Original Article

Volatiles of *Thymus serpyllum* Obtained by Three Different Methods

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Abstract

Volatiles of *Thymus serpyllum* L. were obtained by hydrodistillation (HD), simultaneous hydrodistillation, and extraction (SDE) and static headspace gas chromatography-mass spectrometry analysis (head space [HS]), respectively. Monoterpenes were the most dominant in all 3 techniques (84.8%-94.2%). Essential oil profiles obtained by HD and SDE were similar, with oxygenated monoterpenes being the most abundant (up to 75.4%). In HS volatiles of *T. serpyllum* monoterpene hydrocarbons strongly dominated (94.2%). The main compounds were α-terpinyl acetate (HD and SDE) and myrcene (HS).

Keywords

Thymus serpyllum, essential oil, hydrodistillation, extraction, volatiles, headspace

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Wild thyme (*Thymus serpyllum* L.) (family Lamiaceae) essential oil has antioxidative and antimicrobial properties, and also shows some cytotoxic effects. Besides, it is source of nutritional supplements or components of functional foods in the food industry ([1] and refs. cited therein). Numerous headspace techniques became frequent in the genus *Thymus*,[2-4] etc. Comparison of different techniques in plant species is increasingly used.[5]

Since the *Thymus* sp. essential oil is well examined, the presented study was based on differences in the 2 extraction techniques: HD (hydrodistillation) and SDE (simultaneous distillation and extraction) in comparison with HS (head space). The first 2 techniques require cooking plant material in the water whereby the processes of hydrolysis and thermal decomposition occur. In the HS technique there is no water and the material is shortly exposed to the high temperature, and therefore the most accurate picture of the chemical composition of essential oil is obtained.

The aim of this work is to compare effects of different techniques on chemical composition of volatiles of *Thymus serpyllum*. Thereby, special attention should be paid to HS volatiles, which have not been examined so far for this species. Chemical compounds of *T. serpyllum* obtained by HD, SDE, and HS techniques were summarized in Table 1. A total number of 35, 24, and 25 compounds out of 40, 26, and 28 were identified by HD, SDE, and HS method, respectively, *α*-Terpinyl acetate was the most abundant in HD essential oils and SDE extracts, and myrcene in HS volatiles. Other abundant chemical compounds were also bolded in Table 1.

Dominant terpenes of **HD** essential oil of *T. serpyllum* had following profile: *α*-terpinyl acetate >>>myrcene > *p-mentha*-1,(7),8(10)dien-9-ol > limonene, where >>> denotes differences more than 15% and >differences between 1.1% and 5.0%, after Petrakis et al.[6] Dominant terpenes of **SDE** extracts of *T. serpyllum* had the following profile: α-terpinyl acetate >>>myrcene > *p*-mentha-1,(7),8(10)dien-9-ol >> (*E*)-β-caryophyllene > limonene = 3-octanone. In **HS** volatiles myrcene dominated. In *T. serpyllum* very high similarity between **HD** and **SDE** techniques in terms of the abundance of the terpene classes as well as to the ratio: total monoterpenes/total sesquiterpenes was found. In the **HD** and **SDE** terpene profiles monoterpenes (especially oxygenated) strongly dominated. In **HS** volatiles oxygenated monoterpenes really dominated.

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Table 1. Volatile Compounds (In %) in the Aerial Parts of *T. serpyllum*.

Entry	Compound	RI	HD	SDE	HS
1	Hexanal	804			0.1
2	Tricyclene	921	tr	0.2	1.0
3	α-Thujene	925			0.2
4	α-Pinene	931	0.1	0.5	2.2
5	Camphene	946	0.3	1.0	3.4
6	Sabinene	971	0.3	0.1	0.4
7	β-Pinene	975	0.2	0.4	1.7
8	**3-Octanone**	981	**1.5**	**3.3**	**2.7**
9	**Myrcene**	989	**8.2**	**23.2**	**74.2**
10	Octan-3-ol	992	0.5	1.5	1.4
11	α-Terpinene	1015	tr		
12	*p*-Cymene	1023	0.2	0.2	0.7
13	**Limonene**	1026	**3.5**	**1.0**	**2.8**
14	γ-Terpinene	1056	0.2	0.3	0.8
15	(*Z*)-Sabinene hydrate	1065	tr	0.2	0.3
16	Terpinolene	1086	0.4		0.1
17	Linalool	1098	0.2	0.3	0.3
18	Unknown 1	1099	0.1		0.1
19	1-Octen-3-yl acetate	1109	0.7		0.1
20	3-Octanol acetate	1120	1.2		0.2
21	Unknown 2	1152	0.1		
22	**Borneol**	1161	**0.8**	**2.7**	**0.7**
23	Terpinen-4-ol	1174	0.1		
24	α-Terpineol	1187	0.9		
25	Unknown 3	1217	0.7	0.8	0.2
26	***p*-Mentha-1,(7),8(10)dien-9(ol)**	1223	**6.5**	**19.2**	**1.1**
27	Thymol methyl ether	1230	tr		
28	Bornyl acetate	1281	0.5		
29	Thymol	1289	0.2	0.2	0.1
30	Unknown 4	1302	0.4	0.3	0.1
31	**α-Terpinyl acetate**	1348	**66.2**	**35.3**	**4.2**
32	α-Copaene	1373	0.1		
33	β-Bourbonene	1382	0.1	0.1	
34	**(*E*)-β-Caryophyllene**	1417	**2.7**	**5.1**	**0.7**
35	α-Humulene	1451	0.1	0.1	
36	Germacrene D	1479	0.5	0.9	0.1
37	Bicyclogermacrene	1495	0.2	0.4	
38	**β-Bisabolene**	1508	**1.5**	**2.5**	**0.1**
39	δ-Cadinene	1523	tr		
40	Spathulenol	1575	0.1		
41	Caryophyllene oxide	1580	0.6	0.2	
42	Unknown 5	1634	0.1		
	Total		**100**	**100**	**100**

HD, hydrodistillation; HS, headspace; RT, retention index; SDE, simultaneous distillation and extraction.

Wild thymes from Serbia are also quite different from other at least 10 identified ones in Europe and far beyond it where: thymol,[7] carvacrol,[8] terpinene,[9] γ-terpinene + carvacrol,[10] geraniol,[11] myrcene, caryophyllene oxide, germacrene D, and borneol[12] and 2,4,6-trimethyl-anisol.[13] Furthermore, 5 more dominant compounds: 1,8-cineole, germacrene B, (*E*)-β-ocimene, α-cadinol and (*Z*)-*p*-menth-2-en-1-ol in 3 Indian varieties were found.[14] In the case of dominant (*E*)-nerolidol, beside oxygenated sesquiterpenes,[14] sometimes both sesquiterpene classes,[15] or both oxygenated monoterpenes and oxygenated sesquiterpenes[8] were found to dominate. Sometimes, content of terpenes decreases by prolonging the time of distillation.[16] Analyzed sample of *T. serpyllum* is generally quite similar in volatiles obtained by **HD** and **SD** techniques, respectively, but varies in **HS** volatiles. In **HD** and **SDE** terpene profiles of monoterpenes (especially oxygenated) strongly dominated. In **HS** volatiles monoterpene hydrocarbons strongly dominated (94.2%). The most abundant compounds were α-terpinyl acetate in **HD** and **SDE** techniques, and myrcene in **HS** volatiles.

Experimental

Plant Material

Fresh herbs (as bulk) were collected in September 2015 from South-West Serbia, Mt Zlatar, Gradina locality near the village Pravoševo (43°22′53″, 19°45′52″, elevation: 1320-1427 m). Voucher specimen (TH-SERP 1, 2015) was deposited in Institute of Forestry, Belgrade. Voucher was identified and brought by M. Matović. Natural habitats of this species was described.[17]

Extraction and Isolation

Three different methods of essential oil extraction from air dried above-ground parts of plants were used: (1) Hydrodistillation via Clevenger apparatus, (2) Simultaneous distillation and extraction with dichloromethane via Likens-Nickerson apparatus, and (3) Extraction via Static Headspace sampling apparatus.

Gas Chromatography-flame Ionization Detector and Gas Chromatography-mass Spectrometry Analyses

GC-FID and GC/MS analyses were carried out with an Agilent 7890A apparatus equipped with an 5975C MSD, FID, and a HP-5MSI fused-silica cap. col. 30 m × 0.25 mm × 0.25 μm). For HS analyses 2000 μL of generated vapor was drawn out from the vial and injected directly into the gas chromatograph using a heated gas-tight syringe (105°C). The oven temperature was programmed linearly rising from 60 to 315°C for 15 minutes; injector: 250°C; FID detect.: 300°C; carrier gas, He (1.0 mL/min at 210°C),

injection vol. 1 µL (for CL and LN) or 2 mL (for HS), split ratio,10:1. EI-MS (70 eV), *m*/z range 40 to 550.

Compound Identification

Identification of all compounds in analyses was matched by comparison of their linear retention indices (relative to C8-C36 *n*-alkanes on the HP-5MSI column) and MS spectra with those of authentic standards from NIST11 and home-made MS library data bases.

Declaration of Conflicting Interests

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