Chapter 7
Chamomile Biodiversity of the Essential Oil Qualitative-Quantitative Characteristics

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Abstract Biosynthesis of the special metabolite in ecological system is presented the unique and specific metabolite process, which is placed under general biomass production periodicity of plant. Each plant species has own specific limiting boundaries. There are presented its tolerance to influence of eco-physiological conditions. Extend of this tolerance is called the species ecological amplitude. Chamomile plants, *Matricaria recutita* L., have very wide ecological amplitude and this species geographical occurrence in practical all over the world. Plant habitat and the creation of secondary metabolites in plants are depended on the endogenous and exogenous factors, which can be divided in two groups: (a) morpho-ontogenetic variability, (b) genetic variability respectively genetic determination [1]. In chamomile plant habitat (diploid) is not possible to determinate any principal differences among plants. Impulse for the totally new valuation of the research and development has become if the identification of four chief chemical types of chamomile different by the qualitative – quantitative composition of chemical compounds (sesquiterpenes) in the essential oil was carried out [2]. This very important fact was referred to chamomile biodiversity. This biodiversity was created during long time process (evolution) in regard to influence of eco-physiological conditions (biotic- and abiotic- factors) on the concrete place of chamomile population growth. Contribution presents the results of the chamomile essential oil qualitative-quantitative characteristics of chemo types which are originated from various geographical parts of the world (Slovakia, Ukraine, Poland, Egypt, Malta and Crimea). Nowadays the centre of attention is being devoted to cultivation, breeding (tetraploid plants) and seed production of this special crop [3].

Keywords Biodiversity, **/-/-**α-bisabolol, **/-/-**α-bisabololoxide A and B, chamazulene, chamomile, chemo types, essential oil, sesquiterpenes

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Introduction

Chamomile, *Matricaria recutita* L., is generally known as a weed – a weed with curative power. A medicinal plant, with a history going back to the time of Egyptian pharaohs, is used in phytotherapy today and unequivocally will be used tomorrow.

Chamomile is the most favorite and most used medicinal plant species in Slovakia. A very old folk saying in Slovakia says: "*An individual should always bow before the curative powers of the chamomile flower tea*". This respect resulted from hundreds of years experience in using it as a cure in folk medicine in this country.

About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds [4]. A substantial part of drug effects are determined by the essential oil content. Oil is collected from flower heads, either by steam distillation or solvent extraction, for yields of 0.24–1.90% of fresh or dry plant tissue. Among the essential oil constituents the most active are /-/-α-bisabolol and chamazulene. /-/-α-bisabolol has demonstrated anti-inflammatory, antispasmodic, antimicrobial, antiulcer, sedative and CNS activity. Chamazulene is also anti-inflammatory. Topical applications of chamomile preparation have shown benefit in the treatment of eczema, dermatitis and ulceration [5].

Material and Methods

Plants. Plant material, chamomile anthodia (42 different samples), was collected from natural sites (together 30 localities) in the East-Slovakian Lowland during four years (1995, 1996, 1997 and 1998). The dry flower heads were obtained from additional localities (Ukraine, Poland, Egypt, and Malta abroad Crimea) abroad in 1995, 1997, 1998, 2002, 2004 and 2005.

Flower chamomile drug (*Chamomilae Flos*) were dried in shielded room under laboratory standard temperature.

Steam distillation. Chamomile essential oil was isolated by steam distillation. Hydro distillation lasted for 2 h into n-hexane, sample weights were 2 g of dry drug matter. The modified distillation apparatus by Coocking and Middleton was used [6].

Chromatography. Compounds of essential oil were determined by the means HEWLETT –PACKARD 5890 Series II system, with capillary column HP–5, FID detector, Split-split less system for injection and automatic injector HP 7673.

The operating conditions were: injection temperature 150°C, detector temperature 250°C, carrier gas nitrogen. Sample sizes were used 1.0 μl and manual type of injection.

Composition of Chamomile essential oil was determined by capillary GC analysis: Hewlett-Packard 5890 Series II with FID and Split-split less system for injection. The column was used HP-5 (50 m long x 0.20 mm i.d.). The following temperature program was used: 90°C (0 min), then 10°C min^{-1}–150°C (5 min), then 5°C min^{-1}–180°C (3 min), then 7°C min^{-1} to finally isothermal 280°C for 25 min; nitrogen was used as carrier gas. Detector temperature 250°C, carrier gas nitrogen (flow velocity 274 mm s^{-1}), auxiliary gases were nitrogen (30 ml min^{-1}), hydrogen (30 ml min^{-1}), air (400 ml min^{-1}).

Peak areas and retention times were measured by electronic integration with a Hewlett-Packard 3396 Series II integrator.

Compound identification. Determination of major components of essential oil was realized on the basis of use of standard compounds (/-/-α-bisabolol, chamazulene, cis-/trans-en-in-dicycloether and /-/-α-bisabololoxide A and B). Qualitative identification of selected components was carried out by the comparison the retention times of all detected components with retention time of standard compounds.

Results are presented in the percentage. Percentage of single chromatographic peak areas was measured on the basis of area of the single peaks to the total peak area ratio. Selected results were statistically evaluated using a t-test at the 0.05 level. The intervals of significance are presented in tables.

Results and Discussion

In a natural ecosystem, plant species separated on the basis of their secondary product contribute to the development or modification of the existing ecological balance. However, for a full understanding of their production processes, a detail analysis of the occurrences, and the possible exact circumscription of their role in the phytocenosis, as revealed by studies on geographical distribution of the secondary products, is required [7].

One part of production of the Slovak chamomile is collecting of the free growing plants originated approximately till the half of the 1950s, when large-scale cultivation began to be introduced. Chamomile can be found in the secondary plant communities in the East-Slovakian Lowland, such as trodden societies on dry and moist soils, weed and dump societies. There was large – scale monitoring of chamomile gene pool realized from the species identification of wild – grown population chemo types in 1995–1998 in the East Slovakian Lowland.

The percentage of essential oil content in dry chamomile flower-heads and its qualitative and quantitative characteristics, which were determined by the GC-analysis are presented in comprehensive Table 7.1. Percentage contents of the essential oil from chamomile flower anthodia were ranging 0.63 ± 0.19 over the whole examined samples.

Table 7.1 presents essential oil composition of dry chamomile flowers from individual natural sites and years of their collection in the East-Slovakian Lowland.

The highest contents of /-/-α-bisabololoxide A (39.9 ± 7.5%) and /-/-α-bisabololoxide B (9.75 ± 4.20%) are typical for chamomile plants, which flower anthodia were collected in various places in the East-Slovakian Lowland. In regard to following sesquiterpenes, the chamomile anthodia contain /-/-α-bisabolol (5.09 ± 1.55%) and chamazulene (7.65 ± 3.90%).

Chamomile widely spread in the Ukrainian Carpathians, is a popularly known medicinal herb. In nature, it is predominantly found in fields, meadows, gardens, along the roads growing as weeds, and sometimes forming dense stands. This

Table 7.1 Essential oil content and its composition of dry chamomile flowers from natural sites in the East-Slovakian Lowland

Localities on the East-Slovakian Lowland	Essential	Basic composition of essential oil (%)			
	Oil (%)	Fa	Bo	Ch	BoA
Michalovce ('95,'96,'97,'98)	0.55–0.91	1.2–6.1	2.6–6.8	6.3–7.5	28.9–40.4
Vojany ('95, '96, '97, '98)	0.40–0.71	5.3–10.5	3.4–6.1	5.0–9.1	39.8–48.4
Vysoká n/Uhom ('95, '96, '97)	0.75–0.96	8.1–13.2	4.4–6.8	4.4–10.1	38.5–48.9
Vybuchanec ('95, '96)	0.52–0.97	6.0–6.1	6,6–6,7	5.4–5.5	43.1–43.6
Trebišov ('96,'97)	0.50–0.60	11.7–13.6	2.9–3.0	10.1–10.2	36.1–42.5
Veľké Raškovce ('97,'98)	0.65–0.70	4.2–18.0	3.2–3.5	6.9–10.1	33.1–37.8
Krišovská Liesková ('96,'98)	0.53–0.90	4.0–12.2	2.5–6.6	3.8–4.0	36.7–42.2
Bajany ('95, '98)	0.60–0.74	1.5–3.2	3.8–5.1	4.7–7.4	32.8–52.9
Nižný Hrabovec ('95)	0.61	3.8	4.9	10.8	34.6
Vranov nad Topľou ('95)	0.82	4.1	5.2	7.6	48.3
Moravany ('95)	0.93	4.6	9.6	9.9	37.9
Sírnik ('95)	0.86	4.0	6.2	10.6	40.0
Malé Raškovce ('96)	0.63	9.9	4.8	10.2	36.2
Rakovec nad Ondavou ('96)	0.92	8.0	5.0	11.0	42.3
Čierne Pole ('96)	0.72	8.3	4.5	9.8	43.8
Tibava ('96)	0.85	12.6	5.1	9.0	36.8
Stretava ('96)	0.60	2.9	7.7	10.8	45.6
Beša ('96)	0.62	7.4	6.0	8.3	45.3
Veľké Kapušany ('96)	0.55	7.9	6.9	6.9	48.7
Zálužice ('97)	0.75	15.5	5.2	4.4	33.9
Pavlovce nad Uhom ('97)	0.60	15.2	6.8	6.3	41.6
Pozdišovce ('97)	0.30	17.2	6.8	5.8	26.6
Bracovce ('97)	0.74	16.1	4.7	6.9	36.2
Malčice ('97)	0.82	15.9	2.4	8.9	30.2
Trhovište ('97)	0.90	15.7	5.1	6.2	38.6
Vojčice ('98)	0.97	2.10	5,1	7.6	42.8
Novosad ('98)	0.70	1.5	4.0	12.0	39.9
Hraň ('98)	0.60	2.9	5.5	9.5	35.8
Zemplínska Branč ('98)	0.50	0.4	3.2	7.7	49.2

Fa – farnesene, Bo – /-/-α-bisabolol, Ch – chamazulene, BoA – /-/-α-bisabololoxide A

plant species grows very frequently at abandoned disposal tips, from where the local population usually collects it for medicinal purposes. In Transcarpathia, it comes into flower in the 20th of May – beginning of June; mass flowering and, consequently, bulk collection takes place in the 1st half of June.

Flowers have a highest /-/-α-bisabololoxide A content, about 41% (Table 7.2). The pharmaceutically effective components (/-/-α-bisabolol and chamazulene) have a lower representation.

Chamomile has long been one of the very important medicinal plants collected and cultivated in Poland. A comparison of essential oil yields from the four Polish samples indicates the lowest essential oil content, about 0.3%, in the chamomile

Table 7.2 Quality of essential oil from the chamomile population in Transcarpathia region

Beregovo Rajon (locality, 2005)	Fa	BoB	BnA	Bo	Ch	BoA	Dc	
	% in essential oil						-cis	-trans
Velikaja Bakta	2.9	13.4	6.1	7.5	10.3	41.5	7.3	2.2

Fa – trans-β-farnesene, BoB – /-/-α-bisabololoxide B, BnA – /-/-α-bisabolonoxide A, Bo – /-/-α- bisabolol, Ch – chamazulene, BoA – /-/-α-bisabololoxide A, Dc – en-in-dicycloethers

Table 7.3 Contents (%) of main components in chamomile anthodia from Poland

Composition of essential oil (%)	Areas near Lublin (Poland, 2004)			
	Sample no. 1	Sample no. 2	Sample no. 3	Sample no. 4
/-/-α-bisabolol	17 ± 1	20 ± 2	17 ± 1	18 ± 1
Chamazulene	15 ± 1	16 ± 2	15 ± 1	17 ± 1
/-/-α-bisabololoxide A	15 ± 1	16 ± 2	15 ± 2	17 ± 1
/-/-α-bisabololoxide B	25 ± 2	28 ± 2	23 ± 2	27 ± 2
En-in-dicycloethers	3 ± 0.5	2 ± 0.5	2.5 ± 0.5	1,5 ± 0.5
β-farnesene	10 ± 1	10 ± 1	10 ± 1	10 ± 1

Table 7.4 Quality of essential oil from the Egyptian chamomile cultivation (1997/1998)

Egyptian localities	Fa	BoB	BnA	Bo	Ch	BoA	Dc	
	% in essential oil						-cis	-trans
El Fayoum	18.2	4.9	9.8	5.5	2.0	40.1	9.7	1.3
Beni Suef	5.1	2.7	8.7	2.4	1.7	50.7	8.7	1.5
El Tahrir Sahra	3.5	2.4	7.3	11.2	2.0	51.1	5.9	2.1
El Giza	2.4	1.6	2.7	3.6	2.6	68.2	7.6	1.8

Fa – trans-β-farnesene, BoB – /-/-α- bisabololoxide B, BnA – /-/-α-bisabolonoxide A, Bo – /-/-α- bisabolol, Ch – chamazulene, BoA – /-/-α-bisabololoxide A, Dc – en-in-dicycloethers

tubular flowers. One of the reasons is certainly ontogenetically stage of flower development. The flower heads were in full bloom with a beginning of seed creation. The high content of /-/- α-bisabololoxide B and A is characteristic to the Polish chamomile samples (Table 7.3). The higher part of chamazulene among other components is given a value for this raw material. The high content of chamazulene in the essential oil (15–20%) was presented by Seidler-Lozykowska [8].

One from very important exporter in the world of the chamomile flowers is Egypt. The possibility to visit some chamomile production areas in this African country occured several times in 1997 and 1998. Chamomile flowers from different Egyptian areas have a highest /-/-α-bisabololoxide content, from 40.1% to 68.2% (Table 7.4). The pharmaceutically effective components (/-/-α-bisabolol and chamazulene) have very low representation. It is great pity that a cultivar or variety with the high content of bisabolol and chamazulene has not been yet introduced and a claim "for the future it is important to use selected seeds that yield flowers with a high percentage of azule" [9] remains in force a very long time.

In regard to the sesquiterpenes, original forms mostly show bisabololoxides. A form rich in /-/-α-bisabolol could be found in Spain /Catalonia/. The first time this proclamation was given was in 1973 [10], much earlier. Unfortunately the sufficient documentation material or scientific paper is not enduring. It is not suitable to comment at this time. The endemically, local and isolate chamomile population could be a type rich in /-/-α-bisabolol. The evidence is presented in Table 7.5. The chamomile genetically investigations are clear: /-/-α-bisabolol is inherited recessively and the formation of /-/-α-bisabololoxide A and B is dominant over one [11].

Impulse for the valuation of the chamomile collection has become if the identification of four chemical types of this plant species different by the qualitative – quantitative composition of chemical compounds in the essential oil was carried out by Schilcher in 1987 (Table 7.6). This very important fact was referred to chamomile biodiversity. This biodiversity was created during long time process (evolution) in regard to influence of eco-physiological conditions (biotic- and abiotic- factors) on the concrete place of chamomile population growth.

The results of qualitative-quantitative characteristics of chamomile essential oil from Slovakia, Ukraine and Egypt are showed there is the chemo type B of chamomile population. The results of two chamomile essential oil samples without any

Table 7.5 The /-/-α- bisabolol chamomile from two European Isles

	Fa	BoB	BnA	Bo	Ch	BoA	Dc	
	% in essential oil						-cis	-trans
Malta/1995/	6.3	1.1	Trace	67.3	0.9	1.1	8.8	1.3
Crimea/2002/	4.3	1.2	Trace	68.6	1.8	1.2	7.9	1.7

Fa – Trans-α-farnesene, BoB – /-/-α-bisabololoxide B, BnA – /-/-α-bisabolonoxide A, Bo – /-/- - bisabolol, Ch – chamazulene, BoA – /-/-α-bisabololoxide A, Dc – en-in-dicycloethers

Table 7.6 Four basic chemotypes of chamomile in regard to composition of main components (%) in essential oil [12]

	Type A	Type B	Type C	Type D
α-bisabololoxide A	4.74–15.68	31.07–52.25	2.13–18.50	9.62–25.83
α-bisabolol	4.37–15.41	8.81–12.92	24.18–77.21	8.49–19.58
α-bisabololoxide B	22.43–58.85	5.27–8.79	3.17–34.46	10.43–24.20
En-yn-dicycloethers	2.61–11.27	4.08–9.90	1.92–12.00	5.51–10.68
Chamazulene	2.70–17.69	5.40–7.95	1.45–14.90	1.91–7.89
Type A	α-bisabololoxide B	>α-bisabololoxide A	>α-bisabolol	
Type B	α-bisabololoxide A	>α-bisabololoxide B	>α-bisabolol	
Type C	α-bisabolol	>α-bisabololoxide B	>α-bisabololoxide A	
Type D	α-bisabololoxide B	≈α-bisabololoxide A	≈α-bisabolol	

color are determined the chemo type C on islands: Malta and Crimea. The Polish chamomile belongs to chemo type D.

The chemo type A is typical for chamomile with a high /-/-α-bisabololoxide B content of Argentine origin [13].

This fact was very important in these parts of the world where parts diploid chamomile population characterised by high content of bisabololoxide constituents occurred in large quantities. In regard to this observation began very intensive plant breeding program considering the flower anthodia yield, essential oil production and chamazulene and /-/-α-bisabolol content (an active components with the most precious pharmacological characteristics). Nowadays the centre of attention is being devoted to cultivation, breeding (tetraploid plants) and seed production of this special crop [3].

Chamomile is a very tolerant plant. It grows on areas with different soil and climate characteristics. The plants prefer a light and need intensive sunlight for their growth. They usually have a short vegetation period (90–100 days). Plant habitat is depended on the endogenous and exogenous factors, which can be divided in two groups: (a) morpho-ontogenetic variability, (b) genetic variability respectively genetic determination in order to diploid or tetraploid plants [1]. The evident range of ecological amplitude of chamomile species gives to the chamomile property to adapt to the less sufficient soil-climatic conditions [14]. Morphological variability is very extensive in the chamomile species. The morphological characteristics of chamomile plants are not stable and have direct connection to physio-ecological background of the plant grow. Therefore this plant represents suitable material for the study of variability and morphological variability of features such as plant highness, plant diversity, and biomass production of roots, stems, leaves, flower anthodia and number of the flower anthodia [15].

Conclusions

Chamomile plants, *Matricaria recutita* L., have very wide ecological amplitude. Plant habitat and the creation of secondary metabolites (natural products) in plants are depended on the endogenous and exogenous factors. Impulse for the totally new valuation of the chamomile research and development has become if the identification of four chief chemical types of chamomile differences by the qualitative – quantitative composition of chemical compounds in the essential oil was carried out in 1973. This very important fact is referred to chamomile biodiversity. This biodiversity was created during long time process (evolution) in regard to influence of eco-physiological conditions (biotic- and abiotic-factors) on the concrete place of chamomile population grows. Chamomile is a very tolerant plant. The exogenous morphological characteristics of chamomile plants are not stable and have direct connection to physio-ecological background of the plant growth.

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