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EXPERIMENTAL PAPER

Comparison of chemical compositions of essential oils isolated by hydrodistillation from wild thyme (*Thymus serpyllum* L.) with use of Deryng and Clevenger apparatus

ANETA WESOŁOWSKA[1*], MONIKA GRZESZCZUK[2], DOROTA JADCZAK[2]

[1]Institute of Chemistry and Environmental Protection
West Pomeranian University of Technology
Aleja Piastów 42
71-065 Szczecin, Poland

[2]Department of Horticulture
West Pomeranian University of Technology
Słowackiego 17
71-434 Szczecin, Poland

[*]corresponding author e-mail: anetaw@zut.edu.pl

Summary

The aim of the studies conducted in 2012-2013 was to compare the chemical composition of essential oils isolated from wild thyme (*Thymus serpyllum* L.) by hydrodistillation in Deryng and Clevenger apparatus. GC-MS analysis of the isolated oils revealed that carvacrol (42.81–45.24%), γ-terpinene (7.68–9.04%), β-caryophyllene (5.28-9.10%), β-bisabolene (5.76–6.91%) and carvacrol methyl ether (4.92–6.09%) were the major components of all the samples.
On the basis of the obtained data it was proved that the type of distillation apparatus had no significant effect on the content of the main essential oil constituents of wild thyme. However, based on the means for both years of the study it was proved that hydrodistillation in Deryng apparatus was more effective for carvacrol concentration, while in Clevenger apparatus – for γ-terpinene and carvacrol methyl ether concentration. The type of distillation apparatus had no significant effect on the content of the other essential oil constituents.

Key words: *wild thyme, hydrodistillation, essential oil composition, carvacrol, γ-terpinene, β-caryophyllene*

INTRODUCTION

Wild thyme (*Thymus serpyllum* L.) an aromatic plant belonging to the *Lamiaceae* family, is well known for its biological and pharmacological properties [1]. *Serpylli herba*, which possesses antiseptic, diaphoretic, analgesic, antitussive and spasmolytic activity, is considered as an excellent drug for the preparation of herbal baths and herbal pillows [2-4]. Tea made of aromatic leaves is good against nervous disorders and excellent against cramps [5].

The herb infusion is reputed as useful in the treatment of alcoholism, while herb decoction is usually applied for easing the whooping cough, spasms and to prevent the hair loss [3,6].

Serpylli herba is also used as a source of essential oil. The oil obtained from the leaves and flowering parts of the plant exhibit antiseptic, anthelmintic, carminative and anti-fungal activity [7-9]. *Serpylli aetheroleum* can relieve rheumatism, gout and sciatica. It is used medicinally and in the manufacturing of toothpastes, mouthwashes, gargles and other toilet preparations [3,4,10].

The method commonly applied for the isolation of essential oil from different parts of the plant material is hydrodistillation [11]. The predominant distillation apparatus used all over the world for many years is Clevenger apparatus [12]. This type of apparatus is also recommended by Polish Pharmacopoeia since volume VII [13] was published. However, Deryng apparatus, recommended by Polish Pharmacopoeia earlier than volume VI [14], has been still in use in Poland for essential oils extraction.

The aim of this study was to compare the chemical composition of essential oils isolated by hydrodistillation in Deryng and Clevenger apparatus from wild thyme herb (*Thymus serpyllum* L.) cultivated in North-Western Poland in 2012–2013.

MATERIAL AND METHODS

Plant

The plants (*Thymus serpyllum* L.) were grown in experimental plots with an area of 1.44 m^2, in four replications at the Horticultural Experimental Station in Dołuje near Szczecin. The seedlings, obtained from older plants, after rooting in horticultural substrate, were planted into the open field in the second half of May in the 2010 and 2011, with spacing of 20×20 cm. For laboratory analyses, a herb from two-year-old plants was harvested at the flowering stage (harvest dates: July 24, 2012 and August 26, 2013). The field was prepared according to agrotechnique proper for thyme cultivation. Mineral fertilization was quantified according to the results of the chemical analysis of the soil samples and supplemented to those recommended for thyme level. In the first year of the experiment only nitrogen and potassium fertilization was applied, respectively: 60 g N and 60 g K_2O per 10 m^2, while in the second year – potassium (60 g K_2O per 10 m^2) and phosphorus (60

kg P_2O_5 per 10 m^2) fertilization. The experiment was performed on sandy clay soil characterized by low water-holding capacity.

The plant material was dried in a shady and well ventilated place at a room temperature (20–25°C) for essential oil isolation.

Isolation of the essential oil

Fifty grams of the whole dried aerial parts of wild thyme (*Serpylli herba*) in a 1000 ml round-bottom flask along with 500 ml of distilled water was subjected to hydrodistillation for three hours using Deryng apparatus according to the method recommended by Polish Pharmacopoeia VI [14].

The hydrodistillation was also performed with use of Clevenger apparatus for 2 hours according to the method recommended by Polish Pharmacopoeia VIII [15]. Extracted essential oils were dried over anhydrous sodium sulphate, filtered, weighed and stored in dark sealed vial at 4°C until GC-MS analysis was performed.

Tree replicates were carried out. Essential oil percentage was calculated based on dry weight of plant material and expressed as (% w/w) in table 1.

Table 1.

Essential oil content in *Thymus serpyllum* L. cultivated in North-Western Poland in 2012–2013

Distillation apparatus	Essential oil content (% w/w)		
	2012	2013	Mean
Deryng	1.10	1.10	1.10
Clevenger	1.00	1.00	1.00
Mean	1.05	1.05	1.05
$LSD_{\alpha=0.05}$	n.s.	n.s.	n.s.

n.s. – not significant

GC-MS analysis

The qualitative GC-MS analysis of the oils was carried out using an HP 6890 gas chromatograph, equipped with a HP-5MS ((5%)-phenyl-methylpolysiloxane) capillary column (30 m x 0.25 mm, film thickness 0.25 μm) and coupled with HP 5973N Mass Selective Detector. Helium (1 ml/min) was used as a carrier gas. Samples of 2 μl (30 mg of oil dissolved in 1.5 ml of methylene chloride) were injected in the split mode at a ratio of 5:1. The injector and the transfer line were kept at 280°C. The ion source temperature was 230°C.

The initial temperature of the column was 40°C for 5 minutes, then increased to 60°C at a rate of 30°C/min, next to 230°C at a rate of 6°C/min (kept constant for

10 min), and then increased to a final temperature of 280°C at a rate of 30°C/min. The oven was held at this temperature for 5 minutes. Mass spectra were taken at 70 eV. Mass range was from 40 to 550 m/z. Solvent delay time was 4 min. The total running time for a sample was about 51 minutes.

The relative percentage of the essential oil constituents was evaluated from the total peak area (TIC) by apparatus software.

Compound identification

The components of essential oils were identified by comparison of their mass spectra with those stored in NIST 2002 and Wiley NBS75K.L mass spectral libraries or with authentic compounds (thymol, carvacrol, p-cymene) available in our laboratory and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [16,17].

The retention indices (RI) were calculated for all volatile constituents using a homologous series of n-alkanes (C_7-C_{40}) under the same chromatographic conditions which were used for the analysis of essential oils.

Chemicals

Dichloromethane (pure p.a.) was purchased from Chempur and used after distillation.

Thymol purum, ≥99.0% (GC) and carvacrol purum, ≥ 99.5% (GC) were purchased from Fluka. *p*-Cymene (99%) was purchased from Aldrich.

Statistical analysis

Several results of the study (tab. 1, 3) were subjected to the analysis of variance which was performed with AWAR software (Department of Applied Computer Science, Institute of Soil Science and Plant Cultivation, Puławy, Poland). The means of each year were separated by the Tukey's test at $p=0.05$. The statistical analysis was conducted for these constituents which contained more than 1% of essential oil (tab. 3).

RESULTS AND DISCUSSION

The content of essential oil in wild thyme (*Serpylli herba*) determined by hydrodistillation using two types of distillation apparatus is shown in table 1.

Table 2.

Relative percentage composition of essential oil of wild thyme (*Thymus serpyllum* L.) in dependence on the type of distillation apparatus in 2012-2013

Components	RI	Deryng		Clevenger	
		2012	2013	2012	2013
α-Thujene	927	0.52	0.75	0.69	0.66
α-Pinene	933	0.25	0.40	0.32	0.37
β-Terpinene	973	0.05	0.25	0.05	0.21
β-Pinene	976	0.18	0.29	0.20	0.28
1-Octen-3-ol	981	1.50	1.70	1.55	1.55
3-Octanone	985	0.17	–	0.25	–
β-Myrcene	991	0.91	1.17	1.07	1.13
3-Octanol	996	0.54	0.30	0.83	0.31
α-Phellandrene	1004	0.14	0.23	0.19	0.24
δ-3-Carene	1010	0.05	0.13	0.07	0.16
α-Terpinene	1016	1.06	1.24	1.30	1.26
p-Cymene	1026	3.46	3.30	4.10	3.54
1,8-Cineole	1032	2.25	3.36	2.29	3.65
(Z)-*β*-Ocimene	1039	0.61	1.09	0.72	1.13
(E)-*β*-Ocimene	1049	3.32	4.43	3.38	4.30
γ-Terpinene	1061	7.68	8.90	9.04	8.86
cis-Sabinene hydrate	1068	0.35	1.31	0.46	1.53
α-Terpinolene	1089	0.10	0.21	0.12	0.25
Linalool	1100	0.38	0.48	0.42	0.51
Borneol	1171	0.16	0.21	0.11	0.22
Terpinen-4-ol	1181	0.41	0.30	0.46	0.36
α-Terpineol	1195	0.25	0.46	0.36	0.50
Thymol methyl ether	1237	0.08	0.07	0.09	0.08
Carvacrol methyl ether	1248	5.59	4.92	6.09	5.61
Thymol	1300	0.18	0.12	0.17	0.12
Carvacrol	1315	45.24	44.45	43.18	42.81
α-Copaene	1379	0.11	0.13	0.09	0.19
β-Bourbonene	1392	0.15	0.18	0.13	0.23
β-Caryophyllene	1429	9.10	5.28	8.43	5.30
β-Cubebene	1437	0.21	0.12	0.23	0.13
α-Bergamotene	1444	0.07	0.11	0.15	0.13
trans-*β*-Farnesene	1458	0.11	0.14	0.11	0.14
α-Caryophyllene	1462	0.40	0.28	0.43	0.29

Components	RI	Deryng		Clevenger	
		2012	2013	2012	2013
γ-Muurolene	1484	0.56	0.05	0.51	0.08
Germacrene D	1489	1.56	3.05	1.27	2.89
Bicyclogermacrene	1507	0.12	0.09	0.43	0.09
β-Bisabolene	1515	6.72	6.67	5.76	6.91
γ-Cadinene	1521	0.49	-	0.43	-
δ-Cadinene	1529	1.02	0.44	0.86	0.48
cis-α-Bisabolene	1546	0.18	0.12	0.10	0.13
Caryophyllene oxide	1595	0.86	0.48	0.93	0.59
α-Cadinol	1663	0.17	0.13	0.18	0.13
Octadecanal	2045	0.18	0.60	0.13	0.58
2-Methyleicosane	2060	0.45	0.68	0.33	0.61
3-Methyleicosane	2075	0.21	0.39	0.14	0.35
Nonadecanal	2126	0.42	0.53	0.26	0.51
Heptacosane	2700	0.10	0.11	0.06	0.06
Nonacosane	2900	0.22	0.15	0.12	0.09
Total identified		94.84	99.11	98.59	99.55

RI: retention indices relative to n-alkanes (C7-C40) on the HP-5MS column

Table 3.

Statistical analysis of the content of some constituents of essential oil of wild thyme (*Thymus serpyllum* L.) in dependence on the type of distillation apparatus

Essential oil constituent (*factor I*)	Distillation apparatus (*factor II*)								
	2012			2013			2012–2013		
	Deryng	Clevenger	Mean	Deryng	Clevenger	Mean	Deryng	Clevenger	Mean
1-Octen-3-ol	1.50	1.55	1.53	1.70	1.55	1.62	1.60	1.55	1.58
β-Myrcene	0.91	1.07	0.99	1.17	1.13	1.15	1.04	1.10	1.07
α-Terpinene	1.06	1.30	1.18	1.24	1.26	1.25	1.15	1.28	1.22
p-Cymene	3.46	4.10	3.78	3.30	3.54	3.42	3.38	3.82	3.60
1,8-Cineole	2.25	2.29	2.27	3.36	3.65	3.51	2.81	2.97	2.89
(Z)-β-Ocimene	0.61	0.72	0.66	1.09	1.13	1.11	0.85	0.93	0.89
(E)-β-Ocimene	3.32	3.38	3.35	4.43	4.30	4.36	3.88	3.84	3.86
γ-Terpinene	7.68	9.04	8.36	8.90	8.86	8.88	8.29	8.95	8.62
cis-Sabinene hydrate	0.35	0.46	0.41	1.31	1.53	1.42	0.83	1.00	0.91
Carvacrol methyl ether	5.59	6.09	5.84	4.92	5.61	5.26	5.26	5.85	5.55
Carvacrol	45.24	43.18	44.21	44.45	42.81	43.63	44.85	43.00	43.92
β-Caryophyllene	9.10	8.43	8.76	5.28	5.30	5.29	7.19	6.87	7.03

Essential oil constituent (*factor I*)	Distillation apparatus (*factor II*)								
	2012			2013			2012–2013		
	Deryng	Clevenger	Mean	Deryng	Clevenger	Mean	Deryng	Clevenger	Mean
Germacrene D	1.56	1.27	1.42	3.05	2.89	2.97	2.31	2.08	2.19
β-Bisabolene	6.72	5.76	6.24	6.67	6.91	6.79	6.70	6.34	6.52
δ-Cadinene	1.02	0.86	0.94	0.44	0.48	0.46	0.73	0.67	0.70
Mean	6.02	5.97	5.99	6.09	6.06	6.07	6.06	6.02	6.04
$LSD_{\alpha=0.05}$ for factor I			1.227			0.278			0.574
$LSD_{\alpha=0.05}$ for factor II			n.s.			n.s.			n.s.
$LSD_{\alpha=0.05}$ for interaction I´II			0.848			0.425			0.450

n.s. – not significant

The presented data indicate that the type of distillation apparatus had no significant effect on the content of essential oil which average amount was 1.05%.

The content of volatile oil in *Serpylli herba* depends on the origin of the plants and varied between 0.1 and 0.6% [18]. In India, content ranged from 0.50 to 0.67% in Kyle and Kashmir regions, respectively [19,20], while in Iran, Pakistan and Lithuania it was reported to be 0.95, 0.48 and 0.12-0.27%, respectively [21,10,22]. According to Pióro-Jabrucka and Osińska [23], the oil content among *T. serpyllum* populations in Poland, varied in the range of 0.21–0.60%.

Our results indicate that the content of essential oil in wild thyme cultivated in North-Western Poland was higher than the values reported in other regions worldwide. However, *T. serpyllum* grown at different geographical regions in Jordan (Jeresh in the North and Al-Karak, Al-Aqaba and Al-Shouback in the South) had higher oil content (2.5–5.6%) [24], as compared to our plants.

The relative amounts of the volatile components identified in the essential oils are listed in table 2, in order of their elution from a HP-5MS column.

In total, 48 different compounds were identified in the essential oils of *T. serpyllum* obtained by hydrodistillation in Deryng and Clevenger apparatus, that represented 98.59–99.55% of the oils.

The main components identified in the oil obtained in Deryng apparatus were carvacrol (45.24 and 44.45% in 2012 and 2013, respectively), *β*-caryophyllene (9.10 and 5.28%), *γ*-terpinene (7.68 and 8.90%), *β*-bisabolene (6.72 and 6.67%) and carvacrol methyl ether (5.59 and 4.92%).

Carvacrol (43.18 and 42.81% in 2012 and 2013, respectively) was a predominant component in the oil obtained in Clevenger apparatus. Other abundant constituents were *γ*-terpinene (9.04 and 8.86%), *β*-caryophyllene (8.43 and 5.30%), carvacrol methyl ether (6.09 and 5.61%) and *β*-bisabolene (5.76 and 6.91%).

Generally, the major components identified in both oils were the same. However, the highest amounts of carvacrol (45.24 and 44.45% in 2012 and 2013, respectively) were observed in the oil obtained in Deryng apparatus, while the highest

concentrations of γ-terpinene (9.04 and 8.86%) and carvacrol methyl ether (6.09 and 5.61%) were detected in the oil obtained in Clevenger apparatus.

On the base of the results of statistical analysis it was proved that among the main essential oil constituents of wild thyme herb carvacrol was characterized by the highest concentration (tab. 3).

High content was also found for γ-terpinene, β-caryophyllene, β-bisabolene and carvacrol methyl ether while the least for: α-terpinene, β-myrcene, cis-sabinene hydrate, (Z)-β-ocimene and δ-cadinene. The type of distillation apparatus had no significant effect on the content of the main essential oil constituents of wild thyme. However, the interaction between essential oil constituent and distillation method was statistically significant. It was proved, that in the first year of the study hydrodistillation in Deryng apparatus was more effective according to the concentration of carvacrol and β-bisabolene while in Clevenger apparatus – according to the concentration of γ-terpinene. In the second year of the study hydrodistillation in Deryng apparatus was more effective also for carvacrol concentration while in Clevenger apparatus – for carvacrol methyl ether. Based on the means for both years of the study, it was proved that hydrodistillation in Deryng apparatus was more effective for carvacrol concentration while in Clevenger apparatus – for γ-terpinene and carvacrol methyl ether concentration. On the content of the other essential oil constituents, the type of distillation apparatus had no significant effect.

A literature search showed remarkable differences in the essential oil composition of wild thyme from different origins.

In the essential oil from *T. serpyllum* growing wild in Estonia, (E)-nerolidol (1.7–70.1%), caryophyllene oxide (1.4–45.0%), β-myrcene (tr.–20.2%), β-caryophyllene (1.8–13.3%) and germacrene D (1.7–12.5%) [25] were the major components, while the oil from plants collected in Lithuania, contained mainly 1,8-cineole (0.7–30.3%), (E)-β-ocimene (0.7–34.8%), borneol (0.1–27.1%), (Z)-β-ocimene (0.1-20.0%) and β-myrcene (0.1–16.9%) [16]. Similarly, *T. serpyllum* populations growing wild in Central Poland were characterized by the presence of camphene (8.07–13.91%), β-myrcene (6.53–17.97%), 1,8-cineole (1.41–11.64%), β-caryophyllene (1.06–9.02%) and borneol (0.15–16.91%) [26] in the essential oils. In contrast, the major constituents noted in the oil from *T. serpyllum* growing wild in the western part of Iran (Lorestan area), were carvacrol (14.94%), α-pinene (12.2%), thymol (7.39%) and p-cymene (2.54%) [27]. Thymol (53.33%), carvacrol (10.4%), p-cymene (8.8%), δ-3-carene (5.1%) and camphor (4.9%) dominated in the oil from Pakistan [10]. Wild thyme grown in Kumaon region of Western Himalaya (India), contained thymol (19.4–60.1%), γ-terpinene (0.3–13.8%) and p-cymene (3.5–10.4%) as major oil components [28]. Thymol (46.24–74.92%) and carvacrol (4.69–7.19%) rich essential oils were also isolated from plants collected in Western Nepal and North India [29].

Wild thyme (*Thymus serpyllum* L.) cultivated in North-Western Poland contained mainly carvacrol (42.81–45.24%), γ-terpinene (7.68–9.04%), β-caryophyllene (5.28–9.10%) and β-bisabolene (5.76–6.91%) in the volatile oil. Moreover, the highest concentration of carvacrol (44.45–45.24%) was noted in the oil obtained by hydrodistillation in Deryng apparatus.

Our oils were poor in thymol (0.12–0.18%), compared to that from Pakistan [10], Iran [27] and India [28, 29], but rich in carvacrol. According to literature data [30], essential oils carvacrol containing showed strong antioxidant activity. Based on these findings, it can be stated that oils obtained from wild thyme plants cultivated in North-Western Poland may find wide applications as antioxidant agents in food industry.

CONCLUSIONS

1. Total of 48 components were identified in the essential oil of wild thyme isolated by hydrodistillation in Deryng and Clevenger apparatus.
2. The type of distillation apparatus had no significant effect on essential oil content as well as on the content of the main essential oil constituents.
3. There was a significant interaction between the main essential oil constituents and distillation apparatus found. Hydrodistillation in Deryng apparatus was more effective for carvacrol concentration, while in Clevenger apparatus – for γ-terpinene and carvacrol methyl ether concentration.

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PORÓWNANIE SKŁADU CHEMICZNEGO OLEJKÓW ETERYCZNYCH WYIZOLOWANYCH POPRZEZ HYDRODESTYLACJĘ Z MACIERZANKI PIASKOWEJ (*THYMUS SERPYLLUM* L.) Z WYKORZYSTANIEM APARATU DERYNGA I CLEVENGERA

ANETA WESOŁOWSKA[1*], MONIKA GRZESZCZUK[2], DOROTA JADCZAK[2]

[1]Instytut Chemii i Podstaw Ochrony Środowiska
Zachodniopomorski Uniwersytet Technologiczny
Aleja Piastów 42
71-065 Szczecin

[2]Katedra Ogrodnictwa
Zachodniopomorski Uniwersytet Technologiczny
ul. Słowackiego 17
71-434 Szczecin

[*]autor, do którego należy kierować korespondencję: e-mail: anetaw@zut.edu.pl

S t r e s z c z e n i e

Celem badań prowadzonych w latach 2012–2013 było porównanie składu chemicznego olejków eterycznych wyizolowanych z macierzanki piaskowej (*Thymus serpyllum* L.) poprzez hydrodestylację w aparacie Derynga i Clevengera. Analiza GC-MS otrzymanych olejków wykazała, że karwakrol (42,81–45,24%), *γ*-terpinen (7,68–9,04%), *β*-kariofilen (5,30–9,10%), *β*-bisabolen (5,76–6,91%) i eter metylowy karwakrolu (4,92–6,09%) stanowiły dominujące składniki wszystkich analizowanych próbek. Na podstawie uzyskanych wyników wykazano, że typ wykorzystanego do destylacji aparatu nie miał istotnego wpływu na zawartość głównych składników w olejku z macierzanki piaskowej. Aczkolwiek, na podstawie analizy średnich z dwóch lat badań wykazano, że hydrodestylacja w aparacie Derynga była bardziej efektywna dla zawartości karwakrolu w olejku, natomiast hydrodestylacja w aparacie Clevengera – dla zawartości *γ*-terpinenu i eteru metylowego karwakrolu. Na zawartość pozostałych składników w olejku, typ zastosowanego aparatu nie miał istotnego wpływu.

Słowa kluczowe: *macierzanka piaskowa, hydrodestylacja, skład olejku eterycznego, karwakrol, γ-terpinen, β-kariofilen*