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Qualitative Evaluation of Composition of the Volatile Fraction in Commercial Samples of *Cistus incanus* L.

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Summary. The present research is focused on identification of volatile components of different commercial products containing raw herbs of *Cistus incanus* L. The dried herbal material was hydrodistilled, and the obtained essential oils were analyzed by means of gas chromatography with mass spectrometric detection. Alternatively, the headspace analysis of the volatile sample components was also performed. It was found out that the investigated samples of the *C. incanus* L. species show a wide variation in terms of quality and quantity of the respective essential oils, which might result in their variable biological activity also. In conclusion, a postulate for standardization of chemical composition of the raw plant material used in therapeutic preparations is formulated.

Key Words: *Cistus* sp., volatile fraction, headspace GC–MS, GC–MS, essential oils, Deryng apparatus

Introduction

Herbal medicine, which can replace or support a conventional treatment, is becoming increasingly more popular [1]. Phytotherapeutic agents are used mainly in the treatment of chronic diseases [2]. Despite many benefits of dietary supplements of plant origin, a doubt exists as to the safety of their large scale usage. This is due to the fact that the commercial herbal products are not standardized with respect to the composition and concentration of their ingredients. In extreme cases, the signs of toxicity may occur, which can lead to irreversible damage of certain organs or even to death [3, 4]. The toxicity of a given substance is related to the administered dose, but it also depends on the frequency of usage and susceptibility of an individual person [5]. An interaction of herbal constituents with synthetic drugs can dan-

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gerously strengthen or weaken an effect of the latter ones, often interfering with the drug metabolism [6]. Another problem can be due to contamination of the raw herbal material with various different adulterants which can increase the toxicity of phytotherapeutic agents [7]. Detailed mechanisms of herbal action still remain unproven, although the health-promoting effect of the herbs seems to rely more on the synergism among different plant components than on the effects of individual compounds [8].

Among the herbs with the well-pronounced pharmacological effects, *Cistus incanus* L., or hairy rockrose (a member of the Cistaceae family), has to be named [9]. These plants are native to the Mediterranean zone, the Canary Islands, Madeira, and the Middle Eastern countries. Hairy rockrose is a rather small shrub reaching approximately 1.5 m in height, with the egg-shaped, wrinkled leaves and the five-petal amaranthine flowers.

Traditionally, various kinds of the *Cistus* genus species have been used in folk medicine as antimicrobial, antibacterial, anticancer, antiviral, anti-inflammatory, and soothing agents. Inflammation of the joints, chronic gastrointestinal tract diseases, inflammatory states of the respiratory system, and liver diseases have all been treated with the *Cistus* herbal tea [10–15]. Moreover, preparations of hairy rockrose are considered as active inhibitors in the process of prostate hypertrophy [10, 16]. Water extracts from *C. incanus* L. exhibit high antioxidant activity and influence the DNA splitting [17]. The research confirms also an antiviral in vitro activity of the preparations made of hairy rockrose against the HIV-1, HIV-2, and flu viruses [18, 19].

Among chemical compounds which determine pharmacological properties of *C. incanus* L., polyphenols are the most significant and varied [20, 21]. Herbal essential oils are also known for their therapeutic properties [22]. Essential oil obtained from *C. incanus* L. is known to contain oxygenated monoterpenes, diterpenes, and oxygenated and bicyclic sesquiterpenes [9].

The main purpose of this study is qualitative assessment of the volatile fraction contained in the commercial samples of *C. incanus* L. We also compare an efficiency of the two methods of sample preparation for the further chromatographic analysis. By exposing large compositional variation of the volatile fraction derived from one and the same botanical species of different origin, we wish to attribute particular importance to the need for legal and analytical procedures, which might result in standardization of chemical composition with the raw plant material used for manufacturing of the health promoting products.

Experimental

Chemicals

In our investigations, we employed the following standards of the essential oils components: camphor (96%), (1R)-(−)-fenchone (≥98%), (R)-(−)-carvone (98%), (R)-(+)-limonene (97%), linalool (97%), 1,8-cineole (eucalyptol) (99%), and γ-terpinene (97%) from Sigma-Aldrich (Schnelldorf, Germany); and eugenol (99%) and thymol (99%) from Acros Organics (Geel, Belgium). The other reagents were methyl hexadecanoate (analytical purity) from Poly-Science Corporation (Niles, USA), *o*-xylene (gas chromatographic purity) from PPH POCH (Gliwice, Poland), and anhydrous sodium sulfate (analytical purity) from J.T. Baker (Deventer, Holland). Water was deionized and double-distilled in our laboratory by means of the Elix Advantage model, Millipore system (Millipore, Molsheim, France).

Plant Material

Twelve dried herbal samples of the *Cistus* species commercially obtained from twelve different manufacturers in form of the coarse-grained inhomogeneous particles with easily recognizable leaf and stem parts and blossoms were tested in this work. According to the manufacturers' information, plant material originated from Turkey (samples T1–T5), Albania (samples A1–A4), and Greece (samples G1 and G2). Moreover, one investigated commercial sample was of an unknown geographical origin (sample ND1). Eleven herbal samples were those of *C. incanus* L., and one (G2) was of *Cistus creticus* L.

Sample Preparation

For the sake of comparison, composition of the volatile fraction contained in the analyzed samples was investigated with two methods. The first method was to extract the volatile fraction by employing hydrodistillation (according to the standard procedure described in Polish Pharmacopoeia [23], followed by the analysis by means of gas chromatography coupled with mass spectrometric detection [GC–MS]). Alternatively, the headspace analysis of the volatile sample components was performed.

Hydrodistillation of Herbal Material in the Deryng Apparatus

The dried plant material was weighed (25 g) and placed in a 500-mL round-bottomed flask, and 300 mL redistilled water and 0.5 mL *o*-xylene was added. Vapor distillation was performed for 4 h with use of the Deryng-type apparatus. The contents of essential oils were measured according to an indirect xylene-based procedure described in Polish Pharmacopoeia [23]. The observed essential oil yields (w/w) ranged from 0.04 to 0.12% of the dry herbal material. The isolated fractions were dried over the anhydrous sodium sulfate. Essential oils prepared in that way were stored in the sealed amber glass vials at −20 °C, ready for the analysis. Finally, the 1-μL aliquots of pure essential oils were analyzed by means of GC–MS.

Headspace Analysis of the Volatile Sample Components

The working headspace (HS)-GC–MS conditions were tested for six different incubation periods, namely, 15, 30, 45, 60, 75, and 90 min, in each case testing the temperatures from 60 °C to 150 °C (in the 10 °C intervals). Based on the obtained chromatograms, the best suited HS-GC–MS analysis parameters were selected (which were the incubation period of 60 min at 135 °C).

The 2-g aliquot of each dry herbal sample was placed in a 10-mL glass vial stoppered with a silicon–teflon septum (Thermo Scientific, Waltham, MA), then placed in the thermostatted autosampler of the gas chromatograph, and heated for 60 min at 135 °C. The 750-μL aliquots of gaseous phase from above the surface of botanical material were automatically injected to the GC–MS system.

GC–MS and HS-GC–MS Analysis

The GC–MS and HS-GC–MS analyses were carried out with use of a Trace Model 2000 capillary gas chromatograph with an MS Trace model mass detector (ThermoQuest, Waltham, MA USA), equipped with a CTC Analytics model autosampler (Combi PAL, Basel, Switzerland), working in the HS and the non-HS modes. Compounds were separated on the 30 m × 0.35 mm i.d. TG-35MS capillary column (film thickness, 0.25 μm; by

Table I. Chemical composition of essential oils extracted from the *C. incanus* L. and *C. creticus* L. samples obtained by vapor distillation in the Deryng apparatus and established by means of GC–MS and the retention times (t_R) of individual identified compounds

No.	t_R (min)	Compound	Abundance (%)												ID
			T1	T2	T3	T4	T5	A1	A2	A3	A4	G1	G2	ND1	
1	4.6	Limonene	0.5	–	–	–	–	–	2.0	0.8	–	–	–	–	1,2,3
2	4.7	1,8-Cineole	4.0	–	3.3	3.8	–	0.6	3.1	6.0	3.1	2.7	–	2.5	1,2,3
3	4.9	γ-Terpinene	2.4	–	0.4	1.6	2.7	–	0.7	1.1	0.7	1.1	–	1.0	1,2,3
4	5.5	Linalool	2.6	–	1.1	1.1	1.6	–	1.6	1.2	1.0	0.4	–	–	1,2,3
5	5.7	Fenchone	0.5	–	1.9	0.6	–	0.3	1.3	1,1	1,1	0,5	–	–	1,2,3
6	6.6	Camphor	1.3	1.3	3.6	1.7	1.5	0.4	2.7	4.5	2.3	3.1	–	0.6	1,2,3
7	6.9	α-Terpineol	–	–	0.3	0.6	–	0.2	0.9	–	1.2	0.4	–	0.8	1,3
8	7.1	Estragole	–	–	2.4	0.3	–	–	0.6	–	–	0.3	0.6	–	1
9	7.2	Safranal	–	–	–	1.1	0.5	0.1	–	0.9	0.6	0.3	–	0.5	1,3
10	7.5	3,4,4a,5,6,7-Hexahydro-1,1,4a-trimethyl-2(1*H*)-naphthalenone	–	–	0.5	0.8	–	0.1	–	–	–	–	–	–	1
11	7.6	Nonanoic acid	–	–	0.3	0.3	–	–	–	0.3	–	–	–	–	1,3
12	7.8	α-Ylangene	0.7	–	0.3	0.2	0.8	0.2	–	0.4	–	0.2	–	0.7	1,3
13	7.9	(−)-Carvone	–	–	–	0.2	–	–	–	–	–	–	–	–	1,2
14	8.0	Thymol	1.4	–	0.8	0.7	1.9	0.3	1.1	1.4	4.3	0.4	0.5	0.7	1,2
15	8.2	Carvacrol	12.7	–	4.0	7.4	9.4	3.6	8.5	9.1	6.3	7.3	0.9	13.0	1,3
16	8.7	Caryophyllene	1.3	1.1	–	0.5	1.5	0.5	1.3	1.3	1.6	1.2	1.3	0.6	1,3
17	8.7	Aromadendrene													1,3
18	8.8	Eugenol	–	–	0.3	0.2	–	–	–	–	0.4	0.4	3.5	–	1,2,3
19	9.6	α-Selinene	2.0	–	1.0	0.7	1.9	0.3	0.9	1.0	0.7	–	0.6	2.3	1,3
20	9.9	δ-Cadinene	2.4	–	1.4	0.7	2.7	–	0.7	0.6	0.7	0.2	–	3.7	1,3
21	10.3	Calamenene	1.2	–	1.6	0.7	1.5	–	–	–	–	–	–	2.4	1,3
22	10.6	*n*-Dodecanoic acid	–	–	1.1	1.0	–	0.2	–	–	–	0.5	–	–	1,3
23	11.2	Viridiflorol	0.7	–	1.6	0.5	0.7	7.2	1.2	–	3.3	2.3	0.7	–	1,3
24	11.3	Ledol	–	–	0.3	–	–	0.9	1.5	2.2	2.2	1.3	–	0.5	1,3
25	11.5	Cubenol	1.4	–	1.3	0.7	2.0	–	–	–	–	–	0.5	–	1,3
26	11.6	Himachalene	0.7	–	0.5	0.3	1.0	–	–	–	–	–	0.4	2.6	1,3
27	11.7	Caryophyllene oxide	–	–	0.3	–	0.4	–	–	0.7	–	–	0.4	0.8	1,3
28	12.1	α-Vatirenene	–	–	0.4	–	0.6	–	–	–	–	–	0.3	0.7	1
29	12.6	Tetradecanoic acid	–	1.1	3.0	4.2	–	3.0	–	–	1.9	3.3	–	0.4	1,3
30	12.9	Hexahydrofarnesyl acetone (phytone)	0.6	–	3.1	4.3	0.8	1.6	1.9	1.4	1.8	1.4	0.8	1.0	1,3
31	13.6	Methyl palmitate	–	–	0.4	0.5	–	–	–	–	–	––	–	–	1,2
32	13.9	Sclareoloxide	4.4	5.2	3.1	0.8	4.8	3.5	1.9	3.5	1.2	–	4.1	2.7	1
33	13.9	Farnesyl acetone	–	–	–	–	–	–	–	–	–	1.9	–	–	1,3
34	14.0	Dihydromanoyl oxide	–	–	0.4	0.1	–	–	–	–	–	–	0.7	–	1
35	14.2	Hexadecanoic acid	–	6.9	4.7	6.9	0.6	7.4	0.6	–	4.1	8.0	–	–	1,3
36	14.7	Manoyl oxide	15.0	16.8	7.2	8.6	12.2	8.9	10.4	10.6	4.4	6.5	8.9	13.6	1,3
37	14.9	13-Epi-manoyl oxide	17.0	28.6	7.3	11.7	14.3	12.0	15.7	12.7	10.2	10.1	9.7	19.4	1,3
38	15.3	Sclareol	2.0	–	2.4	0.7	3.2	10.0	2.3	3.0	8.1	6.7	5.2	2.8	1,3
39	15.5	Abietatriene	–	–	2.9	7.8	–	–	–	–	–	–	–	1.6	1,3
40	17.1	1-Heptatriacontanol	–	–	0.4	0.2	–	0.1	–	–	–	–	–	–	1
41	17.1	2-(2-Aceoxy- 2,5,5,8a-tetramethyldecalin-1-yl)-acetic acid	–	–	1.1	0.3	0.6	–	–	–	–	0.2	1.1	–	1
42	19.4	Androsterone	–	–	1.9	0.3	–	0.9	–	0.4	–	0.2	–	–	1
43	21.2	(3α,5β,20S)-Pregnane-3,17,20-triol	2.7	2.1	4.7	1.3	3.4	2.3	–	0.5	–	0.6	–	1.9	1

RSD based on the three replicates never exceeded the ± 7.5% level
Abundance (%) calculated as percentage of the sum of all peak heights
ID: Identification of compounds:
1: based on a comparison of mass spectra recorded for individual chromatographic peaks with those available from the NIST software library
2: using the external standard technique
3: based on refs. [11] and [24]

Table II. Chemical composition of the volatile fraction of the *C. incanus* L. and *C. creticus* L. samples established by means of HS-GC–MS and the retention times (t_R) of individual identified compounds

No.	t_R (min)	Compound	Abundance (%)												ID
			T1	T2	T3	T4	T5	A1	A2	A3	A4	G1	G2	ND1	
1	3.3	Furfural	22.0	4.6	8.0	2.3	3.6	8.1	18.3	4.6	8.1	14.2	11.7	13.6	1
2	4.7	1,8-Cineole	–	–	–	–	1.2	–	–	–	–	–	–	1.8	1,2,3
3	4.9	5-Methyl-2-furaldehyde	15.5	3.6	5.9	4.4	2.5	5.7	23.0	4.5	5.4	17.2	9.8	9.6	1
4	5.5	Linalool	–	–	1.2	–	–	–	–	–	–	–	–	–	1,2,3
5	6.6	Camphor	2.8	2.2	2.1	1.8	1.1	1.4	–	1.4	4.3	–	–	2.1	1,2,3
6	6.9	α-Terpineol	–	–	1.3	–	1.1	–	–	–	2.9	–	–	–	1,3
7	7.2	2-Ethylidene-6-methyl-3,5-heptadienal	1.6	–	2.0	–	1.2	1.4	–	1.0	3.1	–	–	–	1
8	7.5	3,4,4a,5,6,7-Hexahydro-1,1,4a-trimethyl-2(1*H*)-naphthalenone	2.3	1.8	3.3	0.7	1.6	1.9	–	1.1	5.0	–	–	–	1
9	8.0	Thymol	–	–	1.0	–	1.8	–	–	–	4.1	–	–	–	1,2,3
10	8.1	Carvacrol	9.3	1.8	25.0	21.2	31.7	2.5	13.1	7.9	6.5	23.1	–	35.1	1,2
11	8.6	Dehydro-ar-ionene	1.8	1.9	2.1	1.5	1.2	2.6	–	2.0	2.7	–	–	–	1,3
12	8.9	Eugenol	5.3	–	2.3	–	–	1.6	–	–	–	–	1.5	–	1,2,3
13	9.7	α-Muurolene	–	1.8	1.2	0.7	1.4	4.3	–	–	1.7	–	–	–	1,3
14	10.0	δ-Cadinene	1.6	2.1	1.9	1.7	3.5	1.9	–	0.9	1.6	–	–	–	1,3
15	10.1	Calamenene	–	2.3	1.5	2.1	2.0	1.2	–	–	–	–	–	–	1,3
16	10.7	Calacorene	–	–	0.8	0.9	1.2	–	–	1.0	–	–	–	–	1,3
17	11.9	Methyl tetradecanoate	–	–	0.8	5.1	–	–	–	3.0	–	–	–	–	1,2
18	12.4	Phytol	1.7	2.3	2.0	6.2	1.0	4.3	–	7.4	2.6	–	–	–	1,3
19	12.9	Hexahydrofarnesyl acetone (phytone)	1.7	1.3	2.6	2.4	–	2.4	–	1.2	1.8	–	–	–	1,3
20	13.6	Methyl palmitate	–	–	1.2	12.4	–	2.4	–	11.9	–	–	–	–	1,2
21	14.7	Manoyl oxide	4.3	15.4	6.2	6.2	12.8	10.2	–	7.5	7.3	4.8	11.7	7.7	1,3
22	14.9	13-Epi-manoyl oxide	6.7	36.0	8.9	14.2	17.0	18.4	8.1	17.1	13.0	8.2	21.8	13.2	1,3

RSD based on the three replicates never exceeded the ± 7.5% level
Abundance (%) calculated as percentage of the sum of all peak heights
ID: Identification of compounds:
1: based on a comparison of mass spectra recorded for individual chromatographic peaks with those available from the NIST software library
2: using the external standard technique
3: based on refs. [11] and [24]

manufactured Agilent Technologies, Palo Alto, CA, USA). The analyses were run using the following temperature program: 50 °C (2 min), 50–150 °C (15 °C/min), and 150 °C (1 min); 150–220 °C (15 °C/min) and 220 °C (15 min). The temperature of the injector was kept constant at 220 °C. Mass spectrometer was equipped with an electron impact source operated at

70 eV. Samples were injected in the splitless mode, using helium (its pressure 100 kPa) as a carrier gas. Three replicates of each herbal sample were performed in an analogical way.

Identification of Compounds

Identification of individual compounds was based on a comparison of the mass spectra recorded for individual chromatographic peaks with those available from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) software library, or on the data on the components contained in *C. incanus* L., reported in the literature [11, 24]. Moreover, selected compounds were identified using the external standard technique. An overall number of 43 compounds identified by GC–MS are listed in *Table I*. In *Table II*, a list of 22 compounds identified with use of HS-GC–MS is given.

Results and Discussion

Within the framework of this study, we investigated eleven commercially obtained *C. incanus L.* samples and one *C. creticus* L. sample, all of them most probably originating from natural habitats and provided by different manufacturers. As certain confusion exists about the systematics of the *Cistus* species [25], with *C. creticus* L. considered by some as the subspecies of *C. incanus* L., we included this single *C. creticus* L. sample in the general framework of our considerations. The analyzed samples differed in terms of the country of origin. According to the importers' declarations, five samples originated from Turkey, four samples originated from Albania, and two samples were of the Greek origin. With one sample (ND1), no country of origin was specified. Moreover, there is no detailed information regarding the harvesting regions within each country and the harvesting period, the plant drying and storage conditions, and the parts of the plant (leaves, stems, etc.) included in the preparation.

With each investigated sample, composition of the volatile fraction was analyzed, considering first the countries of origin of the investigated *Cistus* samples as a possible common denominator for individual volatile fraction profiles. From the data given in *Tables I* and *II*, considerable differences can be seen in the chemical composition of the volatile fractions, however, quite

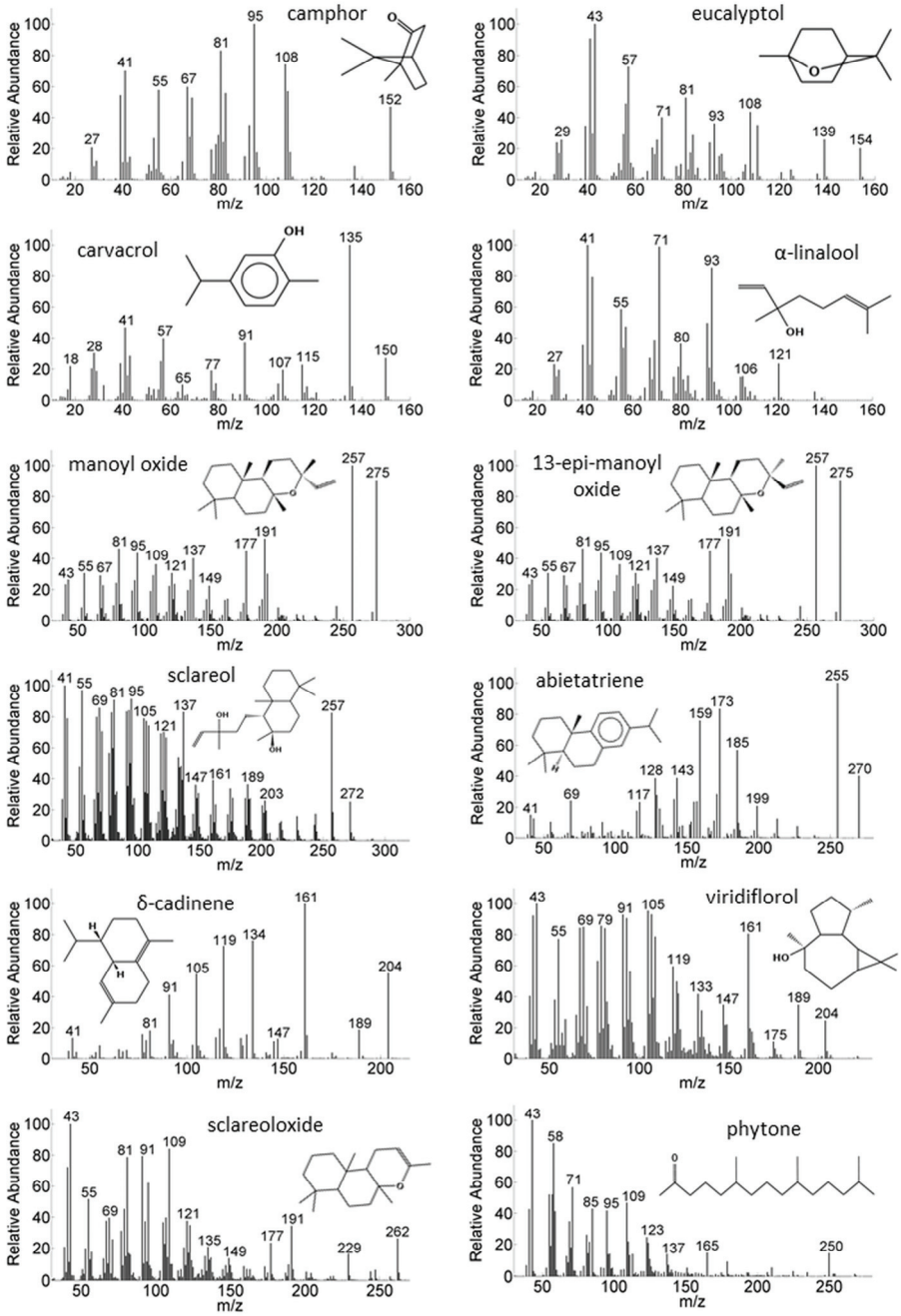

Fig. 1. Twelve most frequently occurring compounds identified in the individual *Cistus* samples with their respective structural formulas and mass spectra

independent of the country of origin of a given herb. In these circumstances, the commercial samples under the discussion could only be compared in terms of the number and the chemical nature of the identified volatile compounds.

Independent of the analytical method assumed, with each analyzed volatile fraction, the presence of the two compounds, namely, manoyl oxide and 13-epi-manoyl oxide, was established. These are the derivatives of the natural bicyclic diterpene labdane, which is the main component of the labdanum resin, characteristic substance excreted by the plants belonging to the *Cistus* genus [26, 27].

Moreover, with the majority of the investigated samples, the following compounds were also identified: eucalyptol (1,8-cineole), linalool, camphor, thymol, carvacrol, viridiflorol, δ-cadinen, sclareol, sclareoloxide, and phytone (hexahydrofarnesyl acetone). In *Fig. 1*, we show chemical structures of the twelve most frequently occurring components identified with the individual *Cistus* samples and their respective mass spectra.

In *Table III*, we divided all volatile fraction components identified with use of HS-GC–MS and GC–MS into the respective chemical classes. In the five samples of Turkish origin (T1–T5), the widest spectrum of the volatile compounds was found. Two of these samples (T3 and T4) proved as the "leaders," with 37 compounds identified in each of them by means of hydrodistillation followed by the GC–MS analysis. Using the HS-GC–MS procedure, in the same two samples, 21 and 16 compounds were identified, respectively. In the least abundant Turkish sample T2, 15 compounds only were identified. Among the four samples of Albanian origin (A1–A4), the contents of the volatile fraction were comparable, with 21 to 24 compounds identified by means of GC–MS, and 4 to 16 compounds identified by means of HS-GC–MS. In this group, the richest composition of the volatile fraction was observed with samples A1 and A3. Considering two samples, G1 and G2 of Greek origin, 27 and 18 compounds, respectively, were identified by means of GC–MS. In the sample ND1 (of an unknown origin), 24 and 7 compounds were identified, respectively, by means of GC–MS and HS-GC–MS.

If we compare the chemical composition of the three *C. incanus* L. samples with the highest numbers of the identified volatile compounds, i.e., T4 (Turkey), A3 (Albania), and G1 (Greece), certain common features can be observed. Based on the results obtained with use of the HS-GC–MS approach, in each respective chromatogram, the peak ascribed to carvacrol was the most intense one. Based on the results of the alternative approach (hydrodistillation followed by the GC–MS analysis) obtained for sample T4,

the most intense peaks were ascribed to manoyl oxide, 13-epi-manoyl oxide, 1,8-cineole, camphor, and – similar to the HS-GC–MS result – to carvacrol. With sample A3 analyzed with use of the alternative approach, the most intense peak originated from 13-epi-manoyl oxide, and the other intense peaks were ascribed – similar to sample T4 – to manoyl oxide, 1,8-cineole, camphor, and carvacrol. With sample G1 analyzed with use of the alternative approach, the most intense peak originated from 13-epi-manoyl oxide, but the other intense peaks also originated from 1,8-cineole, camphor, and carvacrol.

Table III. Classes of compounds identified in the *C. incanus* L. and *C. creticus* L. samples, including their availability with use of each analytical technique

Compound	Availability	
	GC–MS	HS-GC–MS
Monoterpene hydrocarbons		
Limonene	+	–
γ-Terpinene	+	–
Oxygenated monoterpenes		
1,8-Cineole	+	+
Linalool	+	+
Fenchone	+	–
Camphor	+	+
α-Terpineol	+	+
Safranal	+	–
(−)-Carvone	+	–
Phenolic compounds		
Estragole	+	–
Thymol	+	+
Carvacrol	+	+
Eugenol	+	+
Oxygenated sesquiterpenes		
Viridiflorol	+	–
Ledol	+	–
Cubenol	+	–
Caryophyllene oxide	+	–
Sesquiterpene hydrocarbons		
α-Ylangene	+	–
Caryophyllene	+	–
Aromadendrene	+	–
α-Selinene	+	–
α-Muurolene	-	+
δ-Cadinene	+	+
Calamenene	+	+
Calacorene	–	+

Table III. (continued)

Compound	Availability	
	GC–MS	HS-GC–MS
Himachalene	+	–
α-Vatirenene	+	–
Fatty acids and derivatives		
Nonanoic acid	+	–
n-Dodecanoic acid	+	–
Methyl tetradecanoate	–	+
Tetradecanoic acid	+	–
Methyl palmitate	+	+
Hexadecanoic acid	+	–
Diterpenes		
Phytol	–	+
Sclareoloxide	+	–
Dihydromanoyl oxide	+	–
Manoyl oxide	+	+
13-Epi-manoyl oxide	+	+
Sclareol	+	–
Abietatriene	+	–
Carbonylic compounds		
2-Ethylidene-6-methyl-3,5-heptadienal	–	+
3,4,4a,5,6,7-Hexahydro-1,1,4a-trimethyl-2(1*H*)-naphthalenone	+	+
Hexahydrofarnesyl acetone (phytone)	+	+
Farnesyl acetone	+	–
2-(2-Aceoxy-2,5,5,8a-tetramethyldecalin-1-yl)-acetic acid	+	–
Androsterone	+	–
Others		
Furfural	–	+
5-Methyl-2-furaldehyde	–	+
Dehydro-ar-ionene	–	+
1-Heptatriacontanol	+	–
(3α,5β,20S)-Pregnane-3,17,20-triol	+	–

Summing up, a considerable qualitative differentiation of the volatile fraction derived from the commercial *C. incanus* L. samples is observed and the country of origin of a given sample (Turkey, Albania, or Greece) by no means can be a criterion for their classification. To better illustrate this statement, we present the fingerprint gas chromatograms *(Figs. 2* and *3)* for twelve investigated samples. For example, two of the Turkish samples, T2 and T3, considerably differ with the composition of the volatile fraction. In essential oil extracted from sample T2 and analyzed by means of GC–MS, only 8 compounds were identified, and in essential oil extracted from T3, a

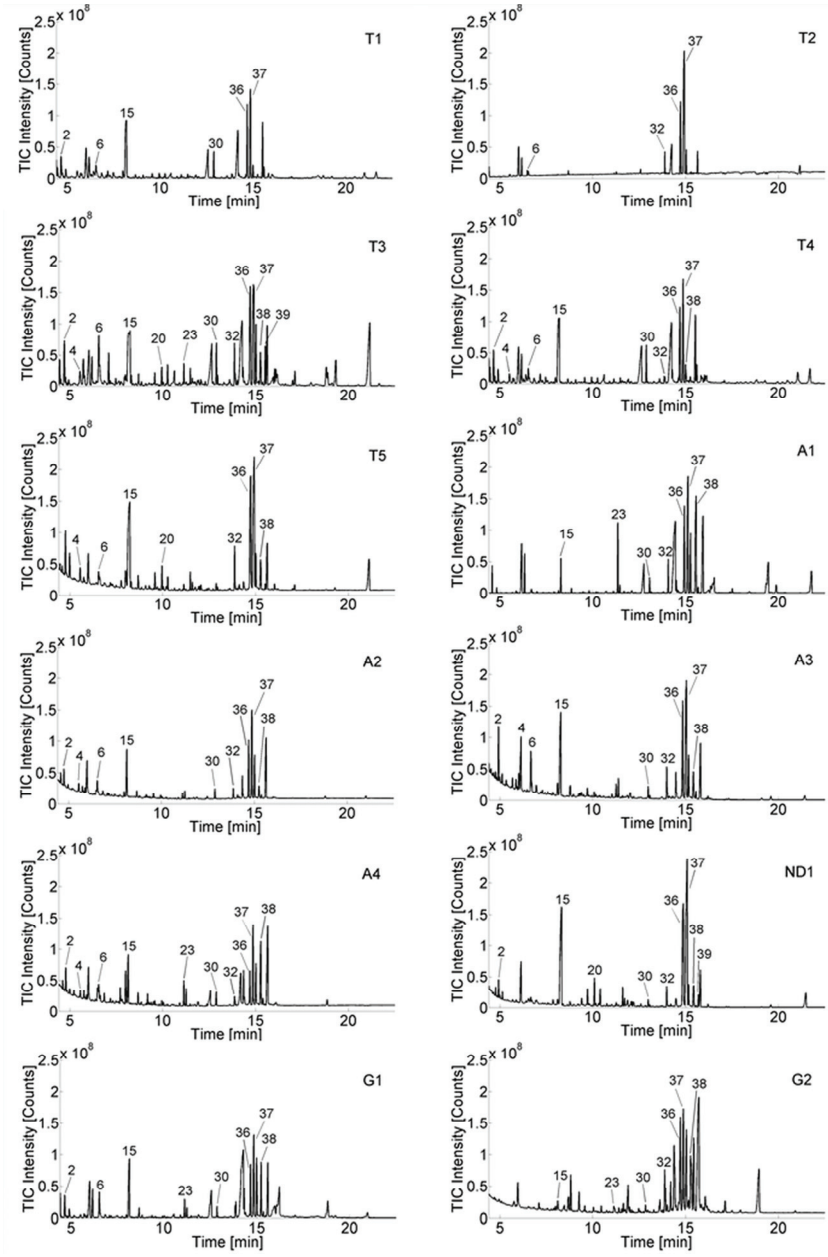

Fig. 2. Fingerprint gas chromatograms of the volatile fraction determined by means of GC–MS obtained for essential oils extracted from twelve *Cistus* samples. Selected peaks (i.e., most abundant and/or most frequently occurring) are labeled according to the numbering attributed to them in *Table I*

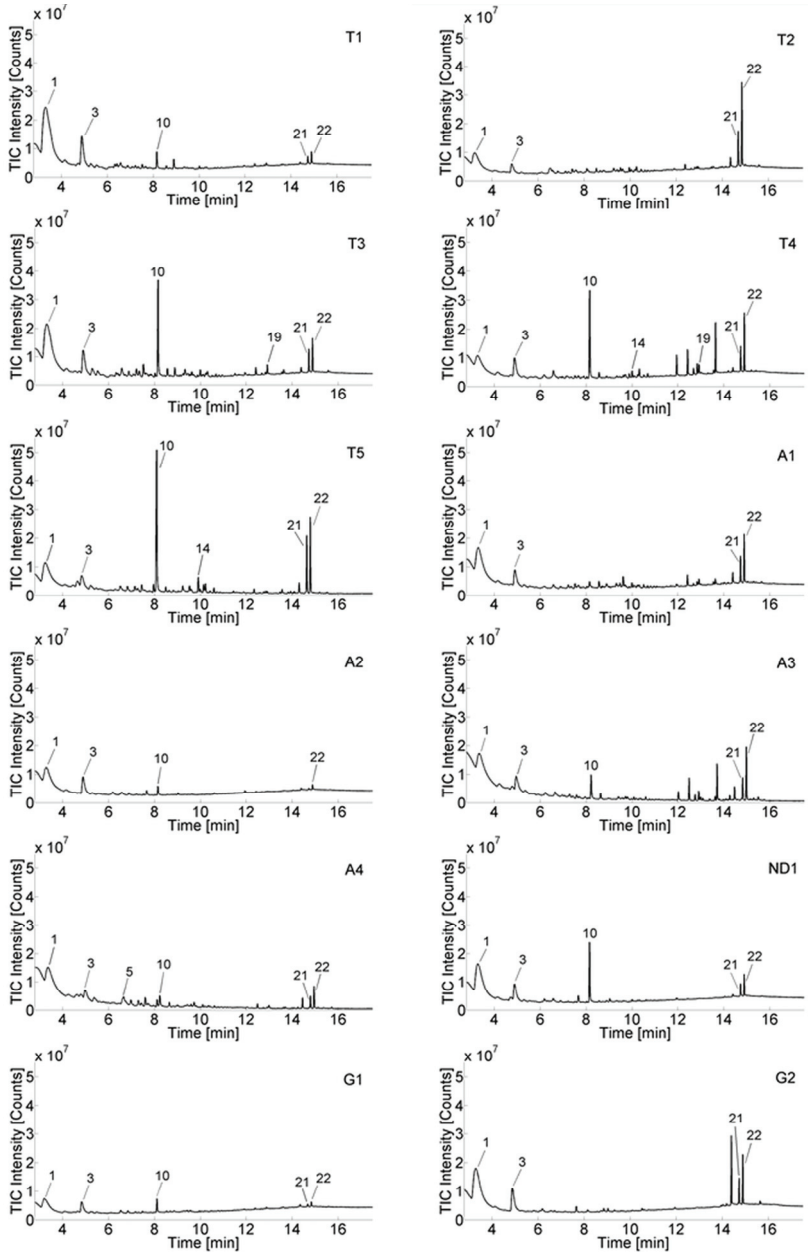

Fig. 3. Fingerprint gas chromatograms of the volatile fraction determined by means of HS-GC–MS obtained for essential oils extracted from twelve *Cistus* samples. Selected peaks (i.e., most abundant and/or most frequently occurring) are labeled according to the numbering attributed to them in *Table II*

wide spectrum of 37 compounds was found. Also, when applying an alternative analytical approach (HS-GC–MS), the number of identified compounds in sample T2 was lower than that in T3.

If we compare the two methods of isolating and identifying volatile compounds from the investigated herbal material, the simpler and faster HS-GC–MS approach proved as less efficient also, as its application resulted in identification of a lower number of volatile compounds, when compared with the hydrodistillation approach followed by the GC–MS analysis (with sample T2 being an only exception). For a number of investigated samples, some volatile compounds were identified, based on one analytical approach only. Using both analytical approaches, the sum of 51 volatile compounds were identified. Eight of these compounds were identified with use of HS-GC–MS only, 22 compounds were identified with use of the alternative approach only, and 14 compounds (1,8-cineole, linalool, camphor, α-terpineol, thymol, carvacrol, eugenol, δ-cadinene, calamenene, methyl tetradecanoate, hexahydrofarnesyl acetone (phytone), methyl palmitate, manoyl oxide, and 13-epi-manoyl oxide) were identified with use of the two analytical methods.

Difference in performance of the two analytical approaches demonstrated in this study could be, to a certain extent, due to a small addition of *o*-xylene to water at the hydrodistillation step, meant to help extracting some less volatile compounds also (like, e.g., hexadecanoic acid). The HS-GC–MS technique certainly allowed identification of the most volatile compounds, which could have been overlapped by the peak of *o*-xylene on the chromatograms of essential oils extracted from plant material by means of hydrodistillation. This can be an important reason of the differences in the chemical composition of the volatile fraction identified by means of the two assumed approaches.

Conclusion

Based on the obtained results, it was found out that the qualitative and quantitative composition of the volatile fraction of the investigated *Cistus* sp. samples significantly differed, depending on the origin of plant material and the manufacturer. Wide variation in terms of qualitative and quantitative composition of the marketed herbal products can result in their variable biological activity, or in extreme cases, even in a complete lack thereof. Thus, the proper and careful quality control and standardization of the raw herbal materials and herbal preparations should be carried out, in order to

maintain known and repeatable characteristics of plant preparations and, hence, the safety thereof. Chromatographic techniques used alone or together with other instrumental methods may prove successful with standardization and quality control of raw botanical materials and the finished herbal medicines. The two complementary techniques, hydrodistillation followed by GC–MS and HS-GC–MS, are very well suited for the extraction of sufficient information on the chemical composition of the volatile fraction of the herbal raw and processed materials.

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