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EVALUATION OF CHEMICAL COMPOSITION OF ESSENTIAL OILS DERIVED FROM DIFFERENT *Pelargonium* SPECIES LEAVES

OCENA SKŁADU CHEMICZNEGO OLEJKÓW ETERYCZNYCH POZYSKANYCH Z LIŚCI RÓŻNYCH GATUNKÓW ROŚLIN Z RODZAJU *Pelargonium*

Abstract: Plants have been used for their aromatic and medicinal purposes for centuries. The genus *Pelargonium* contains about 250-280 species and cultivated around the world for the production of essential oils and absolutes. *Pelargonium* species are remarkable for their odour diversity, from fresh, lemon and minty scent, through floral, rose, fruity up to spicy and camphoreous smell as well as many health beneficial properties, that is why their essential oils are widely used in perfume, cosmetic, food and pharmaceutical industries. The aim of this study was to determine variability in populations of *Pelargonium* genus by evaluating their differences in quantitative and qualitative essential oils compositions. This study determined that of all the examined essential oil, the one of *P. graveolens* indoor cultivar has the closest C:G ratio (1.16) which makes it a very valuable product. In comparison with available literature, beside the geographical region of plant growth, all essential oils from *P. graveolens* contain a diverse range of compounds, nevertheless, in every single one, geraniol and citronellol are present in the most significant fraction of the total oil. The examined *P. roseum* essential oil was characterized by the highest level of citronellol (44.62 % of total oil), but it can be due to the chemical reactions (conversion geraniol into citronellol) occurring in stored product.

Keywords: citronellol, geraniol, essential oil, *P. graveolens*, *P. odoratissimum*, *P. roseum*

Introduction

For centuries plants and their extracts have been used for aromatic and medicinal purposes. Essential oils are an ingredient of perfumes, cosmetics, food and pharmaceuticals. *Pelargonium* species, because of their differential properties and odour diversity are commonly used for essential oil production.

In this study, chemical compositions of different *Pelargonium* species essential oils were compared. A great emphasis was put on dominant components of examined mixtures. Because studied essential oils were derived from plants that had grown in different environmental conditions, gas chromatographic - mass spectral analysis was used to

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determine if the major components of pelargonium essential oils are representative for the whole genus regardless cultivation conditions.

Essential oils

Plants have been used for their aromatic and medicinal purposes for centuries. With the world development and the appearance of many illnesses of the modern civilization, in recent years there has been considerable interest in bioactive agents, especially antimicrobial and antioxidant substances from natural sources, mainly in essential oils, also called volatile odoriferous/volatile oils [1-6]. Essential oils are described by International Organization for Standardization (ISO) as “products obtained from vegetable raw material, either by distillation with water or steam, or from the epicarp of citrus fruits by mechanical process, or by dry distillation” [7]. They are chemically complex mixtures, often containing from only a few up to several hundred of single substances. They are aromatic oily liquids extracted from different parts of plant, mainly the non woody ones, for example, flowers, leaves, peels, buds or seeds, but also cells glands and ducts in, which the essential oils are found, are located in woody parts, such as bark or roots [5, 8]. Volatile oils are widely used in different industries, for example as natural additives in foods and foodstuff packaging materials to prevent them from the oxidative damage and inflammatory conditions, they are added as a natural preservatives or fragrances in majority of cosmetics. Natural antioxidants contained in volatile odoriferous inhibit also aging and prevent inflammatory diseases. Moreover, essential oils replace synthetic substances in the nutritional, pharmaceutical, and agricultural fields due to their reported antiviral, nematocidal, antifungal and insecticidal properties. For medical purposes they need to comply with national or international Pharmacopoeia [3, 5, 8-10]. Essential oils vary in compounds composition, properties, odour, flavour as well as in colour. Depending on plant health, growth stage, habitat and harvest time of starting material, the odoriferous oils are composed of different ratio of lipophilic and highly volatile secondary plant metabolites. Their dominant constituents are terpenes (mono- and sesquiterpenes), aromatic compounds and terpenoids [5, 9]. Essential oils can be obtained by various methods, from which steam distillation has been traditionally applied. Another methods are: extraction with fat and solvents or recently developed techniques, such as extraction with liquefied CO₂ gas and solvent free microwave extraction (SFME) [5, 8].

***Pelargonium* spp. essential oils**

The genus *Pelargonium* is one of the seven genera of the *Geraniaceae* family (*Geranium*, *Pelargonium*, *Erodium*, *Monsonia*, *Hypseocharis*, *Rhynchotheca* and *Sarcocaulon*). This genus contains about 250-280 species and include a range of variation in floral morphologies and life forms [11-13]. Hybrids of *Pelargonium* are cultivated around the world for the production of essential oils and absolutes, but original regions for *Pelargonium* genus are South Africa and nearby countries. It's cultivars grow mainly in Reunion Island, China, Egypt, Algeria and Morocco. While China and Egypt are the major producers of *pelargonium* volatile oils, the Bourbon geranium from Reunion Island is considered as one of the highest quality essential oils [11-16]. The oils are characterized by high levels of citronellol, geraniol, isomenthone, linalool accompanied by wide range of esters, such as citronellyl formate, geranyl formate, geranyl acetate, geranyl propionate and sesquiterpenes, of which 10-epi- γ -eudesmol and guaia-6,9-diene are the most important [16, 17]. Very valuable in high grade perfume chemical aromas obtained from Geraniaceae

family is rhodinol (a mixture of geraniol, citronellol and other alcohols) [18]. *Pelargonium* species are remarkable for their odour diversity, from fresh, lemon and minty scent, through floral, rose, fruity up to spicy and camphoreous smell. The most often commercially used plant in described genus are: *P. capitatum*, *P. graveolens*, *P. odoratissimum* and *P. radens* [12].

P. graveolens is an aromatic and hairy shrub, erect up to 1-1.3 m tall and 1 m spread with soft to the touch and carved leaves, and usually small, pink flowers. It's essential oil yield is high and varies from 0.1-0.9 % v/w [13, 19]. The oil is obtained from leaves, flowers and stalks [21]. Studies show that variety of factors, such as cultivar, method of oil distillation, distilled part of the plant, as well as age of this material, essential oil storage, location of growing and seasonal changes (temperature, light intensity) of the area, also season and time of harvesting, influence the final essential oil composition of rose-scented geranium [17, 22]. Essential oil from *P. graveolens* is very valuable not only for perfumery, food and beverages industry, but it has also a variety of healing properties. It has an antidepressant, anticancer and hemostatic effect, moreover, it regulates bloodstream and stimulates the adrenal glands. This oil has been found to stimulate lymphatic system which in combination with diuretic properties is excellent in the fight against cellulite and fluid retention in the body. *Pelargonium* essential oil helps with heavy menstrual flows or hemorrhoids and many other diseases [19, 20]. Major volatiles in *P. graveolens* essential oil composition are citronellol, geraniol, linalool, citronellyl formate, methone, isomenthone and nerol [21, 22].

Pelargonium roseum is indigenous to Southern Africa and was introduced in Europe in the 18th century. Due to its rose odour, it is widely grown as an ornamental plant almost all over the world. *P. roseum* has woody, straight and branched stem that can reach heights of 120 cm. The leaves are covered in short, rough trichomes which give the plant characteristic rose smell [23, 24].

P. odoratissimum leaves are frequently used not only as a flavouring, an insect repellent, but also in perfumery or aromatherapy in throat infections and gastrointestinal diseases treatments [10]. Another studies show that essential oil from *P. odoratissimum* leaves has antibacterial and spasmolytic properties. Its bioactivity and effectiveness against *Staphylococcus aureus*, *S. Epidermis*, *Proteus vulgaris* and *Bacillus cereus* is proven to be due to the presence of methyl eugenol [25].

The aim of this study was to determine variability in populations of *Pelargonium* genus by evaluating their differences in quantitative and qualitative essential oils compositions. It is the first work where the chemical compositions of oils derived from *P. roseum*, *P. graveolens* and *P. odoratissimum* are compared.

Experimental

Materials and methods

Three commercial pelargonium essential oils: *P. graveolens* from Etja, Poland, *P. roseum* from Aromatika, Ukraine and *P. odoratissimum* from Avicenna oil, Poland were purchased from the manufacturers in quantity of 3 of each oil, and their chemical compositions were determined by gas chromatography - mass spectrometry.

Also an essential oil compounds from freshly harvested leaves of *P. graveolens* indoor cultivation were analysed. 100 g of leaves from one-year old odorous plants were harvested in November. Material was collected from twenty pelargoniums and the leaves were

randomly picked from different plants heights, then cut into small pieces and immediately transferred to round bottom flask of the Deryng apparatus. Water was added till the plant material was completely submerged and then subjected to hydrodistillation. The material has been distilled for 3 hours after reaching the boiling point. The chemical composition of the obtained essential oil was determined by gas chromatography - mass spectrometry as well. Also a citronellol/geraniol ratio (C:G) in studied *Pelargonium* species essential oils was calculated.

Gas Chromatographic-Mass Spectral Analysis

The analyses were performed using a Varian Chrompack CP-3800 gas chromatograph equipped with 4000 GC/MS/MS system and flame ionization detector (FID). The carrier gas was helium with a flow rate of 1.0 cm³/min. The GC oven temperature program was used as follows: 50 °C initial temperature hold for 1 min, increased at 4 °C/min to 240 °C and hold for 10 min. A 1 % w/v solution of the sample in hexane was prepared and 1 mm³ was injected using a splitless injection technique. The split ratio 1:50, scan time 0.8 s; mass range 40-1000 m/z (mass-to-charge ratio). Identification of components in the oils was based on GC retention indices relative to *n*-alkanes and computer matching with the Nits Library and Adams Library.

Phytochemical similarity of studied essential oils

To assess similarity in the oils composition a dendrogram was constructed. In Figure 1 structure of the dendrogram is presented. Among the compounds listed in Table 1. The following were taken for dissimilarity matrix construction: 1-3, 5, 9, 11, 12, 18, 24-26, 29 and 30. Prior to calculations of euclidean distance, concentrations were recalculated using centered logratio (clr) transformation. This transformation ensures appropriate sample space for distance calculation between pairs of points representing different oils [26]. The “complete” agglomeration method in function “hclust” that performs a hierarchical cluster analysis using a set of dissimilarities, provided in R language was applied for dendrogram construction [27]. Also a similarity between relative compounds' concentration in the studied plants was assessed.

Results

Gas Chromatographic-Mass Spectral Analysis

There have been determined the presence of ninety-seven compounds in the commercial *P. graveolens* essential oil, accounting for 99.04 % of the total oil. A component of the largest amount in this EO (essential oil) was citronellol (22.28 %), followed by geraniol (15.50 %), geranyl acetate (13.13 %), limonene (9.27 %), phenyl ethyl alcohol (5.93 %) and linalool (5.59 %). This commercial essential oil was the only one from all examined oils in which a trace of isomenthone was not detected. In *P. odoratissimum* essential oil, ninety-eight compounds were identified representing 99.34 % of the total oil. As in the previous oil, a significant amount of citronellol (29.77 %) was determined. Another dominant components were citronellyl formate (9.22 %), geraniol (6.51 %), isomenthone (3.34 %), linalool (2.45 %), menthone (2.23 %). There was a presence of seventy-six compounds in the essential oil from *P. roseum* determined, accounting for 99.32 % of the total oil. The components identified as principal were citronellol (44.62 %), citronellyl formate (14.42 %), geraniol (10.73 %), linalool (5.39 %),

menthone (3.04 %), isomenthone (0.89 %) and limonene (0.35 %). The essential oil from indoor cultivar of *P. graveolens* was characterized by a light green colour and had a strong odour which in that moment was not very similar to the smell of freshly cut leaves. In this extract, seventy-three compounds were identified representing 99.58 % of the total oil. Essential oil extraction was performed on leaves, as the preliminary experiments showed that the hydrodistillation of stems and petiole was inefficient. The most abundant components in this sample were citronellol (26.62 %) and geraniol (22.92 %). Another significant compounds were linalool (6.37 %), geranyl tiglate (4.37 %), isomenthone (3.39 %) and limonene (0.10 %). Unlike in the previous essential oils, only trace amount of citronellyl formate was detected and no presence of geranyl formate was determined. The major components, which content was bigger than 1 %, constituted from 82.35 % in *P. odoratissimum*, up to 92.33 % in *P. graveolens* of the total oil. This chemical analysis of studied essential oils are presented in Table 1.

As the result of chemical analysis of studied essential oils show, citronellol and geraniol were identified in all EO, but in different ratio. The most preferable ratio (C:G) is 1, because as the literature data shows that pelargonium essential oil is of the best quality, which is very important for perfumery, flavouring and cosmetics industries. In this study the closest ratio was examined in indoor cultivar of *P. graveolens* (1.16). Also the (C:G) ratio in EO from commercial *P. graveolens* was similar (1.44), while in other essential oils this parameter was very different. In *P. odoratissimum* EO it was 4.57 and in *P. roseum*, the ratio was 4.16.

Table 1
Chemical composition of commercial essential oils of *P. graveolens*, *P. odoratissimum*, *P. roseum* and indoor cultivar of *P. graveolens* which content was bigger than 1 %

Serial No.	Component	Indoor cultivar of <i>P. graveolens</i>		<i>P. graveolens</i>		<i>P. odoratissimum</i>		<i>P. roseum</i>	
		[%] [*]	RI	[%] [*]	RI	[%] [*]	RI	[%] [*]	RI
1	Limonene	0.10	1027	9.27	1028	0.15	1028	0.35	1027
2	Linalool	6.37	1092	5.59	1092	2.45	1092	5.39	1091
3	Rose oxide	0.06	1101	0.12	1101	1.63	1011	1.86	1100
4	Phenylethyl alcohol	-	-	5.93	1105	1.96	1105	-	-
5	Menthone	0.07	1153	0.17	1153	2.23	1154	3.04	1153
6	Isoborneol	-	-	1.32	1162	-	-	-	-
7	Borneol	-	-	1.79	1172	-	-	-	-
8	Isomenthone	3.39	1164	-	1190	3.34	1164	0.89	1163
9	α -Terpineol	0.31	1198	2.38	1198	0.31	1198	0.82	1197
10	Endo-Fenchyl acetate	-	-	8.57	1229	1.20	1229	-	-
11	Citronellol	26.62	1234	22.28	1233	29.77	1234	44.62	1234
12	Geraniol	22.92	1259	15.50	1257	6.51	1256	10.73	1256
13	Citronellyl formate	tr.	1278	1.67	1277	9.22	1279	14.42	1278
14	Geranyl formate	-	-	0.59	1303	1.43	1303	2.37	1303
15	Geranyl acetate	-	-	13.13	1382	0.80	1381	0.55	1381
16	β -Bourbonene	tr.	1386	0.09	1386	1.28	1386	0.17	1386
17	Phenyl ethyl isobutanoate	0.10	1396	tr.	1396	1.04	1396	-	-
18	(e)-Caryophyllene	0.23	1425	0.14	1425	1.64	1425	1.92	1424
19	Citronellyl propanoate	-	-	-	-	-	-	3.05	1447

Serial No.	Component	Indoor cultivar of <i>P. graveolens</i>		<i>P. graveolens</i>		<i>P. odoratissimum</i>		<i>P. roseum</i>	
		[%] [*]	RI	[%] [*]	RI	[%] [*]	RI	[%] [*]	RI
20	Allo-Aromadendrene	9.92	1451	0.86	1450	8.08	1451	-	-
21	Geranyl propanoate	-	-	tr.	1481	-	-	1.05	1481
22	Neryl isobutanoate	1.70	1482	-	-	0.54	1482	-	-
23	Epizonarene	3.13	1495	0.08	1495	1.17	1495	-	-
24	Zonarene	0.81	1530	0.13	1530	1.37	-	0.16	1530
25	Citronellyl butanoate	0.18	1536	0.16	1536	1.70	-	0.16	1535
26	Geranyl butanoate	0.43	1563	2.36	1563	2.12	1563	0.08	1563
27	2-Phenyl ethyl tiglate	4.15	1589	0.05	1588	-	-	-	-
28	α-Muurolol	1.72	1654	tr.	1654	0.23	1655	tr.	1654
29	(e)-Citronellyl tiglate	0.90	1676	0.09	1676	1.07	1676	0.10	1675
30	Geranyl tiglate	4.37	1713	0.06	1712	1.11	1712	0.08	1711

tr. - trace (< 0.05 %), RI - retention indices on VF - 5 ms capillary column, [%]^{*} - compounds concentration in a sample, (-) - was not found

Table 2 presents the major components of examined *Pelargonium* spp. EO in comparison with available literature.

Table 2

The main components of essential oil from indoor cultivation of *P. graveolens*, commercial essential oils from *P. graveolens*, *P. odoratissimum* and *P. roseum* in comparison with literature

Source	Component [%]							
	1 [*]	2 [*]	3 [*]	4 [*]	5 [*]	6 [*]	7 [*]	8 [*]
Indoor <i>P. graveolens</i>	26.62	22.92	6.37	0.10	3.39	0.24	tr.	-
<i>P. graveolens</i>	22.28	15.50	5.59	9.27	-	0.25	1.67	0.59
[12, 13, 17, 18, 27, 31, 32]	21-37.5	6.0-26.8	5.2-13	0.08-0.2	0.18-6.3	0.3-8.7	6.9-13.24	1-6.22
<i>P. odoratissimum</i>	29.77	6.51	2.45	0.15	3.34	0.16	9.22	1.43
[25]	8.99	12.69	-	-	-	-	-	-
<i>P. roseum</i>	44.62	10.73	5.39	0.35	0.89	0.09	14.42	2.37
[24]	28.96	18.53	5.72	0.68	4.22	-	-	-

tr. - trace (< 0.05 %), (-) - was not found, 1^{*} - Citronellol, 2^{*} - Geraniol, 3^{*} - Linalool, 4^{*} - Limonene, 5^{*} - Isomethone, 6^{*} - Neral, 7^{*} - Citronellyl 8^{*} - formate, 9^{*} - Geranyl formate

Phytochemical similarity of studied essential oils

The gas chromatographic - mass spectral analysis of the volatile oils revealed significant differences in the number and the type of the identified volatile compounds. It is most likely due to the environmental conditions studied plants had grown in.

The gas chromatographic - mass spectral analysis of the volatile oils was a base for building a dendrogram of phytochemical similarity of all four pelargonium essential oils components with concentrations bigger than the reported limit of quantification (Fig. 1). Two groups of points can be observed in the graph. It can be noted that oils from *P. odoratissimum* and *P. roseum* make separate group that represents the relationship of biggest similarity in their oils compositions. The second group represents commercial extracts from *P. graveolens* and the one distilled from *P. graveolens* from indoor cultivar.

Compositions of these oils were also similar to each other, but in somewhat lower degree than in the first group.

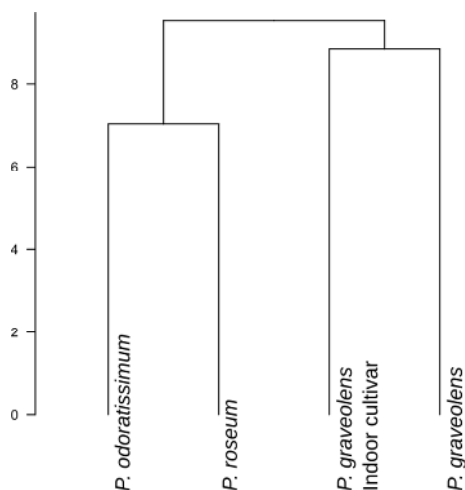


Fig. 1. Dendrogram of phytochemical similarity of commercial essential oils of *P. graveolens*, *P. odoratissimum*, *P. roseum* and indoor cultivar of *P. graveolens*

This dendrogram shows that essential oils from *P. odoratissimum* and *P. roseum* make separate group that represents the similarities in their chemical compositions. The second group contains commercial oils from *P. graveolens* as well as oil distilled from *P. graveolens* from indoor cultivar which means that despite their different starting materials, their compositions are alike.

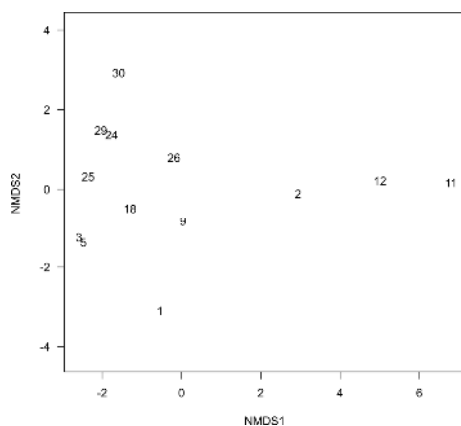


Fig. 2. MDS representation of the clr transformed oil's concentration in the studied pelargoniums essential oils

A similarity between relative compounds' concentration in the plants studied was assessed using nonmetric multidimensional scaling (MDS) method (Fig. 2) [28, 29].

The dissimilarity matrix was calculated for points representing clr transformed concentrations of individual compounds in the plants studied. In computation function “metaMDS” from “vegan” library was used [30]. A similarity between content of compound pairs 3,5 (cis-rose oxide, menthone) and 24, 29 (zonarene, (e)-citronellyl tiglate) in all studied essential oils is observed. The first pair (3,5) belongs to the monoterpene group, while the second pair (24,29) refers to the sesquiterpenes. The relative content of other compounds in plants are different from each other.

In Figure 2 MDS representation of the clr transformed oil's concentration is shown. Similarities in two pairs can be observed. First pair (3,5) belongs to the monoterpene group, while the second pair (24,29) refers to the sesquiterpenes. The relative content of other compounds in studied essential oils are different from each other.

Discussion

The literature report (Table 2) that oxygenated monoterpenes constitute the main group in every obtained essential oil (e.g. citronellol, geraniol, linalool or citronellyl formate) [5, 11, 19]. Comparing the available data (Table 2), all the studies show that for *P. graveolens* essential oil, citronellol is present in the most significant amount, reaching 37.5 % of the total oil of the plant growing in Tajikistan in Sharopov et al. [20] research. Second major component was geraniol, of which *Pelargonium* from Himalayan region of India, that as explored [31] contained 26.8 % of the total oil. A search of the literature reveals that beside the region of plant growth, all essential oils from *P. graveolens* contain a diverse range of compounds, nevertheless, in every single one, geraniol and citronellol are present in the most significant fraction of the total oil. Important compounds are also linalool and citronellyl formate that give the fresh citrusy-floral flavour to the EO. There is not much available data about chemical composition of *P. odoratissimum* and *P. roseum*. However, the data show that in Tabari and Youssefi [24], in examined essential oil form *P. roseum*, major constituents were β -citronellol (28.96 %), geraniol (18.53 %) and linalool (5.72 %). These results are very different from this investigation, where the amount of citronellol equaled 44.62 %, geraniol 10.73 % and linalool 5.39 %, while another compound citronellyl formate was detected in a number of 14.42 %. It cannot be assured if it is due to the starting material properties or chemical reactions occurring in stored product. Main components found in *P. odoratissimum* [32] were geraniol (12.69 %) and citronellol (8.99 %). In present study these two compounds were also detected in the biggest amount, but in the opposite order (citronellol 29.77 % and geraniol 6.51 %). Of all the examined essential oil in this research and from the available literature, the one of *P. graveolens* indoor cultivar has the closest C:G ratio (1.16) which makes it a very valuable product. C:G of the commercial *P. odoratissimum* and *P. roseum* essential oils from this study equals more than 4 which makes them less valuable for the industry. Data from gas chromatography - mass spectrometry shows that composition of the studied essential oils vary in concentration of major constituents and also contain a different total number of compounds where the highest was determined in commercial EO of *P. odoratissimum*. What should be highlight is the fact that due to the relationships of chemicals from the same structural group, they can be easily convert into each other by oxidation, isomerization, cyclization, or dehydrogenation reactions, e.g. geraniol into citronellol, that is why the age of starting material plays a significant role in this aspect.

Conclusions

Studied essential oils from *Pelargonium* genus are mixtures of different amount of chemical components. Every EO has its own unique composition, but all of them are highly similar to one another. However, it is important to remember that due to different environmental conditions, such as rainfall, soil fertility or light intensity, derived essential oils vary in composition. That results in market value of studied mixtures. Because of the fact that cosmetic and perfume industry seek for standardized oils, it is essential to gain the knowledge what cultivation conditions would be the best for this purpose. This issue is going to be the subject of next studies.

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