Analysis of Biologically Active Essential Oil Components of Chamomiles in Hungary (In Vivo – In Vitro)

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Keywords: chamomile (*Chamomilla recutita,* syn.:*Matricaria recutita)*, essential oil, (-)-α-bisabolol, chamazulene, ß-eudesmol, berkheyaradulene, α-selinene, geranyl-isovalerate, cedrol

Abstract

We studied the essential oil production of cultivated (*BK-2*, Degumil) and wild chamomile populations of 4 typical chamomile-rich regions of Hungary. We examined the essential oil composition of flowers, herbs (stem plus leaves) and roots using GC and GC/MS methods. Among cultivated species, the Hungarian BK-2 contains more chamazulene in its essential oil than the German Degumil type, which is mainly cultivated for its α-bisabolol. Both components have important antiinflammatory activities. Wild populations can be easily distinguished from cultivated ones by their high amount of bisaboloides, particularly the flower of *Szabadkígyós* wild type, which contained on average 48 % of the biologically active (-)-α-bisabolol.

The regional wild chamomile samples mentioned above have already been examined previously in our Institute. We found it interesting to compare the content of biologically active components, at the same conditions, of presently promising populations with the results obtained 20 years ago from the same species. While the content of the essential oil of rural *Szabadkígyós* wild type remained unchanged, there was a trend of the essential oil components towards the therapeutically important compounds. The amount of (-)-α-bisabolol in *Szabadkígyós* mounted up to a 3-fold increase and we measured a doublefold increase of chamazulene content in *BK-2* compared with samples 20 years ago. We can conclude that although a change was observed in the essential oil content and also in the proportion of different components, the fundamental characteristics of the oils remained the same.

To keep the genom of *Szabadkígyós* wild type having high (-)-α-bisabolol content, we used biotechnological methods. The sterile roots of organised culture contained also β-eudesmol, wich was firstly identified from the intact roots by us. Our gas chromatographical and mass-spectroscopical studies showed that sterile chamomile cultures generated the most important terpenoid and polyin compounds characteristic of the mother plant. We identified berkheyaradulene, α-selinene, geranyl-isovalerate and cedrol as new components in these sterile cultures.

INTRODUCTION

In Hungary, chamomile, (*Chamomilla recutita,* syn.:*Matricaria recutita* [L.] Rauschert; Asteraceae) is one of the most common medicinal plants. Its importance is widely known in both official and folk medicines. Chamomile owes its therapeutical activity to different groups of effective substances, which make up the complex effect of the drug (Chamomillae anthodium). Essential oils are of greatest importance among all effective substances.

The essential oil content of plant parts under and above ground depends on different chemotypes (Hänsel et al., 1992). According to the bisabololoxide content, commercial chamomile populations are classified as types of bisabolol, bisabololoxide A and B, and bisabolonoxide (Schilcher, 1973, 1987). During the ontogenesis the essential oil content changes, reaching a maximum in the flower just before flowering (0.3-1.5%),

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and decreasing after the process of flowering. This is certainly the case for matricin, bisaboloid content and E-β-farnesene (Franz, 1986).

The root of chamomile contains only traces of essential oil (0.02-0.11%). Despite the fact that some therapeutically active compounds such as chamazulene and (-)-α-bisabolol are missing, other substances such as E-β-farnesene, α-farnesene, chamomilla esther, en-in-dicycloethers, chamomillol, β-caryophyllene and caryophyllene-epoxide can be detected. The other active substances in the flowers of chamomile are flavonoids, coumarins and polysaccharides (Della Loggia et al, 1986; Carle et al., 1987; Sváb, 1993; Tyihák, 1962).

The main anti-inflammatory activity is due to chamazulene, which is formed during the distillation of the oil of matricine, and (-)-α-bisabolol (Ammon and Kaul, 1992) (Fig. 1), but also bisabololoxide A and B play a role (Jakovlev et al., 1983). Spasmolytical effects are attributed to apigenin and bisabololoxides (Achterrath-Tuckermann et al., 1980) and wound healing properties to chamazulene, apigenin and (-)-α-bisabolol (Glowania et al., 1987). In addition, the chamomile oil has an antibacterial effect on Gram-positive germs and a fungicidal effect on *Candida albicans* when used in concentrations of more than 0.025% (Szalontai et al., 1977). It should be noticed that chamomile flowers of the so called bisabololoxide B-type also contain an allergic compound: anthecotulide (Hausen et al., 1984). Both cultivated and wild chamomile types are used in Hungary for therapeutical purposes. In earlier investigations Máthé (1960, 1979) has found an increase of proazulenes in the flower of chamomiles, whereas Marczal and Verzár-Petri (1980) have found an increase in the bisaboloid content over the years. It was established that the content and composition of the essential oil of wild chamomile populations are related to the type of chamomile occurring in different areas of Hungary.

Based on examinations made 20 years ago chamomile populations from the 4 best areas were studied again. Our aim was to study the features of the essential oil production of the chamomile types in these 4 regions of Hungary in order to select chamomile types rich in therapeutically active substances, which can be then kept in a seed bank.

MATERIALS AND METHODS

Plant Material

Wild chamomile populations were obtained from soily areas of *Vésztõ, Szabadkígyós* and the National Park in *Hortobágy*. They have common morphological features and are rich in (-)-α-bisabolol (Marczal, 1982). In addition, another wild type was obtained from the area of *Szeghalom*. The improved German Degumil and Hungaryan polyploid *BK-2* type was cultivated in Kerepes.

The plant material was collected in the end of May on three consecutive years (1997-99) as was done in 1977-79.

Sterile Chamomile Cultures

Sterile chamomile plants were obtained by sterilization of seeds of intact plants with ethanol, then ethanol-mercury-chlorid and methyl-pyridine-chlorid solution. The seeds were then rinsed three times with sterile distilled water (Szőke, 1979). Young plantlets were then cultivated at 2500 Lux (16 hours light, 8 hours dark photoperiod) at 26 ºC, on solid ½ Murashige-Skoog (1962) hormone-free media.

Extraction and Investigation of the Essential Oil

The essential oil from roots, herbs (stem plus leaves), and flowers was extracted by steam distillation with apparatus according to the Ph.Hg.VII. (Pharmacopoea Hungarica, 1986). Fifteen to twenty grams of powdered drug, suspended in 500 ml of water, were distilled for 3 hours. The essential oil content was measured gravimetrically according to the Ph.Hg.VII. Gas chromatography, standard addition and/or GC-MS methods were used to identify the oil components.

Gas chromatographic (GC) parameters:

Gas chromatograph*:* FISONS GC 8000; Column: 30m × 0.32mm, I.D; 0.25μm; Stationary phases: DB-1701 and DEX_m; Column temperature: 60-230°C, 8°C/min, 230°C, isotherm 3 min.; Detector temperature: flame ionisation, 240°C; Carrier gas: Nitrogen, pN_2 = 50 kPa, V = 6.8 cm^3/ min; Injector temperature*:* 200°C; Injection: splitless: 10 sec. Injected solution concentration: 2 μl/2 cm^3 chloroform; Injected solution volume: 0.4 μl. The percentage (%) evaluation of oil components (in the essential oil) was carried out on basis of peak-area by Chrom Card computer programme.

GC-MS parameters (GC-MS: Finnigan GCQ):

Gas chromatographic parameters: chromatograph type: Finnigan GC; Column: 30 m, I.D.: 0.22 mm; Film thickness: 0.25 μm; Stacionary phase: BPX5 (not polar); Column temperature: 60-230°C, 8°C/min, 230 °C isotherm 3 min.; Detector: Finnigan MS; Carrier gas: He, pHe = 40.0 psi; Carrier speed: 40 cm/s; Injector temperature*:* 200°C; Injection: splitless: 6 s; Splitless rate: 1/10; Injected solution volume: 0.4 μl.

MS-parameters: Start: 3 min after injection; Mode: Electron-impact-ionisation (EI) positive ion; Mass rate: 40-650; Scanning: 1 analyse/s; Evaluation: Finnigan GCQ 2.0 computer programme.

RESULTS AND DISCUSSION

Cultivated and Wild Chamomile

The total essential oil content and the percentile distribution of its components were evaluated in selected wild chamomile populations chosen according to the previous investigations (Marczal, 1982). In addition, comparison with cultivated *BK-2* type chamomile concerning the essential oil was done. Table 1 shows that the highest amount of the total essential oil is found in the flowers of wild *Hortobágy* population (0.70%). A similar tendency was observed in the same chamomile type concerning the herbs (0.12%).

According to the GC analysis it was clear that the flowers of the *Szabadkígyós* and *Vésztő* populations (1997) are the richest in (-)-α-bisabolol (35% resp. 41,5%) and the flowers of *BK-2* in chamazulene (23%) (Table 2). The highest bisabololoxide A content is found in *BK-2* (36 %), whereas the *Hortobágy* population has the highest bisabololoxide-B concentration. The *BK-2* type is also important for its chamazulene content. In our samples, the contents of (-)-α-bisabolol and chamazulene are also in inverse proportion to each other as compared with other results found by Máthé (1960). Cycloethers occur in about the same proportion in the studied samples; the content of cis-en-in-dicycloethers shows the double amount of trans-isomers, except for the *BK-2* type where the amount of trans-en-in-dicycloethers exceeds that of cis-isomers. It is remarkable that the (-)-α-bisabolol content is on average 48% (1997-99) in the oil of *Szabadkígyós* population. Therefore we plan to keep the genom of this type using biotechnological methods in order to produce chamomiles with high content of active substances.

Concerning the herbs, its essential oil content is equally low both in cultivated and wild chamomile populations (Table 1). Both in the herbs of the cultivated *BK-2* and the wild populations from *Szabadkígyós,* E-β-farnesene is the main component. Cis-spiroethers exceed the content of trans-isomers in all cases (Table 3).

The characteristic main component of the root is again the E-β-farnesene but its amount is smaller than in herbs. In the essential oil of *BK-2* root it reaches nearly 40 %. Wild populations are also rich in percentile distribution of these components similarly to the cultivated *BK-2* type. In the oil of the roots β-eudesmol was determined and identified by GC/MS, which component is characteristic for wild populations. The highest value of β-eudesmol was found in the oil of roots of *Szeghalom* (Table 4).

We compared the essential oil production of a cultivated and a wild chamomile population of high percentage of therapeutically active components, with the results of the surveys made by our Institute 20 years ago (1977-79) (Fig. 1A, B). The essential oil content in the flowers of wild *Szabadkígyós* was lower 1977-79 (0.36 %) than in 1997-99 (0.47 %) (Fig. 1B). As for the cultivated *BK-2* type, its content decreased in 1997-99 to a

quarter of that made previously (1.13 %) (Fig. 1A), but at the same time the content of the therapeutically important chamazulene almost doubled (Fig. 2). Parallel with a little increase of chamazulene, in wild populations there was an increase of (-)-α-bisabolol in all samples, particularly in the *Szabadkígyós* populations where its value of 15.5 % reached a value of 48 % in 1997-99. This data is noteworthy, since the total essential oil content of *Szabadkígyós* samples showed a little increase as well (Fig. 2). We can conclude that in the *Szabadkigyós* type the (-)-α-bisabolol content still makes up the major part of essential oil components, while in the *BK-2* type the characteristic feature, namely chamazulene remains present in the highest percentage of all essential oil components. Referring to herbs and roots, they both had higher essential oil contents than previously detected (Fig. 1). It is remarkable that in the herbs as well as in the roots of cultivated and wild populations the E-β-farnesene content increased significantly, while the distribution of α-farnesene in the essential oil practically remained unchanged (Fig. 2).

We can conclude that, although a change was observed in the essential oil content and also in the proportion of pharmacologically active compounds in comparison with the results of the earlier survey, the fundamental characteristics of the oil of cultivated and wild chamomile populations remained the same.

Sterile in vitro Chamomile Cultures

Among wild chamomile populations in Hungary, a population was found in the area of Szabadkigyos containing significant amounts - on average 48 % - of α-bisabolol in its inflorescences. We planned to keep the genom of this type using biotechnological methods (Máday et al., 1999) in order to produce chamomiles with high content of active substances.

Sterile organised chamomile cultures were cultivated on solid ½ Murashige-Skoog (1962) hormone-free media. Figure 3 shows the essential oil content (%) of herbs and roots in cultivated (Degumil) and wild chamomile (Szabadkígyós) populations in vivo and in vitro.

The sterile roots contained also no α-bisabolol but a new sesquiterpene alcohol β-eudesmol was firstly identified from the intact roots by us. Gas chromatographical and mass-spectroscopical studies showed that sterile chamomile cultures generated the most important terpenoid and polyin compounds characteristic of the mother plant. We identified berkheyaradulene, α- selinene, geranyl-isovalerate and cedrol as new components in these sterile cultures. Furthermore, in vitro cultures were made from this population to obtain propagation material containing a high number of other active substances too.

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Tables

Table 1. Total essential oil content (%) of flowers, herbs and roots in cultivated and wild chamomile populations

Chamomile type	Total essential oil content (%)		
	Flower	Herb	Root
BK – 2	0.33	0.08	0.13
Degumil	0.78	0.07	0.04
Szeghalom	0.55	0.07	0.05
Vésztő	0.31	-	-
Hortobágy	0.70	0.12	0.02
Szabadkígyós	0.43	0.07	0.12

Table 2. Percentile distribution of oil components in the total essential oil of **flowers** in cultivated and wild chamomile populations (1997)

	Degumil	BK - 2	Szeg-halom	Vésztő	Horto-bágy	Szabad-kígyós
β-caryophyllene	0,15	0,07	0,20	0,65	0,68	0,90
tr-β-farnesene	12,80	8,22	9,53	7,20	11,07	15,28
germacrene-D	0,55	0,16	0,83	0,53	0,62	5,78
α-muurolene	2,86	1,00	0,37	0,24	0,33	1,43
α-farnesene	0,30	0,27	1,62	1,34	2,43	0,15
α-cadinene	0,90	0,85	2,61	1,93	2,99	3,75
spathulenol	0,46	0,90	1,01	0,92	0,81	0,77
bisabolol-oxide B	7,32	8,25	18,37	15,10	20,42	3,61
(-)-α-bisabolol	30,00	1,59	20,72	34,91	24,00	41,45
bisabolon-oxide	0,55	4,13	3,55	2,60	2,51	1,07
chamazulene	24,50	23,41	10,84	5,23	9,31	8,71
bisabolol-oxide A	6,00	36,27	16,67	13,07	11,24	0,42
cis-spiroether	7,48	3,43	5,64	4,12	4,28	6,05
trans-spiroether	0,90	6,01	2,66	1,08	1,87	3,70

Table 3. Percentile distribution of oil components in the total essential oil of **herbs** (stem plus leaves) in cultivated and wild chamomile populations (1997)

	Degumil	**BK - 2**	**Szeg-halom**	**Hortobágy**	**Szabad-kígyós**
M^+ 204	-	0,29	0,16	0,32	0,36
berkheyaradulene	0,20	1,21	0,72	0,98	1,65
α-selinene	+	0,21	0,21	0,21	0,28
β - caryophyllene	+	0,22	0,15	0,22	0,16
tr-β- farnesene	56,74	59,03	52,47	59,01	58,51
germacrene-D	0,43	0,74	0,52	0,70	0,28
α-muurolene	2,17	1,68	1,27	1,68	0,62
α-farnesene	3,60	6,61	4,40	6,02	2,06
α-cadinene	0,50	1,77	2,65	2,32	1,16
spathulenol	0,86	2,85	5,96	3,00	2,81
bisabolol-oxide B	0,45	0,67	1,37	+	3,10
(-)-α-bisabolol	3,93	0,47	3,28	0,47	1,01
bisabolon-oxide	0,20	0,29	0,39	+	0,33
bisabolol-oxide A	0,26	0,28	0,51	0,20	+
cis-spiroether	6,70	9,95	7,23	9,95	4,92
chamomilla ester	0,14	0,11	0,57	0,11	0,39
trans-spiroether	3,70	1,82	3,21	1,82	1,82

+ in traces

Table 4. Percentile distribution of oil components in the total essential oil of **roots** in cultivated and wild chamomile populations (1997)

	Degumil	**BK - 2**	**Szeg-halom**	**Hortobágy**	**Szabad-kígyós**
M^+ 204	0,18	2,02	0,60	0,79	0,60
berkheyaradulene	0,83	7,20	2,27	2,94	1,75
α-selinene	0,21	1,32	0,58	0,65	0,59
β-caryophyllene	0,13	0,91	0,33	0,49	0,40
tr-β-farnesene	25,80	39,8	30,7	35,80	30,2
germacrene-D	0,11	0,42	0,80	0.22	0,73
α-muurolene	0,74	1,97	1,15	2,36	2,98
α -farnesene	1,20	3,06	1,72	2,85	1,17
α-cadinene	0,07	+	0,12	0,17	0,15
geranyl-isovalerate	3,37	2,50	1,53	1,30	1,48
spathulenol	0,56	0,60	0,93	0,48	0,73
cedrol (M^+ 222)	24,9	6,42	24,2	6,95	17,54
bisabolol-oxide B	0,70	1,50	1,12	1,51	2,63
β - eudesmol	8,23	1,10	9,25	3,16	4,87
bisabolon-oxide	0,26	0,18	0,26	0,16	0,22
cis-spiroether	13,85	15,00	11,25	24,50	16,10
chamomilla ester	0,55	1,10	0,14	0.39	0,25
trans-spiroether	4,03	1,12	1,42	2,00	3,10

+ in traces

Table 5. Comparing of percentile distribution of oil components in the total essential oil of sterile organised culture (1/2 MS medium) from cultivated and wild chamomile populations

	Degumil		Szabadkígyós	
Component	**sterile herb**	**sterile root**	**sterile herb**	**sterile root**
M+ 204	0,96	3,20	0,35	1,84
berkheyaradulene	2,89	11,80	1,14	4,17
α-selinene	0,53	2,41	0,25	1,25
β-caryophyllene	0,76	1,80	0,68	1,20
tr-β-farnesene	14,24	26,19	8,42	33,57
germacrene-D	2,57	0,52	2,51	0,50
α-muurolene	-	-	-	-
α-farnesene	35,74	3,93	27,52	0,82
α-cadinene	0,42	-	0,17	-
geranyl-isovalerate (M+238)	1,26	6,50	0,72	9,63
spathulenol	0,68	-	0,55	-
cedrol (M+ 222)	3,62	8,71	0,51	1,40
bisabolol-oxide B	-	+	+	0,24
(-)-α-bisabolol	2,46	-	1,26	-
β-eudesmol	-	2,81		1,23
bisabolon-oxide	+	+	0,4	1,00
cis-spiroether	2,60	0,67	0,64	0,25
chamomilla esther	1,44	+	0,43	2,00
trans- spiroether	1,34	+	1,55	+

+ in traces

Figures

A.: BK-2 **B.: Szabadkígyós**

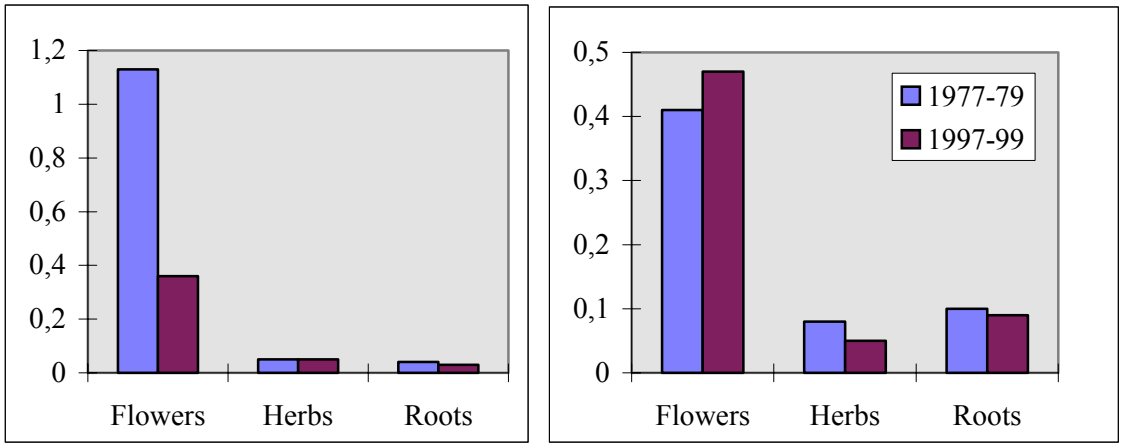
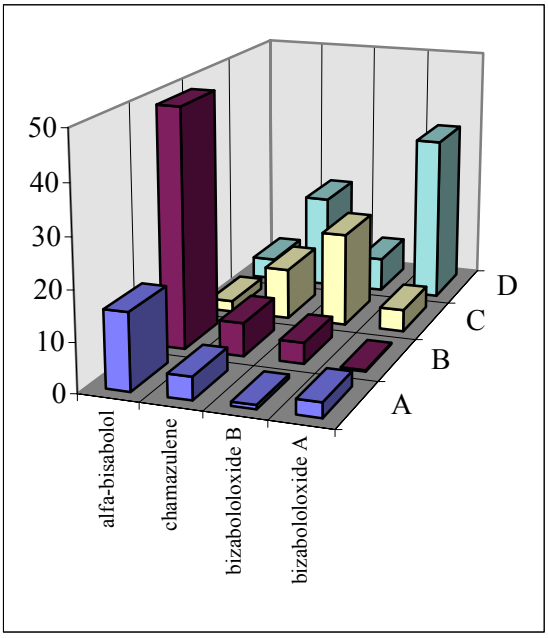
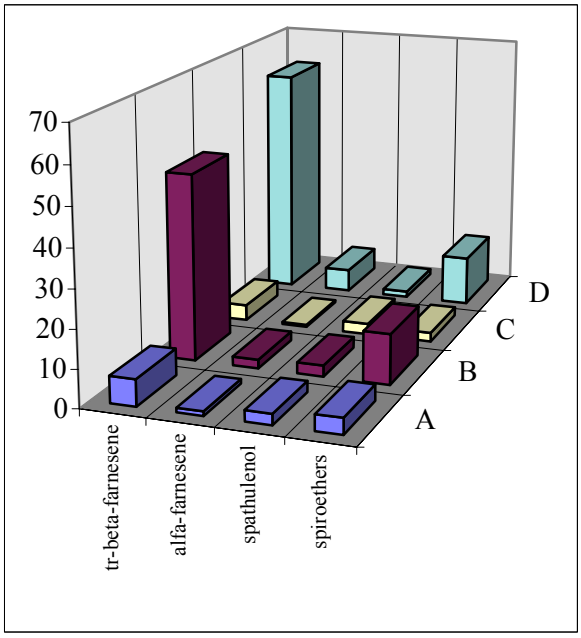


Fig. 1. Total essential oil content (%) of flowers, herbs and roots in cultivated (BK-2) and wild (Szabadkígyós) chamomiles

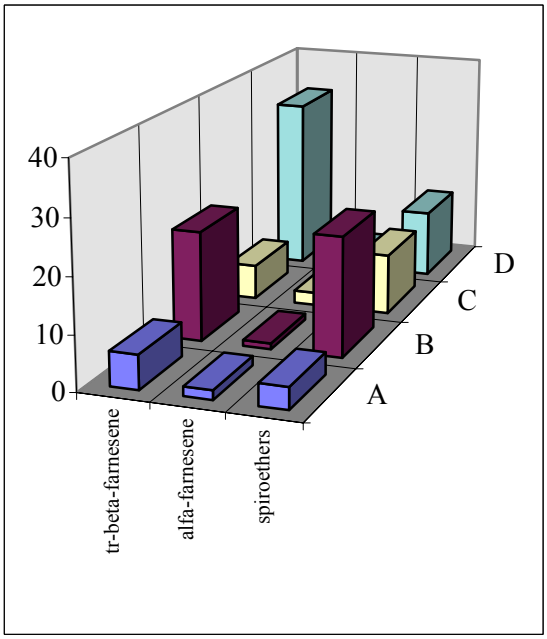
FLOWERS



HERBS



ROOTS



A - chamomile type from *Szabadkígyós* in 1977-79
B - chamomile type from *Szabadkígyós* in 1997-99

C - chamomile type *BK-2* in 1977-79
D - chamomile type *BK-2* in 1997-99

Fig. 2. Percentile distribution of some oil components in the total essential oil of flowers, herbs and roots of cultivated (BK-2) and wild (Szabadkígyós) chamomiles (1977-79 and 1997-99)

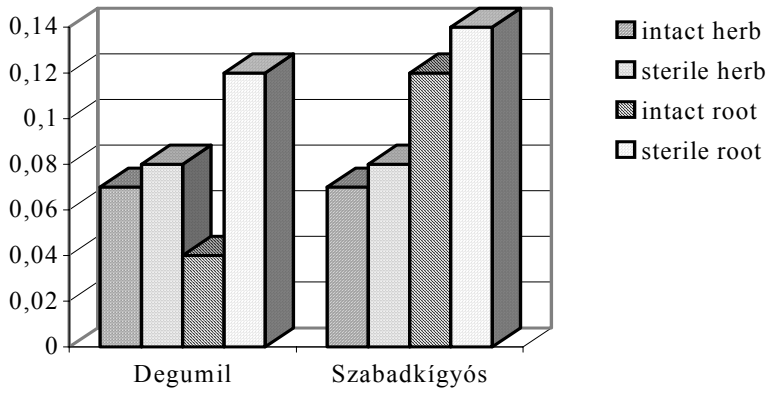


Fig. 3. Total essential oil content (%) of herbs and roots in cultivated (Degumil) and wild chamomile (Szabadkígyós) populations in vivo and in vitro