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The essential oil of Chamomilla recutita (L.) Rausch. cultivated and wild growing in Poland

Olejek eteryczny uprawianego i dzikorosnącego w Polsce *Chamomilla recutita* (L.) Rausch.

INTRODUCTION

German chamomile (*Chamomilla recutita* (L.) Rausch) of the family Asteraceae belongs to the most popular herbal plants cultivated or collected from natural stands in Poland. Chamomile raw material consists of its flower heads – *Chamomille anthodium* (synonym: chamomile flowers – *Chamomille flos*), containing essential oil in the amount of 0.5-1.5% [7, 19,20,22], flavonoids: apigenin, luteolin, and quercetin [29,30], coumarins, carotenoids as well as other compounds: choline, tannins, polysaccharides. Chamomile oil is a dark blue viscous liquid with an intense bitter aroma and its most important constituents are chamazulen, bisabolol, spiroether, and farnesene [15,25,31]. The dark blue colour of chamomile oil is caused by the presence of sesquiterpene compounds such as chamazulene. This compound does not occur in the plant; it is formed by the decomposition of a pigment compound, matricin, during the distillation of raw material [6]. And it is chamazulene, not matricin, which is most probably responsible for the anti-inflammatory activity of chamomile extract, by inhibiting leukotriene synthesis, and for the additional antioxidative effect [24].

The quantitative and qualitative composition of chamomile oil is clearly affected by genetic, ontogenetic and environmental factors [8,12,22,30]. The economic value of Polish chamomile varieties and breeding families, with a high content of essential oil and a good concentration of α-bisabolol and chamazulene, shows significant progress in Polish quality breeding of German chamomile [25]. The use of valuable varieties in chamomile cultivation, with a high content of bisabolol and chamazulene, contributes to the improvement of the quality of domestic raw material, provided that proper agricultural practices are used. It should be stressed that this herbal material, due to its high demand, is also collected from natural stands. The chamomile inflorescence collected from wild-growing plants can be far different from the raw material obtained from cultivation. High morphological and chemical variation, which characterizes this species, is related to its genotype, among others. Diploid and triploid individuals, varying in terms of the chemical composition of raw material, are found both in a natural state and in cultivation. Polyploidization, commonly used in breeding, also occurs spontaneously in wild-growing plants. An increase in the number of chromosomes in chamomile results in enhanced accumulation of essential oil and changes in its composition [26].

The chamomile flower is used both internally and externally in the form of numerous medicinal and cosmetic preparations. This herbal material has anti-inflammatory [2], anti-bacterial [28], relaxant, diuretic, anti-allergic, and immunomodulating activity [10]. The most recent research [17] proves that chamomile, compared to corticosteroids, accelerates the healing process much more. In addition, chamomile oil shows anti-genotoxic and anti-oxidative effect [11]. The chamomile herb, known and valued for centuries as a versatile medicine, is still an official medicine in the pharmacopoeia of 26 countries. Large variations in chamomile raw material in the European pharmaceutical market [19] are associated, among others, with plant origin as well as the method of harvesting, drying and oil extraction [4,5]. The aim of the present study was to compare the number and size of inflorescences as well as the content and chemical composition of essential oil of German chamomile obtained from cultivation and from natural stands.

MATERIALS AND METHODS

This study was carried out from April to July 2007 in Radawiec in the Lublin region. Chamomile raw material was obtained from cultivation (a field with a northern exposure, loamy sandy soil, soil class III, neutral pH) and from a natural stand (meadow area, the stand sheltered from the wind, open land with a northern exposure, loamy sandy soil with a neutral pH). The chamomile cultivar 'Złoty Łan" was grown; this is a Polish tetraploid variety, bred in 1970 at the Herbal Industry Institute in Poznań (currently: Institute of Natural Fibres and Medicinal Plants in Poznań). This cultivar is characterized by good raw material yield: more than 13t· ha^{-1}, and it is suitable for mechanical harvesting. The plants produce large flower heads with a diameter of 2.5-3.0 cm, while the weight of 1000 inflorescences is 210 g. The herbal material contains 1.05% of essential oil, with the percentages of azulene and bisabolol accounting for 11.4% and at 9.3%, respectively [26].

The cultivation experiment was set up using complete randomized design with four replicates. Chamomile seeds were sown in the first decade of April directly into the soil, without cover, in rows 40 cm apart at a rate 2 kg · ha^{-1}. The area of one replicate was 2.8 m^2, while the whole experimental plot covered an area of 11.4 m^2. Potato was the forecrop for chamomile. In the autumn the soil was enriched with compost at a rate of 40 t· ha^{-1}; no additional mineral fertilization was applied. During the growing period, the following tending treatments were performed: soil loosening and hand weeding. The harvest was done manually by picking entire inflorescences at the initial stage of full bloom: the raw material from the wild-growing plants was collected on 6 July, while that from the cultivated crops on 19 July. Right after harvest, the raw material was dried under natural conditions, in a well-aerated and shaded room. The meteorological conditions during the growing period of chamomile are characterized based on observations carried out in a nearby weather station belonging to the Department of Agrometeorology of the Lublin University of Life Sciences and they are presented relative to the long-term averages in Table 1. The obtained results were statistically elaborated using the variance analysis method for single classification at the significance level α=0.05.

Table 1. Average daily temperature and precipitation during the growing period of chamomile in 2007 and in 1951-1995

Temperature (°C)					
Month	10-day mean			Monthly mean	1951-1995
	I	II	III		
April	6.2	9.5	10.6	8.8	7.4
May	9.9	15.1	19.6	14.9	13.0
June	18.2	20.0	16.2	18.1	16.4
July	17.1	21.0	19.3	19.1	17.9
Precipitation (mm)					
Month	10-day total			Monthly total	1951-1995
	I	II	III		
April	8.8	5.6	3.0	17.4	39.1
May	13.5	29.9	37.1	80.5	57.2
June	52.4	25.4	10.0	87.8	65.9
July	48.8	35.0	3.2	87.0	73.6

The dried chamomile inflorescences (30 g of plant material) were subjected to hydrodistillation in a Clevenger-type apparatus, in a 1000 ml round-bottom flask with 500 ml of distilled water with the addition of 0.5 ml of xylene. The distillation time was 3 hours. The extracted essential oil was stored in a dark glass container at a temperature of -10°C, until the time of chromatographic separation.

GC-MS analysis. The GC-MS instrument ITMS Varian 4000 GC-MS/MS (Varian USA) was used, equipment with a CP-8410 auto-injector and a 30 m x 0.25 mm i.d. VF-5 ms column (Varian, USA), film thickness 0.25 μm; carrier gas, helium at a rate of 0.5 ml/min; injector and detector temperature, 220°C and 200°C, respectively; split ratio, 1:20; injector volume, 1μl. A temperature gradient was applied (60°C for 0.5 min, then incremented by 3°C/min to 246°C and held at this temperature for 10 min); ionization energy, 70eV; mass range, 40-1000 Da; scan time, 0.80 s. The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of NIST library [18] and with data available in the literature [1,13]. The identity of the compounds was confirmed by their retention indices, taken from the literature [1,13] and our own data.

RESULTS

The chamomile plants under study significantly differed from one another in terms of the number of flower heads developed (Table 2). The average number of chamomile inflorescences was 41.4 pcs. · plant $^{-1}$. During the harvest period, the largest amount of inflorescences was at full growth (on average 22.2 pcs. · plant $^{-1}$), while the number of overblown inflorescences was the lowest (on average 3.2 pcs. · plant $^{-1}$). The plants collected from the natural stand were characterized by a higher number (44.5 pcs. · plant $^{-1}$) of open (23.3 pcs. · plant $^{-1}$) and closed flower heads (17.8 pcs. · plant $^{-1}$), compared to the cultivated plants (Tab.2). Moreover, the wild-growing plants were found to have a higher number (3.4 pcs. · plant $^{-1}$) of overblown inflorescences compared to the cultivated plants (3.0 pcs. ·

plant $^{-1}$), though this was not statistically proven. The average diameter in the investigated chamomile inflorescences was 0.8cm, and it was larger (0.8 cm) in the wild-growing plants than in the plants of the cultivated chamomile 'Złoty Łan' (0.7 cm). However, the differences were not statistically significant. A significant correlation was shown between the source of origin of raw material and its air-dry weight (ADW) (Table 2). The raw material from the natural stand was characterized by a higher average weight (0.020 g) of the air-dried inflorescence than the raw material obtained from the cultivated plants (0.007 g). Furthermore, a higher weight of air-dried flowers (0.80g · plant $^{-1}$) was found in the wild-growing plants compared to the cultivated plants (0.35· plant $^{-1}$).

Table 2. The number, diameter and weight of chamomile inflorescences in dependence on the origin of raw material

Origin	No. of inflorescences per plant				Inflorescence		
	total	at full bloom	closed	out of bloom	Diameter (cm)	Weight (g)	
						total per plant	individually
Natural	44.5	23.3	17.8	3.4	0.8	0.80	0.020
Cultivation	38.2	21.0	14.1	3.0	0.7	0.35	0.007
Mean	41.4	22.2	16.0	3.2	0.8	0.57	0.013
$LSD_{0.05}$	5.2	2.1	2.7	ns	ns	0.33	0.001

The present study showed a significant effect of the source of raw material on the quantity of essential oil in the chamomile inflorescences (Table 3). The raw material obtained from the chamomile crops 'Złoty Łan' contained more (1.10% ADW) essential oil than the raw material from the naturally growing plants (0.50% ADW). The average essential oil concentration in the investigated raw material was 0.80%. The extracted oil was a dense, dark blue, viscous liquid with an intense aromatic scent.

Table 3 Essential oil content (% ADW) with regard to the origin of raw material

Origin	Essential oil content
Natural	0.50
Cultivation	1.10
Mean	0.80
$LSD_{0.05}$	0.11

The presence of 22 compounds was identified in the essential oil obtained from the inflorescences of wild-growing chamomile, among which sesquiterpene compounds had the highest proportions: the dominant compound *α*-bisabolol oxide A (31.70%), *α*-bisabolol oxide B (17.09 %), and *α*-bisabolone oxide A (15.73%); chamazulene was also found in a large amount (15.58%). Among the other compounds, the presence of (Z)-*β*-farnesene was found in large quantities (4.89%). The oil extracted from the wild-growing plants was marked by a higher concentration of sabinene, *p*-cymene, and a slightly higher content of germacrene D than that in the oil obtained from the 'Złoty Łan'. The

presence of artemisia ketone (1.38%) and dehydro-sesquicineole (0.10%), the compounds not identified in the oil extracted from the cultivated plants, was also found here.

Table 4. The composition of the essential oil from *C. recutita* of different origin

No.	Compound	RI	Natural origin		Cultivation	
			(%)	±SD %	(%)	±SD%
1	*α*-pinene	938	tr.	-	0.08	0.01
2	sabinene	976	0.47	0.01	0.07	0.01
3	yomogi alcohol	997	0.09	0.01	0.17	0.01
4	*p*-cymene	1027	0.35	0.01	0.19	0.03
5	1,8-cineole	1034	0.34	0.00	0.40	0.02
6	(E)-*β*-ocimene	1046	0.22	0.04	0.44	0.04
7	*γ*-terpinene	1053	-	-	1.26	0.16
8	artemisia ketone	1057	1.38	0.06	-	-
9	artemisia alcohol	1079	0.18	0.01	0.30	0.04
10	artemisyl acetate	1162	0.10	0.00	0.08	0.01
11	*α*-terpineol	1200	-	-	0.09	0.02
12	*β*-elemene	1390	-	-	0.08	0.01
13	E-caryophyllene	1428	0.08	-	0.12	0.00
14	(Z)-*β*-farnesene	1459	4.89	0.12	2.62	0.15
15	allo-aromadendrene	1469	-	-	tr.	-
16	dehydro-sesquicineole	1476	0.10	0.01	-	-
17	*α*-acoradiene	1485	-	-	0.05	0.00
18	germacrene D	1495	0.58	0.00	0.45	0.03
19	bicyclogermacrene	1510	0.72	0.03	0.75	1.00
20	E-nerolidol	1564	-	-	0.11	0.01
21	caryophyllene oxide	1568	tr.	-	-	-
22	spathulenol	1587	0.81	0.00	2.02	0.04
23	helifolen-12-al.	1624	-	-	0.29	0.00
24	*α*-bisabolol oxide B	1667	17.09	0.23	24.00	0.69
25	apiole	1689	-	-	0.05	0.00
26	*α*-bisabolone oxide A	1699	15.73	0.12	16.97	0.05
27	ni	1726	0.09	0.01	-	-
28	chamazulene	1744	15.58	0.39	24.85	0.07
29	*α*-bisabolol oxide A	1759	31.70	0.15	17.22	0.04
30	ni	1794	-	-	0.07	0.02
31	ni	1895	6.12	0.06	6.39	0.26
32	ni	1911	2.11	0.05	0.31	0.00
33	ni	1937	0.15	0.01	0.30	0.02
34	ni	1969	0.36	-	-	-
35	ni	2148	tr.	-	-	-
Total			99.87%		99.97%	

ni - compound not identified

The presence of 25 compounds was identified in the chamomile oil obtained from the inflorescences of the 'Złoty Łan' plants, among which chamazulene was predominant (24.85%). Other sesquiterpene compounds were also found in large quantities: *α*-bisabolol oxide B (24.00%), *α*- bisabolol oxide A (17.22%), and *α*-bisabolone oxide (16.97%). The proportion of (Z)-*β*-farnesene in the essential oil from chamomile 'Złoty Łan' was 2.62%. The essential oil extracted from the inflorescences of the 'Złoty Łan' plants had a higher concentration of E-*β*-ocimen and spathulenol as well as a slightly higher content of 1,8-cineole than the oil obtained from the wild-growing plants.

In addition, the presence of γ-terpinen (1.26%), α-acoradiene (0.05%), α–terpinolene (0.09%), β-elemene (0.08%), E-nerolidol (0.115), helifolen-12-al. (0.29%), and apiol (0.05%), the compounds not identified in the wild-growing plants, was found in the oil extracted from the cultivated plants.

DISCUSSION

The variations in the number and weight of chamomile inflorescences depending on plant origin show high morphological variability of this species, which is attributable to genetic and environmental factors. The wild-growing plants under study developed a higher number of inflorescences with a larger weight and diameter than the cultivated plants. 'Złoty Łan' is characterized by large inflorescences with a diameter from 2 to 3 cm and the weight of a single fresh flower head amounting to 0.21 g [25,26]. The present study showed much lower values of the morphological plant traits in question, which could have resulted from the less favourable weather conditions during the growing season. Even though the thermal conditions were close to the long-term average, but the moisture conditions were much less favourable at the initial stage of plant growth. Chamomile belongs to drought-sensitive plants and moisture conditions greatly affect its growth and yield [8,23]. It should be also added that the raw material from the natural stand was collected almost 2 weeks earlier than that from cultivation. The later flowering of the cultivated chamomile plants should be attributable to the slower growth and development of the plants caused by the spring sowing time, which is less favourable for this species as the moisture conditions are usually worse at that time than in the case of autumn sowing.

The essential oil content found in the studied plant material indicates a smaller influence of environmental variability and a higher effect of genetic variability on the chemical composition of the raw material. The inflorescences of the chamomile cultivar 'Złoty Łan' are distinguished by a high concentration of essential oil (1.05%), which was confirmed in this study (1.10%) in spite of the less favourable cultivation conditions for the plants. Chamomile is characterized by a great variability of the chemical composition, and its numerous types and varieties have a different potential for the accumulation of essential oil and its composition [3,20,21]. The accumulation of essential oil and the increased concentration of α-bisabolol compounds are promoted by high temperature and a large amount of rainfall [8]. However, on the other hand, the quantity and composition of chamomile oil changes adversely in the conditions of temperate and subtropical climate [14] as well as under the influence of high temperature and strong insolation [27], which results from the climatic requirements of this species.

The essential oil of the chamomile inflorescences under investigation was characterized by a high concentration of the compounds of bisabolol and chamazulene, which was reflected in the deep dark blue colour of the extracted substance. The obtained results show the German type of chamomile, also called blue chamomile; it is marked by a high proportion of chamazulene, farnesene, bisabolol, bisabolol oxide, and *cis*-spiroether in its oil [6]. The tetraploid varieties of German chamomile are distinguished by a high content of α-bisabolol and chamazulene [21,25], while most of the varieties grown in Europe belong to the chemical type A, characterized by an increased content of bisabolol oxide A and chamazulene [12,20]. The content of α-bisabolol and chamazulene in the oil of the 'Złoty Łan' found in the present study was comparable to the proportion of these components in Slovakian varieties [21] as well as in Egyptian and Brazilian

varieties [22], whereas it much exceeded the amounts reported for this variety [25] and for other chamomile varieties described in the literature [7,31]. This divergence can be explained by the different methods of raw material collection and drying as well as of essential oil extraction. The composition of essential oil differs depending on the distillation method [5,20] and the type of distilled material, but the variance in the analysis results obtained can result even from the different position of leaves or flowers on the plant [16].

The essential oil isolated from the inflorescences of wild-growing chamomile was characterized by a nearly twofold higher proportion of *α*-bisabolol oxide A, a comparable proportion of *α*-bisabolol oxide B and *α*-bisabolon oxide A as well as a slightly lower percentage of chamazulene than these proportions in the essential oil of the 'Złoty Łan'. This shows a great potential of wild-growing chamomile plants, sometimes substantially differing in the oil composition from the chemical profile of cultivated varieties. This phenomenon has been described in the context of the content of isoprenoids in chamomile volatile oil [9] and the accumulation of derivatives of apigenin, compounds of high biological activity [30]. Taking into account the results of the present study and those reported in the papers of the above cited authors, it can be concluded that habitat conditions, together with genetic factors, largely affect the growth and development of German chamomile as well as the composition of its essential oil and that the proportions of *α*-bisabolol oxide A, *α*-bisabolol oxide B and chamazulene varied to the greatest extent among the dominant constituents.

CONCLUSIONS

The studied plants of German chamomile growing in a natural state and the cultivated plants of the 'Złoty Łan' showed high variation in some morphological features, earliness of flowering as well as in the accumulation and composition of essential oil. The flower heads of the cultivated chamomile contained more essential oil with a higher concentration of chamazulene than those picked from the naturally growing plants. The oil of chamomile collected from the natural stand was in turn characterized by a higher proportion of *α*-bisabolol and (Z)-*β*-farnesene compared to the oil from the cultivated plants.

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SUMMARY

This study was carried out from April to July 2007 in Radawiec in the Lublin region. Chamomile raw material was obtained from cultivation (a field with a northern exposure, loamy sandy soil, soil class III, neutral pH) and from a natural stand (meadow area, the stand sheltered from the wind, open land with a northern exposure, loamy sandy soil with a neutral pH). The chamomile cultivar 'Złoty Łan' was grown, which was seeded directly in the field in the spring. Seeds were sown in the first decade of April in rows 40 cm apart at a rate 2 kg per 1 ha. The harvest was done in July by picking entire inflorescences; they were subsequently dried under natural conditions. Essential oil content in the raw material was determined using the hydrodistillation method with the addition of xylene in a Clevenger-type apparatus. The number of components identified in the oil was determined by the GC/MS method. The raw material obtained from the cultivated plants had twice more (1.1%) essential oil than the raw material from the wild-growing plants (0.5%). There were identified 22 (natural state) and 25 (cultivation) compounds making up the chamomile oil under study. The source of origin of chamomile raw material was shown to have a significant effect on the amount and quality of essential oil. The dominant compound in the oil extracted from the raw material obtained from the wild-growing plants was α-bisabolol oxide A (31.70%), while in that from the cultivated plants it was chamazulene (24.85%).

Keywords: German chamomile, origin of raw material, chamazulene, *α*-bisabolol oxide A

STRESZCZENIE

Badania przeprowadzono w okresie od kwietnia do lipca 2007 roku w Radawcu, woj. lubelskie. Surowiec rumianku pozyskiwano z uprawy (pole o wystawie północnej, gleba gliniasto-piaszczysta, klasa bonitacyjna III, odczyn obojętny) i ze stanowiska naturalnego (obszar łąkowy, stanowisko osłonięte od wiatru, teren otwarty o wystawie północnej, gleba gliniasto-piaszczysta o odczynie obojętnym). Uprawiano rumianek odmiany Złoty Łan, z siewu wiosennego bezpośrednio w pole. Nasiona wysiano w pierwszej dekadzie kwietnia, w rzędy odległe o 40 cm, w ilości 2 kg na 1 ha. Zbiór przeprowadzono w lipcu, zbierając całe kwiatostany, które następnie suszono w warunkach naturalnych. Zawartość olejku eterycznego w surowcu określona metodą hydrodestylacji z dodatkiem ksylenu, w aparacie typu Clevenger. Ilość składników zidentyfikowanych w olejku wyznaczono metodą GC/MS. Surowiec pochodzący z uprawy zawierał dwukrotnie więcej (1,1%) olejku eterycznego, niż surowiec pochodzący z roślin dzikorosnących (0,5%). Zidentyfikowano 22 (stan naturalny) i 25 (uprawa) związków wchodzących w skład badanego olejku rumiankowego. Wykazano istotny wpływ źródła pochodzenia surowca rumianku na ilość i jakość olejku eterycznego. Związkiem dominującym olejku ekstrahowanego z surowca roślin dzikorosnacych był tlenek *α*-bisabololu A (31,70%), a u roślin uprawianych chamazulen (24,85%).

Słowa kluczowe: rumianek pospolity, pochodzenie surowca, chamazulen, tlenek *α*-bisabololu A