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This is an **author produced version** of a paper published in:

The Journal of Supercritical Fluids 119 (2017): 283-288

DOI: <https://doi.org/10.1016/j.supflu.2016.10.005>

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Supercritical Fluid Extraction of Bulgarian *Achillea millefolium*

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Abstract

Achillea millefolium L. (yarrow) from Bulgaria was extracted in a supercritical pilot-scale plant using carbon dioxide (CO₂). First, the extraction yield was studied as a function of time at 14 MPa, 40 °C and 70 g/min of CO₂. Then, the extraction time was set in order to study the effect of different process parameters (pressure, temperature, CO₂ flow and use of ethanol as co-solvent) on the extraction yield. The composition of the volatile oil obtained in yarrow extracts was thoroughly studied by GC-MS.

Overall extraction yield increased with pressure, temperature and amount of co-solvent. Nevertheless, minor effect of the studied process parameters on yarrow volatile oil profile was found. On the other hand, important differences were found when comparing the volatile oil composition of Bulgarian *Achillea millefolium* L. extracts with those obtained from other origins.

Keywords: yarrow; volatile oil; supercritical fluid extraction; pilot-scale.

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1. Introduction

Yarrow, Old Man's Pepper, Soldier's Woundwort, Milfoil, Knight's Milfoil, Thousand Weed, Nose Bleed, Carpenter's Weed, Bloodwort, Stauchweed, Sanguinary, Devil's Nettle are common synonyms for *Achillea millefolium* L. It is a flowering plant in the Asteraceae family, native to the Northern Hemisphere and widely spread in Europe and Asia [1]. The name *Achillea* comes from the tale of *Achilles* using this plant to heal his warriors because it staunches blood flow. The genus *Achillea* is composed by a group of species and subspecies with different essential oil compositions [2-4].

Achillea millefolium (yarrow) is a substantial medicinal plant with wide range of pharmaceutical applications. It has been used for centuries to treat various diseases including malaria, hepatitis and jaundice as well as treatment of wounds, hemorrhages, headaches, inflammation, pain, spasmodic diseases, flatulence, and dyspepsia [5-8].

Certain compounds present in genus *Achillea millefolium* have been associated with health benefits, such as phenolic compounds, especially flavonoids [9], and those belonging to the essential oil fraction. The biological properties of yarrow essential oil include anti-inflammatory [10], antimicrobial, antioxidant [11] and anticancer [12] activities.

Extraction of plant volatile oil using supercritical carbon dioxide (CO₂) offers many advantages for pharmaceutical, perfume and food industries [13]. Due to the low critical temperature of CO₂, thermal degradation of natural products and the subsequent generation of undesirable compounds are minimized or avoided. Indeed, the low temperatures required, the absence of oxygen during extraction, and the advantage of recovering the extract with high purity, free of solvent, contribute to produce high quality extracts [14-16]. The available literature shows there are five articles [17-21]

dealing with the supercritical extraction of the volatile oil of *Achillea millefolium* and were performed in a lab-scale apparatus.

Bocevska and Sovová [17] worked on improving the volatile oil / wax ratio in the extract obtained from yarrow white flowers (cultivated in Macedonia) with supercritical CO₂ at 10 MPa, temperatures of 40–60 °C and CO₂ flow rate of 0.57 g/min, using a 150 mL extractor loaded with 25 g of the plant. The extraction at 10 MPa and 60 °C was most selective with respect to unwanted cuticular waxes but the best conditions with respect to extraction rate were 10 MPa and 40 °C. The yield of total extract, measured in dependence on extraction time, was affected by extraction temperature but not by particle size of ground flowers. The capture of more volatile compounds in cooled trap was not sufficient to prevent their loss.

Bimbiraitė et al. [18] determined the total content of flavonoids in four *Achillea millefolium* L. morphotypes: white, pink, deep pink and red cultivated in Lithuania. The highest content was determined in the deep pink (70 mg/g dry matter) yarrow morphotype and the lowest in the pink yarrow morphotype (50 mg/g dry matter). Furthermore, authors extracted the volatile oil with supercritical CO₂ using a small extractor loaded with 0.5 g of the flowers at 40 °C and 9.1 MPa. The highest content of volatile oil was determined in the white yarrow morphotype; the content of essential oil in flowers of the white yarrow morphotype was more than twice as high as in the pink morphotype.

Barghamadi et al. [19] obtained the volatiles of *Achillea millefolium* L., cultivated in Iran, using supercritical CO₂. They investigated the effect of different parameters on the supercritical fluid extraction of *Achillea millefolium* with respect to extraction yield. An 8 mL extraction vessel loaded with 2 g of the plant was conducted at pressures of 10.13, 20.26 and 30.39 MPa and temperatures of 35 °C, 45 °C and 55 °C for duration of 20

min, static, followed by 10-30 min dynamic extraction. Their results showed that the extraction yield increases with increasing the working pressure and temperature. The highest yield was 3.6 %, obtained under a pressure of 30.39 MPa and 45 °C with presence of methanol cosolvent.

Falconieri et al. [20] used flowering aerial parts of wild *Achillea millefolium* growing on the Mediterranean coast (Sardinia Island, Italy) and on the Atlantic coast (Portugal-Serra de Montemuro) as matrix for supercritical CO₂ extraction of the volatile oil. Extraction was performed in a 400 mL vessel loaded by 180 g of the flowers at 9.0 MPa and 40 °C. They observed a strong chemical variability in essential oils depending on the origin of the samples. The Italian volatile extracts are predominantly composed by α -asarone (25.6-33.3 %), β -bisabolene (27.3-16.6 %) and α -pinene (10.0-17.0 %); whereas the main components of the Portuguese extracts are trans-thujone (31.4-29.0 %), trans-caryophyllenyl acetate (19.8-15.8 %) and α -pinene (1.2-11.1 %).

Marzouki et al. [21] were used flowering aerial parts of *Achillea millefolium* growing on Lithuania for supercritical CO₂ extraction of the volatile oil. Extraction was performed with 320 mL extraction vessel at 9.0 MPa and 40 °C. The supercritical extract was dominated by (E)-caryophyllene (26.0 %), γ -muurolene (22.0 %), and caryophyllene oxide (8.1 %).

This work aims to provide new data about the supercritical pilot-scale extraction of *Achillea millefolium* L. cultivated in Bulgaria. To our knowledge, no previous work related with the extraction of Bulgarian yarrow is reported in the literature. The influence of different working parameters on overall yield and the volatile oil composition was analyzed. Furthermore, considering the information available in the literature, differences with respect to the supercritical extracts obtained from yarrow of other origins were established.

2. Materials and methods

2.1 Chemicals

Ethanol was purchased from Panreac (Barcelona, Spain). CO₂ (N38) was supplied from Carburos Metálicos (Madrid, Spain). Analytical standards for GC-MS identification of compounds (eucalyptol, β -linalool, camphor, borneol, α -curcumene, β -caryophyllene, caryophyllene oxide and β -eudesmol) were all from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sample preparation

Achillea millefolium sample from Bulgaria was obtained from an herbalist's local supplier (Murcia, Spain) and the water content was lower than 5 % wt. *Achillea millefolium* included inflorescences and upper leaves of the plant. The sample was ground using a Premill 250 hammer mill (Lleal S.A., Granollers, Spain) and the mean particle size was 500 μ m. All samples were stored in polyethylene bags and kept at 4 °C until extraction.

2.3 Supercritical fluid extraction

Supercritical fluid extraction was carried out using a pilot-plant supercritical fluid extractor (model SF2000; Thar Technology, Pittsburgh, PA, USA), comprising a 2-L cylinder extraction cell and two separators (S1 and S2), each of 0.5 L capacity, with independent control of temperature and pressure (Figure 1). The extraction device also includes a recirculation system where CO₂ is condensed, pumped and heated up to working conditions, and a cascade decompression system comprising two separators.

A kinetic study was performed using 400 g of *Achillea millefolium* at a constant pressure of 14 MPa and temperature of 40 °C to select a reasonably extraction time with respect to yield. In this case, the CO₂ flow was set to 70 g/min and the oleoresin

fractions that precipitated in the first separator ($P = 5.4$ MPa) were collected at 15, 30, 60, 90, 120, 180, 240 and 300 min of extraction time. The material precipitated in the second separator ($P = 0.10$ MPa) was also recuperated after the 300 min of extraction, and represented less than 0.2 % of the material recovered in the first separator.

Additional to the kinetic study, different extractions were accomplished at temperatures of 40 and 60 °C, pressures of 9, 14, 20 and 35 MPa and CO₂ flow of 70 and 120 g/min, representing a CO₂/plant ratio of 42 and 72 kg/kg, respectively. Also, ethanol was used as a co-solvent with ethanol/CO₂ ratio of 5 % and 10 % (mass %). The mass loaded in the extraction cell was 400 g for each experiment. The extract of each experimental assay was collected by depressurization up to recirculation pressure ($P = 5.4$ MPa) in the first separator. The experimental conditions explored are summarized in Table 1. Extraction 2 was carried out by duplicate, and the standard deviation obtained in yield was 0.16 (see Table 1).

The oleoresin fractions precipitated in the separators were collected using ethanol, which was eliminated by rotary evaporation at 30 °C. Samples were kept at -20 °C under darkness until analysis.

2.4 GC-MS analysis

SFE samples were analyzed in an Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) comprising a split/splitless injector, a flame ionization detector, an electronic pressure control, a G4513A auto-injector, a 5975C triple-axis mass spectrometer detector and GC/MS Solution software. The column used was an Agilent HP-5MS capillary column (30 m \times 0.25 mm i.d., 0.25 μ m phase thickness). The chromatographic method was as follows. The oven temperature started at 40 °C, then increased to 150 °C at 3 °C min⁻¹ and was held at 150 °C for 10 min, then from 150 to 300 °C at 6 °C min⁻¹ and finally held at 300 °C for 1min. Sample injections (1 μ L) were

performed in splitless mode. Chromatographic separation was carried out at a flow rate of 1 mL/min and helium (99.996 mass %) was used as carrier gas. The injector temperature was 250 °C and the mass spectrometer ion source, interface and quadrupole temperatures were 230, 280 and 150 °C, respectively. The mass spectrometer operated under electron impact mode (70 eV), was used in total ion current (TIC) mode and scanned the mass range from m/z 40 to 500.

Around 45 peaks were detected in the Bulgarian yarrow supercritical extracts. Several components of their volatile oil were identified by comparing their mass spectra and retention indices with standards; others were identified by matching the mass spectral fragmentation patterns with the Wiley 229 mass spectral library and also by comparing their corresponding retention indices and mass spectra with literature data.

3. Results

3.1 Effect of process parameters on overall extraction yield

Part I: The kinetic behavior of the extraction at 14 MPa and 40 °C is shown in Figure 2. The CO₂/plant ratio, after 300 min of extraction, was 52.5 kg/kg. The material precipitated in the first separator was used to calculate extraction yield (mass of extract * 100 / mass of loaded plant). As mentioned before, the mass recovered in the second separator after the 300 min of extraction represented less than 0.2 % of the material recovered in the first separator and thus, was not considered in the kinetic study of the extraction yield.

The extraction yield increased rapidly during the first 60 min reaching 2.22 % and slightly increased to 2.58 % and 2.77 % at 120 min and 180 min, respectively. After 240 min, the yield is almost constant around 2.90 %. This time was selected as the working extraction time for the rest of the extraction experiments.

Part II: Further experiments were carried out to investigate the effect of pressure, temperature, CO₂ flow and use of a polar co-solvent on the overall extraction yield and the volatile oil profile. The experimental conditions and overall yield obtained are reported in Table 1.

Experiments 1-4 in Table 1 illustrate the influence of pressure on the extraction yield at 40 °C and CO₂ flow of 70 g/min obtained in the pilot-scale extractor. The amount of extract recovered highly increases as pressure rises from 9 to 14 MPa reaching 2.82 %; as the density of supercritical carbon dioxide highly increased from 0.4855 to 0.7633 g/mL [22] it results in more solvation power of CO₂. Then, extraction yield slightly increased up to 3.18 % with respect to the large difference in pressure between 14 and 35 MPa. On the other hand, by raising temperature to 60 °C (Exp. 6) the extraction yield moderately increased with respect to the yield obtained at the same pressure. These results show that extraction yield increases with increasing the pressure and temperature, as reported by Barghamadi et al. [19] for Iranian yarrow variety. Whereas, the extraction yield obtained from Macedonia yarrow variety decreases with increasing temperature, possibly due to the loss of the volatile compounds at higher temperatures [17].

Increasing the CO₂/plant ratio from 42 to 72 kg/kg has no effect on the extraction yield at the working conditions as shown in Exp. 2 and 5. Furthermore, as expected, a significant increase in the extraction yield from *Achillea millefolium* was obtained by using ethanol as co-solvent and it is due to extraction of more polar phytochemicals along with volatile oil compounds as described in Exp. 7 and 8, representing around 1.4 and 1.8 fold increase, respectively, in comparison with Exp. 2.

Figures 3 and 4 show the effect of the most important variables affecting extraction yield. As mentioned before, the effect of pressure is noteworthy only when pressure

increases from 9 to 14 MPa (Figure 3). On the other hand, the increase of the amount of cosolvent shows a linear increase of yield (Figure 4) in the range studied (0-10 % ethanol).

3.2 Volatile oil composition of the Bulgarian yarrow supercritical extracts

The volatile oil of the supercritical extracts obtained from Bulgarian yarrow variety is given in Table 2. Also is included in the table, the composition obtained for duplicate experiments (2a and 2b), which resulted in standard deviations lower than or close to 0.1. As can be observed in the table, the volatile oil is characterized for a large amount of different compounds: a total of 40 different monoterpenes and sesquiterpenes were identified and 4 non-identified compounds were found in all samples. Total chromatographic area identified was close to 90 %.

In general, very similar volatile oil GC profile was obtained despite the extraction conditions applied, and major compounds identified were Borneol, Camphor, Artemisia ketone and Eucalyptol. Also, considerable larger amounts of oxygenated terpenes, particularly alcohols, were identified in comparison with hydrocarbons (see Table 3).

3.3. Comparison of the volatile oil of yarrow from different origins

Regarding the phytochemical profile of the extracted volatile oil and by way of comparison with the data obtained in the present study, Table 4 shows the main terpenoids reported in the literature obtained by the SFE of yarrow from different origins.

Extracts from yarrow cultivated in Portugal [20] and in different habitats of Lithuania [18, 21] were all obtained by SFE at low pressure (9 MPa) and moderate temperature (40 °C) but the composition of the corresponding extracted volatile oils considerably differs. The volatile oil from Lithuania mainly contains monoterpene hydrocarbons, being β -pinene the most important (42.2 %) in the yarrow studied by Bimbiraitė et al.

[18], while *E*-caryophyllene (26.0%) and γ -muurolene (22.0%) sesquiterpene hydrocarbons were the most abundant in the volatile oil extracted by Marzouki et al. [21]. Also sesquiterpene hydrocarbons were identified in the supercritical extracts of Italian yarrow [20], although in this case β -bisabolene (27.3 %) was the most abundant compound. In contrast, the volatile oil from Portugal [20] had high content of oxygenated compounds, such as *trans*-thujone (31.4 %) and *trans*-C₁₅h₂₂o₂ (19.8 %).

Regarding the variety from Macedonia [17], the volatile oil mainly contains oxygenated monoterpenes, being camphor (26.4-31.6 %), bornyl acetate (15.8-16.7 %) and 1,8-cineole (9.6-12.5) the most abundant constituents. These compounds were also recognized as abundant compounds in yarrow from Iran [19] and from Bulgaria (this work). Furthermore, in Bulgarian yarrow, no pinene isomers were detected in contrast to other origins (see Table 4).

Conclusion

Yarrow supercritical extracts are a very complex and multicomponent oleoresin, and the origin of yarrow plant has a strong effect in volatile oil composition. In this work the effect of the extraction conditions on yield and volatile oil composition of Bulgarian yarrow was established. Pressure and amount of cosolvent were the most important variables affecting extraction yield. Yet, pressures higher than 14 MPa did not increase meaningfully the amount of extracted material. On the other hand, the amount of ethanol used affected linearly the extraction yield in the range of conditions explored, and almost duplicated the extracted material with 10 % cosolvent.

The volatile oil composition of Bulgarian yarrow supercritical extracts was slightly affected varying the extraction conditions in the range examined in this work. Camphor

(8.6-12.6 %), borneol (9.3-12.5 %) and artemisia ketone (5.7-14.4 %) were the most abundant compounds identified. High amounts of oxygenated monoterpenes and sesquiterpenes (85-87 %) were identified, representing the terpene alcohols 37-54 % of total oxygenated monoterpenes.

Acknowledges

The authors gratefully acknowledge the financial support from Ministerio de Economía y Competitividad of Spain (project AGL2013-48943-C2) and the Comunidad Autónoma de Madrid (ALIBIRD, project number S2013/ABI-2728).

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Table 1. Experimental conditions and extraction yield of yarrow supercritical extracts recovered in first separator. Extraction time was set to 240 min.

Exp.	Temperature (°C)	Pressure (MPa)	CO ₂ Flow (g/min)	Co-solvent ratio (%)	Weight recovered (g)	Yield (%)
1	40	9	70	0	6.33	1.58
2	40	14	70	0	11.28*	2.82*
3	40	20	70	0	12.21	3.05
4	40	35	70	0	12.71	3.18
5	40	14	120	0	11.23	2.81
6	60	14	70	0	13.70	3.42
7	40	14	70	5	16.16	4.04
8	40	14	70	10	20.59	5.15

*Mean value between duplicate experiments; standard deviation in extraction yield was 0.16.

Table 2. Volatile oil composition (% area) of Bulgarian yarrow supercritical extracts recovered in first separator.

RI	Compound	Exp.								
		1	2a*	2b*	3	4	5	6	7	8
999	Yomogi alcohol	2.21	2.86	2.73	2.82	2.57	3.07	2.05	2.63	2.50
1021	Cymene	0.51	0.84	0.75	0.69	0.73	0.94	0.69	0.66	0.74
1026	Eucalyptol	3.86	7.48	7.55	5.86	5.96	9.36	4.53	5.63	4.64
1035	γ -Vinyl- γ -valerolactone	0.81	0.92	1.05	1.07	1.10	1.16	1.06	1.30	1.31
1058	Artemisia ketone	5.73	11.20	11.32	10.19	9.29	14.38	6.46	9.79	7.76
1068	1,2-Epoxylinool	0.52	0.54	0.43	0.62	0.66	0.67	0.58	0.69	0.78
1081	Artemisia alcohol	1.07	1.16	1.25	1.41	1.29	1.46	1.00	1.40	1.26
1084	cis-Linalool oxide	0.50	0.59	0.6	0.65	0.61	0.61	0.55	0.68	0.75
1097	β -Linalool	0.76	0.57	0.72	0.82	0.79	0.81	0.79	1.15	1.20
1101	Thujone	1.59	2.25	2.32	2.33	2.19	2.74	1.70	2.06	1.92
1111	Chrysanthone	0.25	0.33	0.31	0.38	0.34	0.40	0.25	0.31	0.27
1119	n.i.	2.61	2.73	2.77	2.81	2.81	2.69	2.62	3.18	3.24
1138	Camphor	8.21	12.54	12.42	11.80	10.03	12.62	8.18	9.78	8.92
1140	cis-Verbenol	0.76	0.40	0.49	0.69	0.68	0.60	0.65	0.64	0.66
1160	Borneol	12.46	11.76	11.52	11.42	11.25	10.07	11.51	9.30	9.90
1164	Lavandulol	1.08	0.73	0.82	1.04	0.96	0.85	1.10	1.14	1.32
1171	2-Methyl-2-octen-4-ol	1.01	1.14	1.00	1.18	1.10	1.37	0.99	1.12	1.09
1172	Terpinene-4-ol	0.44	0.42	0.58	0.45	0.43	0.44	0.41	0.51	0.57
1181	p-Cymen-8-ol	0.63	0.33	0.37	0.55	0.63	0.62	0.77	0.79	0.91
1186	3,7-dimethyl-1,5-Octadiene-3,7-diol	4.64	3.85	3.66	3.40	4.24	3.13	5.63	5.61	6.71
1203	Verbenone	0.49	0.41	0.52	0.54	0.52	0.52	0.63	0.71	0.66
1210	Grandisol	3.76	2.81	2.78	3.13	3.40	2.67	3.77	2.86	3.19
1218	2-Hydroxycineole	0.67	0.48	0.42	0.54	0.61	0.46	0.63	0.63	0.74
1231	trans-Chrysanthenyl acetate	1.60	1.82	1.89	1.95	1.65	1.98	1.44	1.57	1.56
1248	Piperitone	0.98	0.79	0.99	0.88	0.91	0.84	0.99	1.14	1.18
1263	(5E)-5,9-Dimethyl-5,8-decadien-2-one	0.74	0.30	0.38	0.45	0.88	0.64	1.09	1.32	1.81
1271	2,6-Dimethyl-1,7-octadiene-3,6-diol	5.96	4.13	4.21	3.66	5.37	3.64	7.24	6.76	7.89
1281	Bornyl acetate	0.71	0.53	0.48	0.66	0.78	0.69	0.74	1.01	0.85

(Continued)

RI	Compound	Exp.								
		1	2a	2b	3	4	5	6	7	8
1282	n.i.	2.42	1.85	1.82	1.95	2.19	2.07	3.08	3.46	4.06
1288	Lavandulyl acetate	1.33	0.96	1.05	1.22	1.16	1.03	1.32	0.91	0.78
1292	Thymol	0.64	0.41	0.60	0.47	0.58	0.43	0.71	0.59	0.64
1299	n.i.	3.43	3.11	2.99	4.25	3.27	2.93	2.99	3.98	3.59
1353	Eugenol	0.37	0.16	0.15	0.23	0.30	0.28	0.42	0.38	0.47
1393	Jasmone	0.88	0.67	0.69	0.65	0.76	0.46	0.89	1.54	1.02
1412	β -Caryophyllene	1.58	1.21	1.23	1.33	1.23	1.05	1.25	0.98	0.84
1474	Germacrene D	1.12	0.68	0.69	0.73	0.82	0.65	0.78	0.68	0.58
1478	α -Curcumene	1.00	0.61	0.61	0.66	0.73	0.52	0.64	0.78	0.71
1570	Spathulenol	1.66	0.86	0.88	1.15	1.34	0.79	1.61	0.85	0.72
1575	Caryophyllene oxide	5.40	3.91	4.05	4.12	3.91	2.81	4.41	2.93	2.95
1607	n.i.	4.21	3.44	3.39	3.25	3.39	2.18	3.99	2.56	2.93
1623	n.i.	2.95	2.21	2.25	2.25	2.18	1.39	2.46	1.43	1.60
1637	β -Eudesmol	1.63	1.27	1.29	1.08	1.13	0.81	1.20	0.95	0.88
1811	Saussurea lactone	3.33	2.67	2.57	2.31	2.62	1.48	3.15	1.81	1.92
1848	Hexahydrofarnesyl acetone	3.51	2.07	2.12	2.39	2.60	1.68	3.07	1.82	1.97

* 2a and 2b correspond to the composition obtained for the extracts obtained by duplicate at 14 MPa, 40 °C, 70 g CO₂/min and without co-solvent.

Table 3. Distribution of terpenes and oxygenated derivatives in Bulgarian yarrow supercritical extracts recovered in first separator.

	Exp.							
	1	2	3	4	5	6	7	8
Monoterpene hydrocarbons	0.5	0.8	0.7	0.7	0.9	0.7	0.7	0.7
Oxygenated monoterpenes*	66.3	73.6	72.9	72.3	78.3	68.9	75.4	74.5
Sesquiterpene hydrocarbons	3.7	2.5	2.7	2.7	2.2	2.7	2.5	2.1
Oxygenated sesquiterpenes**	18.5	13.1	13.4	13.7	9.0	15.9	9.7	10.1
Other compounds	11.0	10.0	10.3	10.6	9.6	11.8	11.7	12.6

* Alcohols representing 37.4-53.5 % of total oxygenated monoterpenes identified.

** Alcohols representing 6.9-10.1 % of total oxygenated sesquiterpenes identified.

Table 4. Prevailing constituents (area %) obtained from different yarrow varieties by SFE.

Plant source	Extraction conditions	Major constituents	Reference
Macedonia	10-20 MPa	Camphor (26.4-31.6 %)	Bocevska and Sovová [17]
	40-60 °C	Bornyl acetate (15.8-16.7 %)	
	No co-solvent	1,8-Cineole (9.6-12.5 %)	
Lithuania	9.1 MPa	β -Pinene (42.2 %)	Bimbiraitė et al. [18]
	40 °C	Limonene (6.4 %)	
	No co-solvent	α -Pinene (5.2 %)	
Iran	10-30 MPa	Germacrene (32.1-40.1 %)	Barghamadi et al. [19]
	35-55 °C	1,8-Cineole (13.2-17.6 %)	
	Co-solvent	Camphor (3.7-4.7 %)	
	(methanol)	β -Cubebene (3.1-4.9 %)	
Italy	9.0 MPa	β -Bisabolene (27.3 %)	Falconieri et al. [20]
	40 °C	α -Asarone (25.6 %)	
	No co-solvent	α -Pinene (10.0 %)	
		<i>trans</i> - α -Bergamotene (9.1 %)	
Portugal	9.0 MPa	<i>trans</i> -Thujone (31.4 %)	Falconieri et al. [20]
	40 °C	<i>trans</i> -Chrysanthenyl acetate (19.8 %)	
	No co-solvent	Germacrene D (11.0 %)	
Lithuania	9.0 MPa	E-Caryophyllene (26.0 %)	Marzouki et al. [21]
	40 °C	γ -Murolene (22.0 %)	
	No co-solvent	Caryophyllene oxide (8.1 %)	
Bulgaria	9-35 MPa	Camphor (8.2-12.6 %)	This study
	40 °C and 60 °C	Borneol (9.3-12.5 %)	
	Co-solvent (ethanol)	Artemisia ketone (5.7-14.4 %)	

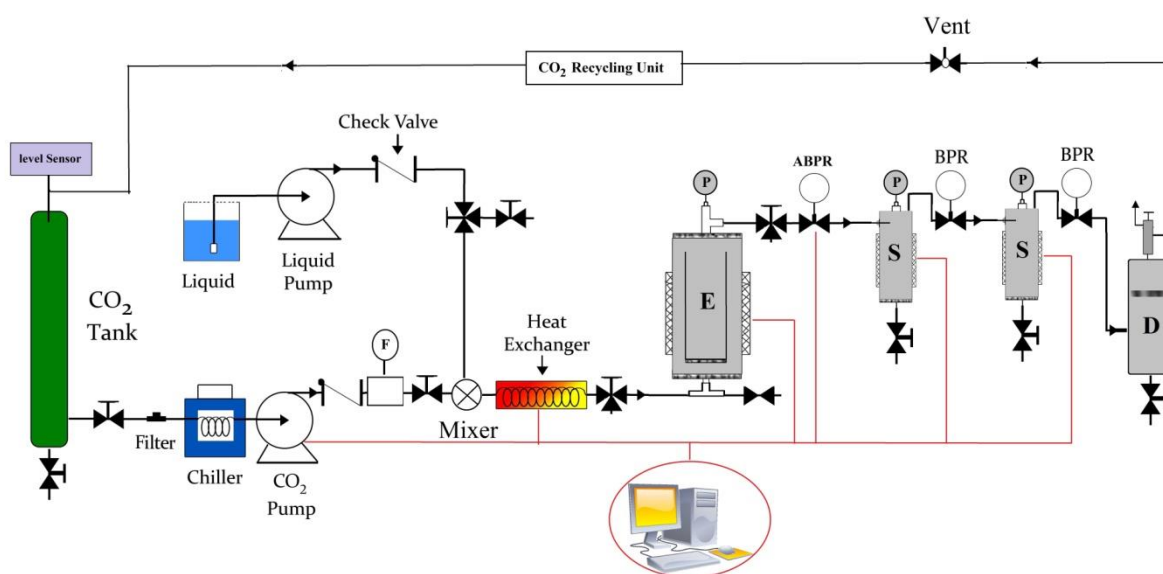


Figure 1. Schematic diagram of the pilot-scale SFE apparatus used in this study. (F) mass flow meter, (E) extraction vessel, (ABPR) automatic back pressure regulator, (BPR) manual back pressure regulator, (P) manometer; (S) separator and (D) demister.

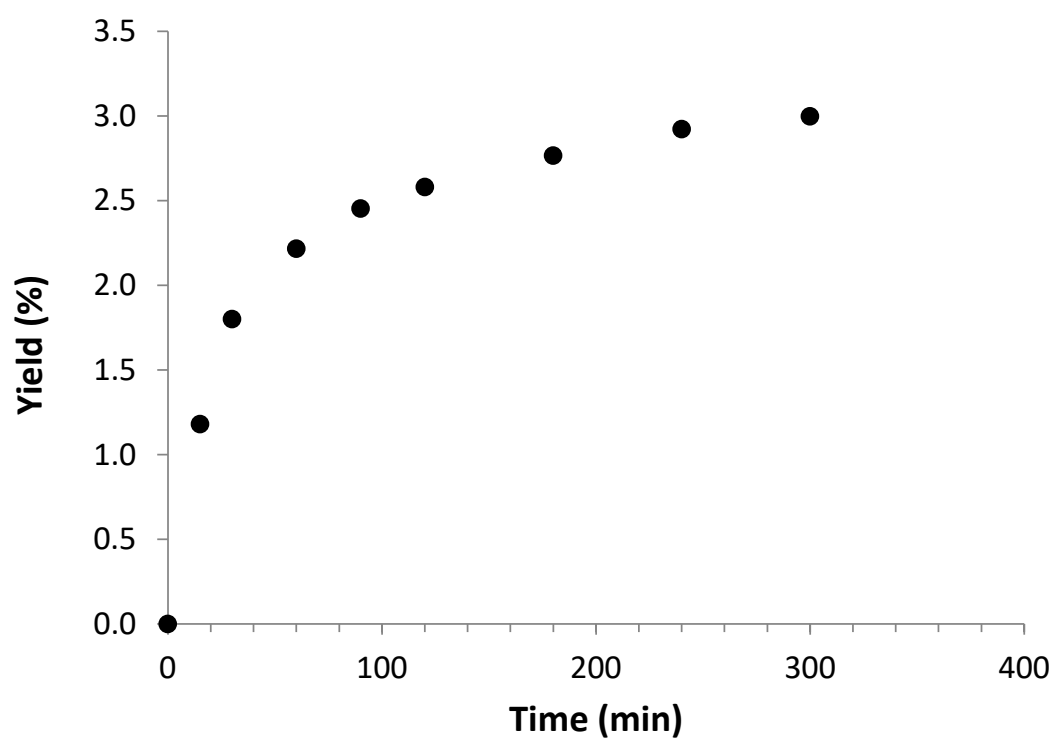


Figure 2. Extraction yield vs. extraction time obtained at 14 MPa, 40 °C and CO₂ flow of 70 g/min.

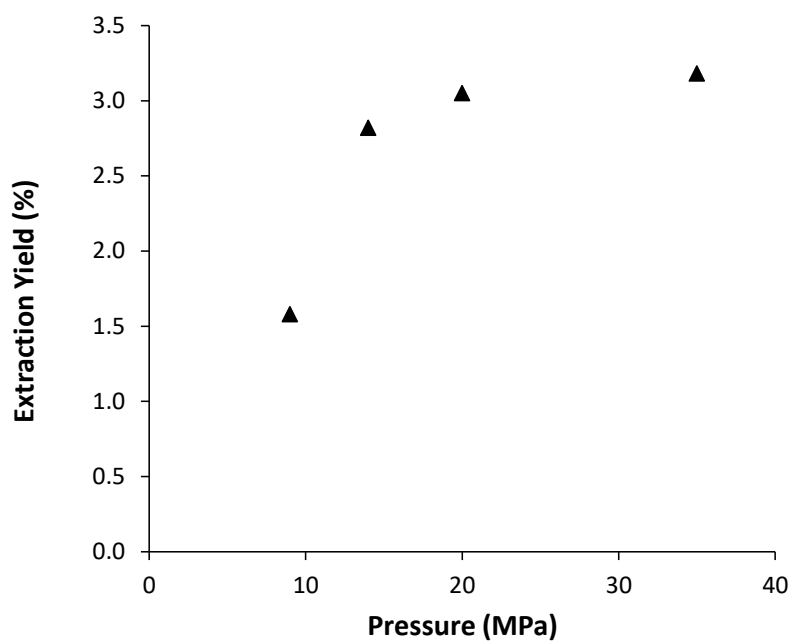


Figure 3. Effect of pressure on the extraction yield of yarrow obtained at 40 °C and CO₂ flow of 70 g/min.

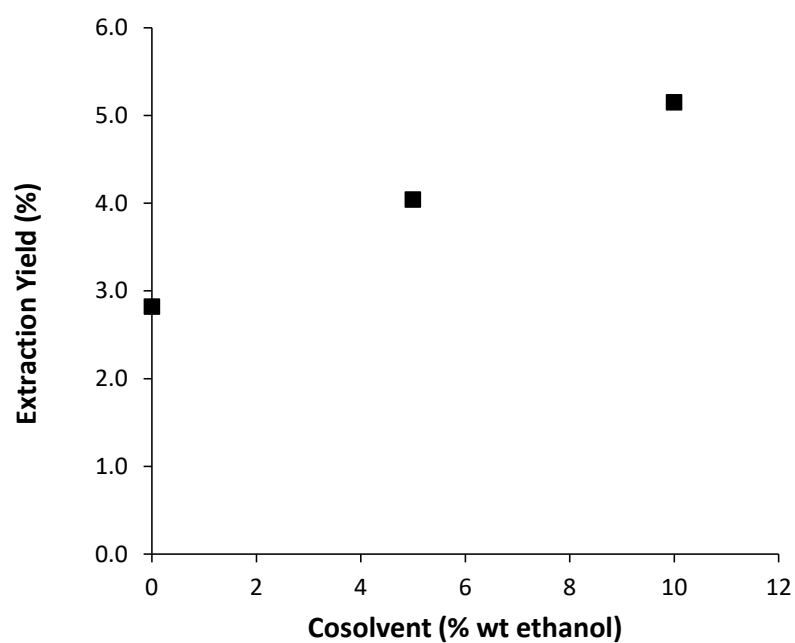


Figure 4. Effect of cosolvent on the extraction yield of yarrow obtained at 40 °C and CO₂ flow of 70 g/min.