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Influence of Extraction Methods on the Composition of Essential Oils of *Achillea millefolium* L. from Lithuania

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Abstract

In this study, flowering aerial parts of *Achillea millefolium* were used as a matrix for supercritical CO_2 extraction (SFE) of volatile oil. The collected extracts were analyzed by GC-FID and GC-MS methods and their composition were compared with that of the essential oil isolated by hydrodistillation (HD). The composition of the essential oil obtained by hydrodistillation and SFE methods is widely different. Indeed, the SFE volatile oil had a pale yellow color whereas the HD oil had a blue color due to the presence of chamazulene (48.0% *vs.* 4.3%). Other important constituents of HD oil were (E)-caryophyllene (19.5 %) and γ-muurolene (13.1%). The CO_2 supercritical extract was dominated by (E)-caryophyllene (26.0%), γ-muurolene (22.0%), and caryophyllene oxide (8.1%).

Keywords: *Achillea millefolium* L.; Hydrodistillation; Supercritical CO_2 extraction; Essential oil; Chamazulene

Introduction

Achillea millefolium L. is a well-known aromatic and medicinal plant, widely used in folk medicine against gastrointestinal disorders and lack of appetite. The chemical composition of *A. millefolium* essential oil was investigated in several countries and a high chemical variability was observed [1-3].

Different studies showed that the chemical composition depends on many parameters as the geographical origin of the plant, the part of the plant analyzed [4,5] and the technique of extraction [6,7]. Although distillation is a very convenient method for extracting essential oils, the artefacts produced during the process, especially when thermolabile components are involved cause some disadvantages. Some authors have reported that *A. millefolium* from some habitats of Lithuania contain proazulenes, which produced azulenes during hydrodistillation. Several previous phytochemical studies focused mainly on azulenes and proazulenes (sesquiterpenic lactones) as the most characteristic compounds in yarrow plants [8,9]. However, the inflorescence oils of pink flowers collected in 14 habitats from Lithuania did not contain chamazulene among the main constituents. Nine oils were azulene-free, four samples contained ≤ 0.5% of chamazulene, and only a single oil sample contained 5.7% of it [10]. Also Orav et al. [11] found in that chamazulene was within the three principal constituents only in some simples of investigated *A. millefolium* essential oils.

Extraction by carbon dioxide in the supercritical state is a process that offers many advantages in obtaining volatile extracts or aroma substances for human nutrition and for the pharmaceutical and perfume industries. Indeed, the mild extraction conditions give assurance against chemical reactions not taking place during the process. Oil of superior quality, free of hydrolysis and thermal degradation products, can be isolated using supercritical carbon dioxide (SFE) as a solvent. The low temperature in SFE prevents thermal degradation, and the low water content limits the hydrolytic process. This technique avoids the degradative thermal processes, hydrolysis, isomerization and racemization [12-14].

This paper reports the composition of the volatile oils of Lithuanian *A. millefolium* extracted by mean of supercritical CO_2, in comparison with that of the HD oil.

Experimental

Plants Materials

The flowering aerial parts of *Achillea millefolium* were collected in July 2013 from its natural habitat in Lithuania. The plant has been identified in the Botany and Botanical Garden Division, Department of Life and Environment Sciences, University of Cagliari, Italy. Before utilization, the vegetable matter was ground with a Malavasi mill (Bologna, Italy) taking care to avoid overheating and the particles sizes were in the range (250-425) μm.

Hydrodistillation

Hydrodistillation (HD) was performed for 3 h in a circulatory Clevenger-type apparatus according to the procedure described in the European Pharmacopoeia [15].

SFE extraction

Supercritical CO_2 extractions were performed in a laboratory apparatus equipped with a 320-cm^3 extraction vessel and two separator vessels of 300- and 200-cm^3 respectively connected in series. Extraction was carried out in a semi-batch mode: batch charging of vegetable matter and continuous flow solvent. The *Achillea millefolium* oil was obtained working at 90 bar and 40°C (CO_2 density, ρ_{CO2} = 0.287 g cm^{-3}) in the extraction vessel, at 90 bar and –10°C in the first separator and at 20 bar and 15°C in the second one.

GC/FID and GC/MS analysis of the volatile extracts

Qualitative analysis was carried out by means of gas chromatography-mass spectrometry (GC/MS) and the quantitative composition was accomplished by gas chromatography (GC/FID).

GC/MS analyses were carried out in a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA) equipped with a split-splitless injector, an autosampler Agilent model 7683 and two different Agilent fused silica capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 µm) of different polarities (HP-5, 5% phenyl-methylpolysiloxane; DB-WAXetr, polyethylene glycol).

GC conditions used were: programmed heating from 60 to 250°C at 3°C/min followed by 20 min under isothermal conditions. The injector was maintained at 250°C. Helium was the carrier gas at 1.0 mL/min; the sample (1 µL) was injected in the split mode (1:10). The GC was fitted with a quadrupole mass spectrometer, MS, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200°C, quadrupole temperature 150°C, scan rate 3.2 scan/sec, mass range 30÷480 u. Software adopted to handle mass spectra and chromatograms was ChemStation. Samples were run in chloroform with a dilution ratio of 1:100. The volatile compounds were identified by both their retention indices and their mass spectra. Retention indices [16], calculated by linear interpolation relative to retention times of a series of *n*-alkanes, were compared with those of authenticated samples from our database. Mass spectra were compared with reference spectra from a homemade library or from literature data [17,18].

Analytical GC/FID was carried out in a gas chromatograph (Agilent, Model 7890A, Palo Alto, CA), equipped with a flame ionization detector (FID), an autosampler (Agilent, Model 7683B), Agilent HP5 fused silica column (5% phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., film thickness 0.25 µm, and a Agilent ChemStation software system. Oven temperature was settled at 60°C, raising at 3°C/min to 250°C and then held 20 min at 250°C; injector temperature: 250°C; carrier gas: helium at 1.0 mL/min; splitting ratio 1:10; detectors temperature: 300°C.

The percentage of individual components was calculated based on GC/FID peak areas without FID response factor correction.3. Results and Discussion

Table 1 shows the detailed identification and area percentage of compounds found in the oil isolated by supercritical extraction and by hydrodistillation. The main compounds responsible for the fragrance of *A. millefolium* oil were: (E)-caryophyllene, 26.0% in SFE versus 19.5% in HD; γ-muurolene, 22.0% in SFE versus 13.1% in HD; caryophyllene oxide, 8.1% in SFE versus 5.6 % in HD and chamazulene, 4.3% in SFE versus 48.2% in HD. The oil obtained by supercritical extraction gave a yield (w/w) of 0.12% while the one obtained by hydrodistillation a yield of 0.07%.

The oil composition is variable according to the method of extraction and the main differences in oil quality are clearly distinguished by their color: the HD oil had a blue color due to chamazulene in aqueous medium, whereas the SFE extract was yellow. In confirmation of this degradative phenomenon, the SFE oil was water diluted and heated, in bringing a change of its color from yellow to blue. Indeed, the chamazulene has sub-basic properties and in acid medium, CO_2 medium, it originates a chamazulenio cation colorless [19]. Literature data reported variations in the essential oil composition of Achillea millefolium L. growing in different countries. Previous investigations reported fourteen of the twenty oils collected from five habitats in Lithuania did not contain chamazulene [3]. This is not surprising as there are some studies supporting that environmental variations affect content and composition of volatile oil in medicinal and aromatic plants [20,21].

COMPOUND	RI	HD	SFE
sabinene	976	tr	tr
β-pinene	979	1.5	0.6
1,8-cineole	1034	1.9	1.5
cis-chrysanthenol	1164	1.4	3.0
borneol	1167	tr	1.1
α-terpineol	1191	tr	2.6
trans-carveol	1210	tr	1.5
cis-chrysanthenyl acetate	1263	tr	tr
β-bourbonene	1384	tr	1.0
(E)-caryophyllene	1418	19.5	26.0
α-humulene	1453	2.6	3.7
γ-muurolene	1480	13.1	22.0
α-zingiberene	1495	1.5	2.4
δ-cadinene	1523	1.9	2.5
(E)-nerolidol	1564	1.3	1.8
germacrene D-4-ol	1574	tr	1.0
caryophyllene oxide	1581	5.6	8.1
viridflorol	1589	1.5	2.4
10-epi-gamma-eudesmol	1622	tr	1.1
3-iso-thujopsanone	1644	tr	1.2
selin-11-en-4-α-ol	1652	tr	2.0
14-hydroxy-9-epi-(E)-caryophyllene	1670	tr	1.4
n.i.	1691	tr	5.8
chamazulene	1726	48.2	4.3
n.i.	1755	tr	2.8
Total identified		100.0	91.1
Hydrocarbon monoterpenes, HM		1.5	0.6
Oxygenated monoterpenes, OM		3.3	9.7
Hydrocarbon sesquiterpenes, HS		38.7	57.6
Oxygenated sesquiterpenes, OS		8.4	18.9
Azulenes		48.2	4.3
Note: tr: trace, i.e., percentage lower than 0.1%. n.i.: not identified compound			

Table 1: Retention index and chromatographic area percentages of compounds identified in *Achillea millefolium* volatile oil extracted by SFE at 90 bar 40°C (SFE) and by hydrodistillation (HD)

Samples from Estonia contained high amounts of monoterpenes and chamazulene. High amounts of monoterpenes and chamazulene were also found in samples from Hungary, Greek, Moldavia, Latvia,

Lithuania and Germany. The oils from France, Belgium, Russia, Armenia, Spain and Italy were rich in oxygenated monoterpenes and contained a little amount of chamazulene. Comparing our results with *A. millefolium* oils earlier reported from other countries, it was evident that these oils are quite different from the others. Recently, Falconieri et al. [22] found that the volatile extracts obtained from the Sardinian *A. millefolium* were characterized by high content of α-asarone 25.6-33.3%, in the SFE extract and in the HD oil, respectively), β-bisabolene (27.3-16.6%) and α-pinene (10.0-17.0%). Whereas in the work of Jaimand et al. (2006) [23], the major constituents of the leaf oil of Iranian ssp obtained by different methods were: chamazulene, isoborneol, p-cymene and germacerene. Essential oils of *A. millefolium* ssp. elbursensis flowers isolated by hydrodistillation indicated chamazulene (54 %), camphor (8 %) and isoborneol (7.6%) as main constituents. While the leaf oil contained chamazulene (35 %), isoborneol (18%) and p-cymene (15%) [23]. The essential oil of *A. millefolium* grown under tropical conditions was rich in sabinene, 1,8-cineole, borneol, bornyl acetate, α-pinene, β-pinene, terpinine-4-ol and chamazulene [24]. The drugs from Greece, Estonia, Moldavia and Scotland were rich in sesquiterpenes. Some authors have reported that *A. millefolium* from some habitats of Lithuania contain proazulenes, which produced azulenes during hydrodistillation. As a rule, the essential oil quality depend on genetic, climatic and soil conditions, plant age and phase of vegetation, anatomical part of plant and harvesting season.In conclusion, the data obtained in this study showed a remarkable quantitative variation of constituents in the oil according to the method of extraction. Essential oils that contain chamazulene are important in therapeutic applications because of its apparent radical scavenging activity [25]. Essential oils that contain chamazulene are important in therapeutic applications because of its apparent radical scavenging activity. Interesting research supporting this activity was carried out by Rekka et al. [26]., who investigated the role of chamazulene in vitro experiments using an iron (II)/ascorbate system to generate hydroxyl radicals inducing membrane lipid peroxidation in liver microsomes, so in a second step biological activity potential must be estimated.

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