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EFFECTIVENESS OF THE DERYNG AND CLEVENGER-TYPE APPARATUS IN ISOLATION OF VARIOUS TYPES OF COMPONENTS OF ESSENTIAL OIL FROM THE *MUTELINA PURPUREA* THELL. FLOWERS

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Abstract: In this study, both qualitative and quantitative analyses of chemical composition of *M. purpurea* essential oil obtained in the Deryng and Clevenger-type apparatuses were compared. As a result, content of volatile compounds were: 785.67 mg/mL and 833.33 mg/mL in the oil obtained in the Deryng (D-EO) and Clevenger-type apparatuses (C-EO), respectively. The major components of both essential oils from *M. purpurea* were: a-pinene, sabinene, myrcene, (Z)-sesquisabinene hydrate, (E)-sesquisabinene hydrate, and a-bisabolol. The correlation coefficients values are not determined by the differences in the concentrations of the components resulting from the application of two different methods of distillation.

Keywords: essential oil, hydrodistillation, Deryng apparatus, Clevenger-type apparatus, *Mutellina purpurea*

Plant extracts, especially essential oils, have been employed in pharmaceutical, agronomic, food, cosmetic, and perfume industries due to several reported biological properties. From among 3000, approximately 300 isolated oils are commonly used. The main methods to obtain essential oils from the plant materials are steam or water distillation, cold expression or dry distillation. (1-3).

The composition of essential oil (EO), even within one specie, depends on many factors such as growing conditions, time and place of the collection of raw material, drying and storage. The method of determination also has an impact on the qualitative and quantitative analysis of chemical compounds present in the tested EO. Methods for assessing the composition of essential oils can be broadly divided into two research directions. The solid phase microextraction (SPME) method analyzes the composition of the volatile compounds around the plant (4, 5). SPME is a preliminary analysis that requires further action in order to obtain the essential oil in the form that can be applied. The most common method for obtaining the essential oil from the plant material is distillation. Most often it is carried out using glass Clevenger-type or Deryng apparatus. Both devices are recommended pharmacopoeial apparatuses for determining the essential oil content, the former one is described by the European Pharmacopoeia (6) and the latter by the Polish Pharmacopoeia (7).

The volatile composition of the essential oils from different parts of the *Mutelins purpurea* Thell. has been studied earlier (8, 9). However, there is no detailed information about the effect of chosen distillation apparatus on types of isolated compounds. The objective of this study was to compare the effectiveness of Deryng and Clevenger-type apparatus in isolation of various types of components of essential oil from the *Mutelins purpurea* Thell. flowers.

The present research is a continuation of studies comparing essential oils extraction in different

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apparatuses. In an earlier work it was shown that there are differences in the percentage of the chemical composition in the essential oils of sage (10). As the authors found, these differences may result from different times of EO distillation in the Deryng and Clevenger-type apparatuses. In the present paper, the same distillation time was applied to obtain the *M. purpurea* EO in both apparatuses. This could have an impact on the content of the identified compounds, as was evidenced by the high correlation coefficients.

EXPERIMENTAL

Plant material

M. purpurea flowers were collected in the Botanical Garden of the Medical University in Lublin in June 2010. The voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Medical University in Lublin (ES032011M).

Isolation procedure

The fresh plant material (50.0 g) was placed in a round-bottomed flask and 500 mL distilled water was added. Hydrodistillation was performed simultaneously for 3 h by means of the Deryng apparatus and the Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulfate and stored at 4°C before the GC analysis. Analyses were repeated three times.

Chemical analysis of essential oil

GC/MS and GC/FID conditions

The gas chromatograph Varian 450-GC with the type triple quadrupole Varian 320-MS was used. The analytes were separated on a 30 m × 0.25 mm VF-5ms capillary column coated with a 0.25 μm film of 5% phenyl methylpolysiloxane, and were inserted directly into the ion source of the MS. The split injection 1 : 100 was used for the samples. The column oven temperature was programmed at 4°C/min from an initial temperature of 50°C (held for 1 min) to 250°C, which was held for 10 min. The injection temperature was 250°C and the injection volume was 1 μL. Helium (99.999%) was used as carrier gas at a flow rate of 0.5 mL/min. The ionizing electron energy was 70 eV and the mass range scanned was 40-1000 m/z with 0.8 s/scan. Manifold temp. was 45°C, transfer line temp. was 289.5°C and the ion source temp. was 271.2°C.

The range of concentrations of constituents outlier in this group GC Varian 3800 (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm DB-5 column (J&W Scientific, USA), film thickness 0.25 μm, carrier gas - helium 0.5 mL/min, injector and detector FID temperatures of 260°C; split ratio 1 : 100; injection volume 5 μL. A temperature gradient was applied (50°C for 1 min, then incremented by 4°C/min to 250°C, 250°C for 10 min).

Qualitative and quantitative analysis of essential oils

The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra of the NIST/EPA/NIH Mass Spectral Library Search Program (11) and with the data available in the literature (12, 13). The identity of the compounds was confirmed by their retention indices (14), taken from the literature (12, 13) and our own data for standards (α-pinene, *p*-cymene, limonene, γ-terpinene, linalool, (*E*)-caryophyllene, caryophyllene oxide).

The quantitative analysis was performed by means of the internal standard addition method (alkanes C_{12} and C_{19}) according to previously described procedures (15). Essential oil was diluted 1000 times using n-hexane to achieve 1 mL volume, then 1 mg of C_{12}, and 1 mg C_{19} was added into the diluted oil. Samples so prepared were subjected to GC-MS and GC-FID determinations. The quantitative analysis was performed on the basis of calibration curves plotted to find the dependence between the ratio of the peak area for the analyte to the area for internal standard ($A_{analyte} : A_{i.s.}$) *vs*. the analyte concentration ($C_{analyte}$), for α-pinene, *p*-cymene, limonene, γ-terpinene, linalool, (*E*)-caryophyllene, caryophyllene oxide, in the appropriate concentration range (15). The following alkanes were applied as internal standards: C12 (for compounds with retention index < 1300, α-pinene, *p*-cymene, γ-terpinene, linalool); and C19 (for compounds with retention index > 1300, *(E)*-caryophyllene, caryophyllene oxide). The contents of the analyzed substances were read from the achieved calibration curves, the data for which originated from peak areas for *M. purpurea* oil components and internal standard peak areas from GC separation. The final result took into account all dilutions during the whole analytical procedure.

Statistical analysis

All calculations were done using a Statistica 7.1 (StatSoft®, Kraków, Poland) software. Average values were calculated. The results are expressed as the mean ± SD. The chemical composition of the oils was evaluated by Wilcoxon signed-rank test and the sign test.

Table 1. Comparison of the composition of *M. purpurea* flowers essential oil obtained by means of the Deryng and Clevenger's type apparatus.

Compound	RI_{exp}[1]	Concentration in the oil[2] (mg/mL)			
		DERYNG	+/- SD	CLEVENGER	+/- SD
Monoterpenes					
α-thujene	932	1.16	0.25	1.49	0.28
α-pinene	939	76.60	3.40	105.83	2.65
camphene	954	8.58	0.32	11.01	0.16
sabinene	975	189.90	6.33	161.11	4.53
β-pinene	980	13.83	0.96	12.56	0.16
myrcene	989	70.16	1.54	65.76	1.28
limonene	1028	26.26	2.27	30.06	0.59
β-phellandrene	1030	9.76	0.82	4.56	0.12
(*Z*)-b-ocimene	1033	7.58	0.36	8.10	0.18
(*E*)-b-ocimene	1044	18.25	0.26	18.78	0.02
γ-terpinene	1056	10.84	0.11	4.04	0.02
terpinolene	1082	2.41	0.02	1.19	0.10
Total		435.33		424.49	
Oxygenated monotrpenes					
(*Z*)-sabinene hydrate	1068	1.69	0.07	1.40	0.07
linalool	1095	1.00	0.14	1.39	0.00
(*E*) sabinene hydrate	1098	1.11	0.11	1.27	0.00
(*Z*)-p-menth-2-en-1-ol	1122	0.76	0.12	1.05	0.06
terpinen-4-ol	1182	12.89	0.87	6.21	0.48
carvacrol methyl ether	1242	1.88	0.40	1.06	0.02
lavandulyl acetate	1287	1.66	0.05	1.32	0.01
bornyl acetate	1290	4.61	0.20	2.64	0.14
Total		25.60		16.34	
Sesquiterpenes					
β-elemene	1393	19.73	2.84	15.51	0.35
(*E*)-caryophyllene	1427	11.43	0.06	20.28	0.71
γ-elemene	1436	0.28	0.04	0.65	0.13
(*E*)-α-bergamotene	1440	0.12	0.02	0.31	0.01
(Z)-β-farnesene	1460	5.20	0.24	6.03	0.28
α-humulene	1467	1.01	0.16	1.52	0.16
β-acoradiene	1489	0.50	0.16	0.59	0.07
germacrene D	1495	11.50	0.20	20.00	0.93
α-selinene	1503	0.23	0.04	0.31	0.01
bicyclogermacrene	1510	11.75	0.08	13.10	0.60
β-bisabolene	1515	0.38	0.02	0.36	0.04
germacrene A	1521	10.50	0.95	7.18	0.03
δ-amorphene	1531	1.15	0.15	1.10	0.05
β-sesquiphellandrene	1554	0.74	0.06	0.77	0.04
germacrene B	1571	6.89	1.24	10.06	0.21
viridiflorene	1605	0.20	0.03	0.53	0.03
Total		81.61		98.30	

Table 1. cont.

Compound	RI_{exp} [1]	Concentration in the oil [2] (mg/mL)			
		DERYNG	+/- SD	CLEVENGER	+/- SD
Oxygenated sesquiterpenes					
sesquicineole	1525	1.04	0.42	1.32	0.10
(Z)-sesquisabinene hydrate	1564	54.59	1.38	63.06	0.93
spathulenol	1588	6.73	0.07	12.10	0.16
caryophyllene oxide	1593	0.87	0.07	2.91	0.14
(E)-sesquisabinene hydrate	1598	67.76	1.13	75.79	1.55
β-atlantol	1624	1.03	0.17	1.34	0.02
1-*epi*-cubenol	1638	1.80	0.18	1.72	0.01
α-acorenol	1647	8.79	0.49	8.80	0.22
epi-α-muurolol	1658	1.46	0.61	1.88	0.07
β-acorenol	1666	2.39	0.14	2.23	0.06
α-cadinol	1671	4.34	0.17	5.10	0.03
neo-intermedeol	1675	1.54	0.20	1.50	0.04
β-bisabolol	1685	12.42	0.39	12.16	0.32
bulnesol	1691	0.90	0.10	1.30	0.10
α-bisabolol	1703	37.20	1.17	56.59	0.62
(Z)-farnesol	1712	6.97	0.55	8.09	0.27
(Z)-α-bisabolene epoxide	1726	0.23	0.04	0.75	0.01
Total		210.06		256.64	
Aromatic compounds					
p-cymene	1024	16.87	0.75	15.34	0.26
m-cresol	1071	1.34	0.57	2.26	0.08
methyl eugenol	1404	1.38	0.18	1.49	0.22
2.5-dimethoxy-*p*-cymene	1418	1.30	0.10	2.19	0.04
Total		20.89		21.28	

[1] – retention time on the column VF - 5 ms. [2] – concentration of the compound in the sample (mg) on the basis of the internal standard comparison.

RESULTS AND DISCUSSION

The obtained content of volatile compounds were: 785.67 mg/mL and 833.33 mg/mL in the oil obtained in the Deryng (D-EO) and Clevenger-type apparatuses (C-EO), respectively. In both essential oils 58 identical compounds were identified. The quantitative analysis revealed differences between the methods. Table 1 shows the qualitative and quantitative comparison of the components of the essential oils obtained by means of two methods.

Composition of essential oils obtained in Deryng and Clevenger-type apparatuses

The dominant components of D-EO were sabinene > α-pinene > myrcene > *(E)*-sesquisabinene hydrate > *(Z)*-sesquisabinene hydrate > α-bisabolol. In the case of C-EO the sequence was similar. Only myrcene content differed quantitatively. The structures and the quantitative comparison of the major essential oils components are shown in Figs. 1 and 2.

Analyzing the groups of chemical compounds present in the oils obtained in both apparatuses, it should be noted that in both cases monoterpenes were predominant: D-EO 55.4% (435.33 mg/mL), and C-EO 50.1% (424.49 mg/mL). The lowest amount was recorded for oxygenated monoterpenes: D-EO 3.3% (25.60 mg/mL), and C-EO 2.0% (16.34 mg/mL).

A high amount of sabinene and α-pinene in the essential oils is characteristic of Apiaceae family

(16). The dominant components were sabinene and α-pinene. In our study, sabinene and α-pinene, content were 189.9 and 76.60 mg/mL, respectively, in the Deryng, and 161.11 and 105.83 mg/mL, respectively, in the Clevenger-type apparatus, confirming the literature data on the chemotaxonomic characteristics of Apiaceae family.

According to the European Pharmacopoeia (6) and the Polish Pharmacopoeia VI (7) an essential oil is the plant extract obtained just by distillation processes, like hydrodistillation, with the exception of the *Citrus* sp. peel oil, which is isolated by cold expression. When other isolation techniques are employed, other designations, such as volatiles or

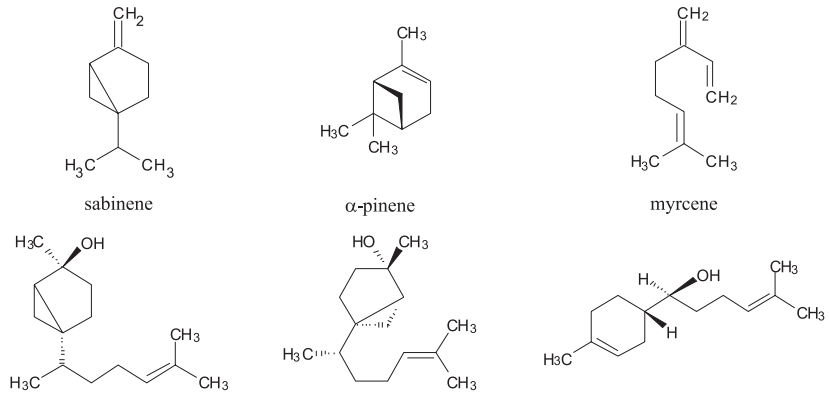

Figure 1. Structures of main compounds of essential oils of *M. purpurea* flowers

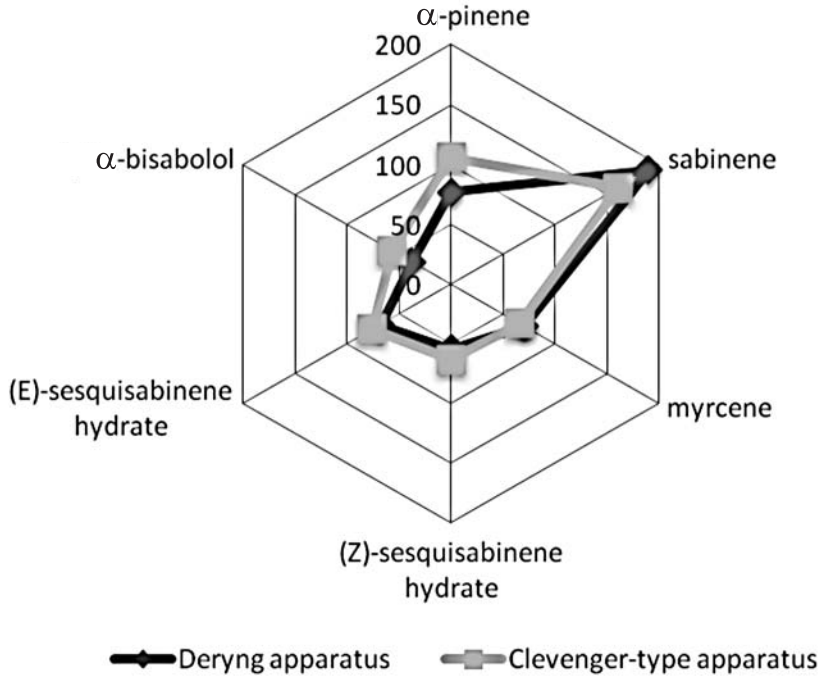

Figure 2. Comparison of the main components of essential oils from *M. purpurea* received by means of the Deryng and Clevenger-type apparatus

volatile oil, must be used (17). Hydrodistillation is a conventional method used to extract essential oils from aromatic plants. It can be used in industry and gives no chemical pollutions (18). The essential oil is obtained using the equipment usually based on the circulatory distillation approach using Deryng and Clevenger-type apparatuses. In theory, the recoveries of volatiles are quantitative for an infinite distillation time; thermal artifacts can be produced but they are accepted as a result of the traditional process.

Most hydrodistillation methods used in order to standardize essential oils apply a Clevenger-type apparatus as a Pharmacopoeial apparatus. Alternatively, a Deryng apparatus can be used. This device was included for many years in the Polish Pharmacopoeia and formed the basis of the qualitative evaluation of aromatic plant materials for use in the pharmaceutical industry. So far, it has been still used in many laboratories. Its compact design makes it even more convenient to use, whether to modify hydrodistillation methods or for the pre-screening of raw aromatic materials.

The qualitative analysis showed that the essential oils obtained in both apparatuses did not differ in the number of chemical compounds present. The quantitative differences between the main components were small.

Statistical analysis

The results presented in Table 1 were submitted to statistical analysis using tests assessing the significance of the difference between two dependent samples, which were essential oils obtained from the same plant material by hydrodistillation in the Deryng and the Clevenger-type apparatuses.

A null hypothesis was formed that there was no difference between the results of quantitative and qualitative analyses of oils distilled by means of those two different methods. The sign test showed that the calculated significance level $p = 0.0256$ was lower than the accepted one ($p = 0.05$), thus the hypothesis was rejected. Wilcoxon test confirmed those results and calculated the significance level also below 0.05 and it amounted to 0.0454. The results of both tests indicate that the quantitative and qualitative composition of essential oils obtained from the same raw material is dependent on the method by means of which it was obtained. Therefore, there is no basis to determine which type of apparatus is more suitable for oil extraction.

As shown in Fig. 3, the components found in the oil in the highest quantity are as follows: α-pinene, sabinene, myrcene, *(Z)*-sesquisabinene hydrate, *(E)*-sesquisabinene hydrate and α-bisabolol.

Despite the fact that the composition of essential oils acquired from the same raw material is

Table 2. The values of correlation coefficients and their interpretation (n = number of components).

Group of volatile compounds	Correlation	Range of constituents in similar quantities	Range of concentrations of constituents outlier in this group
Monoterpenes (n = 15)	0.9732	1.16 - 30.06 (n = 12)	65.76 - 189.90 (n = 3) sabinene, α-pinene, myrcene
Oxygenated monoterpenes (n = 8)	0.9935	0.76 - 4.61 (n = 7)	6.21 - 12.89 (n = 1) terpinen-4-ol
Sesquiterpenes (n = 16)	0.8878	0.12 - 1.52 (n = 9)	5.20 - 20.28 (n = 7) β-elemene, (*E*)-caryophyllene, (*Z*)-β-farnesene, germacrene D, bicyclogermecrene, germacrene A, germacrene B
Oxygenated sesquiterpenes (n = 17)	0.9891	0.23 - 12.42 (n = 14)	37.20 - 75.79 (n = 3) (*Z*)-sesquisabinene hydrate, (*E*)-sesquisabinene hydrate, α-bisabolol
Aromatic compounds (n = 4)	0.9985	1.30 - 2.26 (n = 3)	15.34 - 16.87 (n = 1) p-cymene

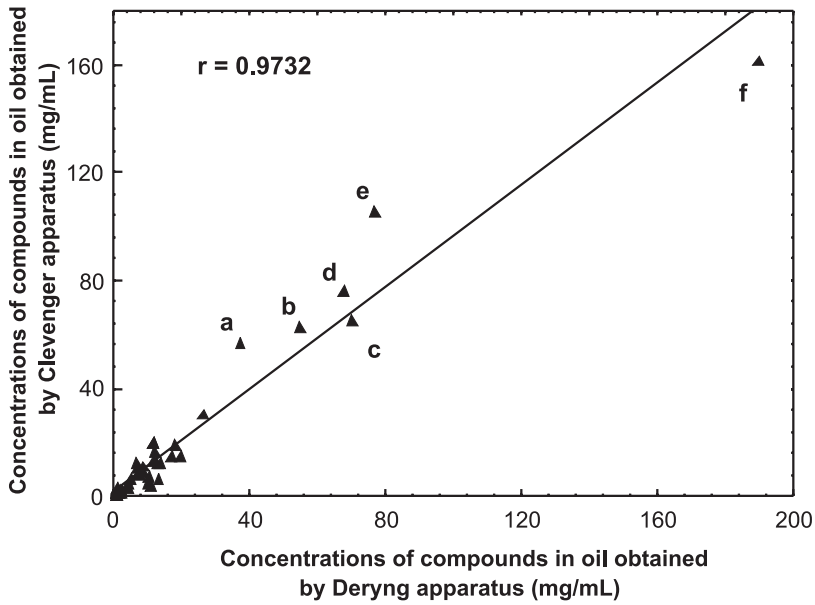

Figure 3. Correlation between the content of compounds of essential oils obtained in the Deryng and Clevenger-type apparatus from *M. purpurea* flowers. (a) α-bisabolol, (b) (*Z*)-sesquisabinene hydrate, (c) myrcene, (d) (*E*)-sesquisabinene hydrate, (e) α-pinene, (f) sabinene

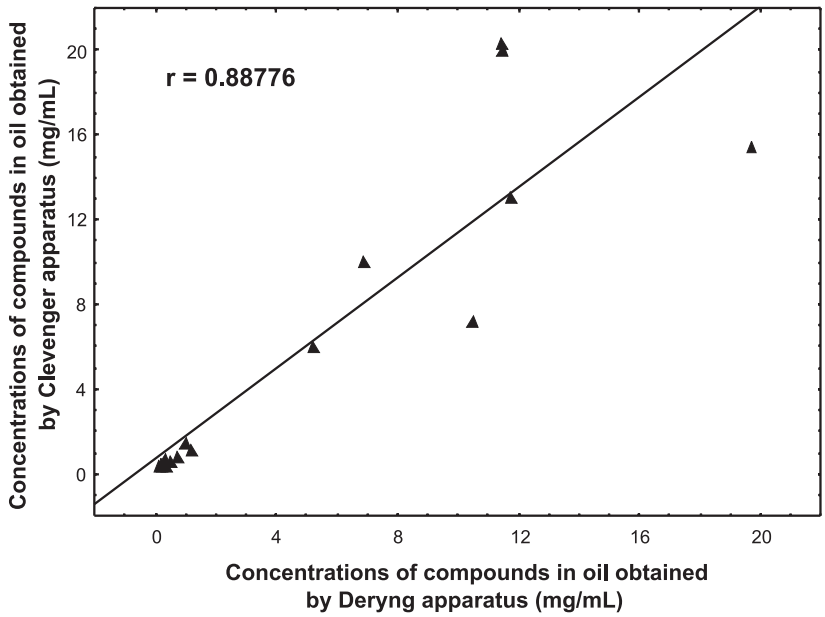

Figure 4. Correlation between the content of sesquiterpenes obtained in the Deryng and Clevenger-type apparatus from *M. purpurea* flowers

dependent on the distillation method, a high value of the correlation coefficient (r = 0.9732) can be found between the concentrations of the compounds in both oils. The following equation y = 1.4794 + 0.9515 x describes this relationship. It suggests that both groups of results are highly correlated and only a few components of the oils differ significantly in concentration.

The next step of comparing the composition of the essential oil obtained in both apparatuses was

determination of the relationship between the groups of identified compounds.

Table 2 shows the relationship between the groups of components of essential oils obtained during the distillation process in the both apparatuses.

The linear relationship for all groups presents a very similar picture: a large number of components grouped in the lowest concentration range, then a cluster with the highest concentration of one or three components. For sesquiterpenes, seven components with higher concentrations are grouped in a wide range of concentrations (see Fig. 4).

The highest correlation coefficients were obtained for the following two groups: aromatic compounds (n = 4, r = 0.9985) and oxygenated monoterpenes (n = 8, r = 0.9935). However, both groups are characterized by two features:

- a small number of components,
- one component at a concentration exceeding several times the average concentration of the other ingredients in the group.

In this situation, the differences in the concentrations of other constituents resulting from the application of two different methods of distillation have a negligible effect on the value of the correlation coefficients. They have very small values comparing to the component with an extreme concentration value.

Slightly lower values of correlation coefficients were obtained for the following two groups: oxygenated sesquiterpenes (n = 17, r = 0.9891) and monoterpenes (n = 15, r = 0.9732). They contain three ingredients in terms of concentrations, from a few to several times exceeding the average concentration of the other ingredients in the group. Three components which give direction of the correlation reduce its value.

The lowest value of the correlation coefficient was obtained for sesquiterpenes (n = 16, r = 0.8878). Regardless of the method of the oil production, the concentrations of 9 components are located in a narrow range of values, thus creating the initial section of the linear relationship. The direction of the linear relationship gives 7 other components present in a wide concentration range from a few to several times exceeding the average concentration of the other ingredients in the group. The result of a lack of a single component at a high concentration comparing to the other ingredients is that the direction of the correlation is given by a few components. The dispersion of their concentrations reduces the value of the correlation coefficient.

The correlation coefficients values are not determined by the differences in the concentrations of the components resulting from the application of two different methods of distillation. They are determined by the individual components at concentrations much higher than the average concentration of the other ingredients in the group. They give the correlation direction, and the fewer such components in the group, the higher the value of the correlation coefficient is achieved.

The present work is a detailed analysis of the essential oils content and it reflects the impact of the construction of the apparatus on the composition of the essential oil, but the differences can be acceptable for future biological research. Hydrodistillation in the Deryng and Clevenger-type apparatuses yielded 10.09 mL/kg and 16.80 mL/kg (dry weight) of essential oil, respectively. Applying the same conditions it was possible to obtain 60.1% more essential oil in a Clevenger apparatus. Analyzing the quantitative composition of both essential oils it cannot be said that the differences between individual compounds were proportional. The oil obtained in a Deryng appaeatus was abundant in monoterpenes whereas the dominant group in a Clevenger-type apparatus were sesquiterpenes. The statistical analysis suggests that both groups of essentials oils are highly correlated and only a few components of the oils differ significantly in concentration.

REFERENCES

1. Handa S.S., Khanuja S.P.S., G. Longo, Rakesh D.D.: Extraction Technologies for Medicinal and Aromatic Plants. International Centre For Science and High Technology, Trieste 2008.
2. Rubiolo P., Sgorbini B., Liberto E., Cordero C., Bicchi C.: Flavour Fragr. J. 25, 282 (2010).
3. Cardoso-Ugarte G.A., Juárez-Becerra G.P., Sosa-Morales M.E., López-Malo A.: JMPEE 47, 63 (2013).
4. Murat Ch., Gourrat K., Jerosh H., Cayot N.: Food Chem. 135, 913 (2012).
5. Prakash Om, Rout P.K., Chanotyia C.S., Misra L.N.: Ind. Crop Prod. 37, 195 (2012).
6. European Pharmacopoeia 6.0., Vol. 1, Council of Europe, Strasbourg Cedex 2007.
7. Polish Pharmacopoeia, Vol. VI, PTFarm, Warszawa 2002 (in Polish).
8. Sieniawska E., Baj T., Kowalski R., Glowniak K.: Rec. Nat. Prod. 2014. (In press).
9. Sieniawska E., Baj T., Ulewicz-Magulska B., Wesolowski M., Glowniak K.: Biochem. Syst. Ecol. 49, 125 (2013).
10. Baj T., Ludwiczuk A., Sieniawska E., Skalicka-Woźniak K., Widelski J., Zięba K., Glowniak K.: Acta Pol. Pharm. Drug Res. 70, 35 (2013).

11. NIST/EPA/NIH Mass Spectral Library with Search Program: Data Version, NIST 08, Software Version 2.0f, National Institute of Standards and Technology 2005.
12. Joulain D., Konig W.A.: The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B. Verlag, Hamburg 1998.
13. Adams R.P.: Identification of Essential Oil Compounds by Gas Chromatography/ Quadrupole Mass Spectroscopy, Allured Publishing Corp., Carol Stream, IL 2001.
14. Van Den Dool H., Kratz P.D.: J. Chromatogr. 11, 463 (1963).
15. Kowalski R., Wawrzykowski J.: Flavour Fragr. J. 24, 69 (2009).
16. Evergetis E., Michaelakis A., Haroutounian S.A.: Essential Oils of Umbelliferae (Apiaceae) Family Taxa as Emerging Potent Agents for Mosquito Control, in Integrated Pest Management and Pest Control - Current and Future Tactics, Larramendy, M.L., Soloneski S. Eds., InTech, Rijeka 2012.
17. Zhu M., Li E., He H.: Chromatographia 68, 603 (2008).
18. Huang B., Lei Y., Tang Y., Hang J., Qin L., Liu J.: Food Chem. 125, 268 (2011).

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