and triterpenes.^{11–14}

In this study, CO₂ extraction of marigold flower (*Calendula officinalis* L.) under various conditions (supercritical and subcritical) was investigated. The differences between the essential oil obtained from plant material and CO₂ extracts were determined using GC-FID and GC-MS chromatography. The extraction kinetics was investigated at 200 bar and 40 °C.

EXPERIMENTAL

Plant material

Marigold (*Calendula officinalis* L.) flowers were obtained from the Institute of Field and Vegetable Corps, Novi Sad, Serbia (2000). Before utilizations, the flowers were milled in a blender to a particle size diameter of 703 µm as determined by sieving.

Commercial carbon dioxide was used as the extraction agent.

All other chemicals were of p.a. grade.

Oil isolation

The essential oil from the plant material (50 g) and CO₂ extracts (100–200 mg) was obtained using the DAB 8 procedure¹⁵ and its content is given in % (w/w) with respect to the drug or extract.

CO₂ extraction

The extraction was carried out on a laboratory-scale High Pressure Extraction Plant-HPEP (Nova-Swiss, Switzerland) and the extraction yield are given in % (w/w).

The samples (50 g) were extracted for 3 h under different conditions: at 100, 200 and 300 bar and 40 °C (supercritical conditions) and at 60, 90 and 120 bar and 15 °C (subcritical conditions). The flow rate of CO₂ was 0.19 kg/h. The separation of the extracts was carried out at 25 bar at room temperature. For the investigation of the extraction kinetics and the determination of the total extract content, the extraction was carried out at 200 bar and 40 °C for 10 h. The yield was determined after extraction times of 1, 3, 5, 7.5 and 10 hour.

Oil characterization

The determination of the compounds present in the oil was performed using a Varian 3400 gas chromatograph equipped with a flame ionization detector (FID) and split/splitless injector. The separation was achieved using a DB-5 (5 % diphenyl and 95 % dimethylpolysiloxane) fused silica capillary column, 30 m x 0.25 mm i.d., and 0.25 µm film thicknesses. GC oven temperature was programmed from 50 °C (6 min) to 285 °C at a rate of 4.3 °C/min. Hydrogen was used as the carrier gas

(flow rate 1.6 ml/min at 45 °C). The injector temperature was 250 °C; the detector temperature 300 °C; the injection mode was splitless. The injection volume was 1.0 µL.

The gas chromatographic-mass spectrometric (GC-MS) analysis was performed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 Network mass selective detector (MSD), in the positive ion electron impact (EI) mode. The separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C (6 min) to 285 °C at rate of 4.3 °C/min. Helium was used as the carrier gas; the inlet pressure was 25 kPa; the linear velocity was 1 ml/min at 210 °C. The injector temperature was 250 °C. The injection mode: pulsed splitless, injection pulse pressure 30 psi until 1 min, purg follow to split went 15 ml/min at 0.75 min, gas saver 20 ml/min at 3 min. MS scan conditions: source temperature 200 °C; interface temperature 250 °C; *E* energy 70 eV; mass scan range 40–350 amu.

Identification procedure

The constituents of the essential oils were identified by their retention index and comparison with reference spectra (Wiley and NIST databases). Percentage (relative) of the compounds was computed from the GC/FID peak area.

RESULTS AND DISCUSSION

The oil content of the essential oil of marigold flowers was 0.050 %. The major constituents of the oil, shown in Table I, were α -cadinol (26.54 %), T-cadinol and T-muurolol (9.80 %), γ -cadinene (2.99 %), hexadecanoic acid (2.95 %), ledane (2.45 %), leden (1.63 %), and α -muurolene (1.18 %). Chalchat *et al.*¹⁶ reported that *Calendula officinalis* L. flower oil contained mainly sesquiterpene alcohols of the muurolol and cadinol families (main compound α -cadinol), which agree with the present results.

TABLE I. Composition of marigold essential oil obtained by steam distillation of the plant material and CO₂ extract

No.	Compound name	RT min	Essential oil compounds content, %, obtained from						
			Plant mat. (steam dist.)	Supercritical CO ₂ extracts/(40 °C)			Subcritical CO ₂ extracts/(15 °C)		
				100 bar	200 bar	300 bar	60 bar	90 bar	120 bar
1	Thymol	18.5		0.65	0.57				
2	α -Cubebene	20.3			0.35	0.32	0.33		
3	α -Copaene	21.2			0.56	0.58	0.61	0.52	
4	(-)- α -Grujunene	22.5					0.44		
5	β -Caryophyllene	22.6			0.44	0.31			
6	β -Grujunene	22.9					0.30		
7	α -Humulene	23.4		0.66	0.95	0.87	0.30	0.34	0.56
8	5-diene cis-Muurolo-4-(14)	23.7					0.3		
9	n.i*	23.8		0.68	0.66	1.00	0.58	0.67	
10	γ -Muurolen	24.2	0.72	1.09	1.52	0.48	1.42	1.44	
11	D-Germacrene	24.3		0.67	1.44		1.41		
12	β -Ionone	24.4		0.71	1.03	1.66	1.39	1.03	1.26

TABLE I. Continued

No.	Compound name	RT min	Essential oil compounds content, %, obtained from						
			Plant mat. (steam dist.)	Supercritical CO ₂ extracts/(40 °C)			Subcritical CO ₂ extracts/(15 °C)		
				100 bar	200 bar	300 bar	60 bar	90 bar	120 bar
13	Leden	24.7	1.63	1.04	3.28	3.36	1.88	2.11	2.32
14	α -Muurokene	24.9	1.18	1.08	3.36	3.04	3.56	3.51	3.84
15	γ -Cadinene	25.4	2.99	2.05	7.24	9.55	11.28	11.43	10.69
16	δ -Cadinene	25.6		6.50	19.87	15.69	19.36	19.41	16.09
17	Cadina-1,4-diene	25.8				0.58	0.80	0.90	0.45
18	α -Cadinene	26.0		0.56	1.50	1.58	2.06	1.99	1.90
19	α -Calacorene	26.4				0.40		0.27	0.38
20	Ledane	27.7	2.45	1.75	3.54	3.81	1.50	1.80	2.97
21	Ledol	27.9	0.72		0.96	1.00	0.41	0.38	0.83
22	Cubenole	28.6	1.38	0.48	0.85	0.87	0.56	0.58	0.76
23	T-Cadinole+T-Muurolol	29.0	9.8	4.87	8.36	8.17	7.60	7.61	8.54
24	β -Eudesmol	29.1	1.58	0.79	0.83				0.79
25	α -Cadinol	29.5	26.54	14.84	22.41	19.1	16.95	17.62	22.45
26	Hexadecanoic acid	33.5	2.95			0.28			
27	n.i.	33.7	2.95						t
28	2-Pentadecanone	34.1	1.67	1.53	0.87	0.45	0.40	0.76	0.67
29	n.i.	34.3	1.95			t	t	t	
30	n.i.	35.0	1.86						
31	n.i.	35.2	2.87						
32	n.i.	35.4	1.50						
33	Heptadecane	35.5	1.50	5.43	2.88	2.55	2.68	2.67	2.34
34	n.i.	35.6	1.70						
35	n.i.	35.7	1.73						
36	n.i.	36.5	4.55						
37	n.i.	36.7	2.09						
38	n.i.	37.2	2.80	4.53	1.73	2.61	3.87	6.84	3.01
39	n.i.	37.4		4.56	2.65	1.17	2.02		1.05
40	Octadecane	37.5	0.85	2.08	1.58	1.05	1.16	1.20	0.93
41	Nonadecane	40.2	1.88	7.11	2.79	1.81	3.26	2.73	2.24
42	n.i.	41.4		2.47	t	t	1.19	t	
43	n-Eicosane	44.5	1.15	2.51	1.95	1.18	2.66	2.16	1.89
44	n.i.	44.7		8.56				t	
45	n.i.	46.6		1.03					
46	n.i.	48.7		5.87	t	t	1.48	1.05	1.18

t, Less than 0.1%. * unidentified compounds less than 1.0 % are not reported

Comparing the composition of the oils obtained from the plant material and the CO₂ extracts, it can be observed that the oil from the plant material has two groups of unidentified compounds (*RT* 34.3–35.4 min and *RT* 35.6–36.7 min), which were not found in the oils obtained from the extracts. Compounds with higher *RT* (37.2 min and more) were present in higher amount in the oils obtained from the extracts.

TABLE II. Yield of Marigold extracts and essential oil obtained under supercritical and subcritical conditions

Pressure/temperature bar/°C	Extraction yield, %	Essential oil content count to extract, %	Essential oil content count to plant material, %
100/40	0.56	13.44	0.076
200/40	3.69	3.72	0.124
300/40	4.27	2.94	0.126
60/15	1.47	8.07	0.119
90/15	1.87	7.23	0.135
120/15	2.03	5.55	0.113

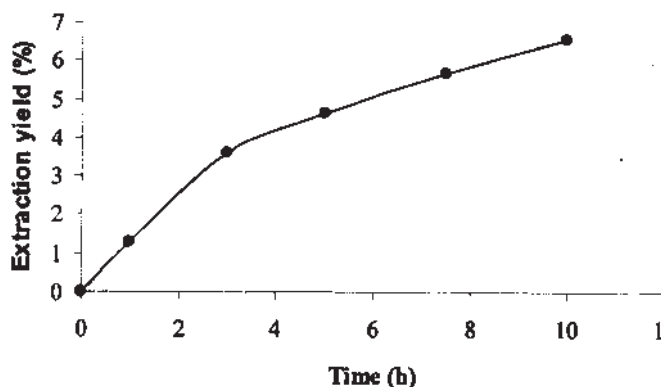
The main compound of the essential oils from the CO₂ extracts shown in Table I was, besides mentioned, δ -cadinene (6.50–19.87 % under supercritical and 16.09–19.41 % under subcritical conditions). The essential oils from the CO₂ extracts also contained a low *RT* group of compounds (α -humulene, β -caryophyllene, α -copaene *etc.*) in smaller percentage (less than 1.0 %). As can be observed, increasing the pressure under supercritical extraction conditions influenced the composition of the oil (the composition was more complex), which was not the case under subcritical extraction conditions.

The extraction yield and the content of essential oil obtained under the investigated extraction conditions are presented in Table II. It can be observed that increasing the pressure promoted an increase in the yield under supercritical conditions. This could be the result of an increase of the solubility of high molar mass compounds (fatty oil, cuticular waxes and ester). This increase was less pronounced under subcritical conditions.

The amounts of essential oil with respect to the plant material obtained from the CO₂ extracts under the investigated conditions were considerably higher (1.52–2.70 times) than the one determined by the official procedure. In our opinion, this could be explained by the fact that *Calendula officinalis* L., besides the essential oil, contains fatty oil, waxes and resins, in which a part of the essential oil is dissolved. Using steam distillation, the official procedure for the determination of the content of essential oil according to all world Pharmacopoeias, only the "free" essential oil from the plant, accessible to steam, is extracted. Application of high pressure CO₂ extraction enables, beside the "free" essential oil extraction, fatty oil, waxes and resins (carrying essential oil dissolved in them) to be extracted. It is obvious that steam distillation of the CO₂

extracts, used to allow the determination of the content of essential oil, could show higher values. From the results shown in Table II, it can be seen that increasing the applied pressure increases the content of essential oil with respect to the plant material. This could be explained by an increase of the extraction yield at higher pressures, more exactly, an increase in the amount of extracted fatty oil, waxes and resins, in which a certain amount of essential oil is dissolved.

Considering the fact that the extraction yield obtained at 200 bar at 40 °C (supercritical conditions) with a three-hour extraction time and that the content of essential oil in the extract ("free" and dissolved in waxes and fatty oil) were satisfactory high, investigation of extraction kinetics were performed under those conditions. The kinetic curve of extraction under the investigated conditions is shown in Fig. 1.



12 Fig. 1. Kinetic curve of mari-gold extraction using carbon dioxide at 200 bar and 40 °C.

As it can be observed the yield increased with increasing extraction time. The total yield obtained under these conditions after 10 h of extraction was 6.54 % and the content of essential oil with respect to the extract was 3.194 %. The amount of essential oil with respect to the plant material was 0.209 %, which is 1.67 times higher than after 3 h of extraction and 4.16 times higher than one determined by the official steam distillation procedure. It can be concluded that 10 h of extraction time was sufficient to extract all the essential oil contained in *Calendula officinalis* L.

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ИЗВОД

ИСПИТИВАЊЕ CO₂ ЕКСТРАКЦИЈЕ НЕВЕНА (*Calendula officinalis* L.)

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Одређен је садржај етарског уља невена (*Calendula officinalis* L.) применом поступка дестилације воденом паром (0.05 %). Испитивана је екстракција угљен-диоксидом под високим притиском у суперкритичној (100, 200 и 300 бар и 40 °C, и течной (60, 90 и 120 бар и 15 °C) области екстракције. Нађено је да се принос екстракције повећава са повећањем примењеног притиска. Садржај етарског уља у испитиваним CO₂ екстрактима био је већи (1,52–2,70 пута) од оног добијеног применом поступка дестилације воденом паром и повећавао се са порастом притиска. Главне компоненте етарског уља, одређене применом GC-MS и GC-FID, биле су α-cadinol (26,54 %), T-cadinol и T-murolol (9,80 % γ-cadinene (2,99 %), хексадеканоична киселина (2,95 %) и ledane (2,45 %). Поред наведених компоненти, етарска уља из CO₂ екстраката садржала су и δ-cadinene (6,50–19,87 % у суперкритичној и 16,09–19,41 % у течной области екстракције) који није нађен у етарском уљу добијеном применом поступка дестилације воденом паром. Кинетика екстракције испитивана је на 200 бар и 40 °C. Укупни екстракт добијен након 10 сати екстракције био је 6,54 % рачунато на биљни материјал, док је садржај етарског уља у њему био 0,209 %, што је 4,16 пута више од садржаја уља одређеног дестилацијом биљног материјала.

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