

DOCTORAL THESIS

FACTORS CAN INFLUENCE THE ACCUMULATION OF ANTIOXIDANT COMPOUNDS IN SELF-HEAL (*PRUNELLA VULGARIS* L.) AND GARDEN THYME (*THYMUS VULGARIS* L.)

SZILVIA SÁROSI

BUDAPEST, 2009

CORVINUS UNIVERSITY OF BUDAPEST FACULTY OF HORTICULTURAL SCIENCES DEPARTMENT OF MEDICINAL AND AROMATIC PLANTS

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Literature background and the aims of the study

Nowadays plant species, accumulating antioxidant compounds, have raised the interest of several scientists. Because of the free radical scavenging effect their role both in the modern pharmacy and in the food industry is becoming more important.

Making a survey on the literature data confirms that this scientific field was one of the most developing one in the last few years. As an example between 1993 and 2003 the number of the publications focused on the consequences of the oxidative stress as well as the effect of antioxidant compounds increased fourfold (1993: 1684 articles, 2003: 6510 articles) (Huang et al., 2005). Actually, on the Sciencedirect database, the word "antioxidant" as a searching target results in 25161 articles.

The usage of plant originated secondary metabolites as food preservatives is not a new invent but a rediscovered possibility. Ancient Egyptians had already used spices for this purpose (anise, caraway, cinnamon, mustard, and saffron) (Wilson, 1993). Today it has been also revealed that the antioxidant effect is due to the high level of phenolic compounds, moreover the structural conditions are also clarified (o-hydroxi-group, conjugated double bonds at the 4-oxo-group, presence of 3-OH and 5-OH groups) (Bors et al., 1999). Since phenolic compounds are good quenchers of singlet oxygen, they can decrease the local oxygen concentration (Beutner at al., 2001). These compounds are proven to act as metal chelators as well (Brown et al., 1998). They can regenerate the endogen α -tocopherol in the cell membrane therefore they increase LDL resistence to oxidation both in vitro and in vivo (Viana et al., 1996). Phenolic compounds are known to react as oxidative enzyme inhibitors, too (Cos et al., 1998).

As it was confirmed that natural antioxidants can be regarded as "multifunctional antioxidant" (Shahidi and Wanasundara, 1992), the number of the evaluated plant species increased significantly. Therefore, in the last few years, besides medicinal and aromatic plants, vegetables, fruits and nuts were also analysed testing their possible advantageous effect on human health (Lugasi et al., 1999; Dorman et al., 1995). Not only phenolic acids (rosmarinic acid, caffeic acid, chlorogenic acid), triterpenes (oleanolic acid, ursolic acid) and flavonoids (quercetin, rutin, isoquercitrin) were studied for their antioxidant effect, but several essential oils (Dorman et al., 1995; Sacchetti et al., 2005) and also their components were involved in the research work (Dorman et al., 2000; Ruberto and Baratta, 2000).

Phenolic compounds having antioxidant effect attract the attention of many researchers since their importance will be more emphasized not only in the field of medical sciences but also in food industry as natural food preservatives. Based on this aspect two plant species accumulating phenolic compounds were chosen to our studies *Prunella vulgaris* L. and *Thymus vulgaris* L.

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Self-heal (*Prunella vulgaris* L.) from the *Lamiaceae* plant family is native to Eurasia, used traditionally in the Chinese and Indian medicine. In the plant extract several active compounds are found possessing antioxidant effect: ursolic acid, oleanolic acid, rosmarinic acid, flavonoids, and antocyans (Senra, 1963). As the extract is almost taste and odourless its usage in the food industry seems to be perspective as natural food preservative.

Garden thyme (*Thymus vulgarus* L.) also from the *Lamiaceae* family shows one of the strongest antioxidant activity among medicinal plants. Its essential oil (mainly the components thymol and carvacrol) and the non volatile compounds (rosmarinic acid, carnosol, carnosic acid, flavonoids) are both responsible for the radical chain reaction inhibitor and the free radical scavenging effect (Deans et al., 1993). Several studies confirmed its strong antioxidant activity (Mantle et al., 1998; Dorman et al., 2000; Katalinic et al., 2006). Referring to the literature data garden thyme was chosen as a reference plant material for the evaluation of the results.

The aims of the study were the following:

- comparison of the total phenol content and total antioxidant capacity in both species by using different extraction methods (ethanol and water) to define the most proper way for the extraction,
- making morphological and chemical investigations on the Hungarian wild self-heal populations have not been analysed before,
- evaluation of the possible effect of different ecological and climatic conditions on the morphological and chemical characteristics of self-heal,
- making a standing self-heal culture in our experimental field describing the main morphological and chemical changes during the cultivation,
- evaluation of the possible effect of the different cultivation years and the plant age on the total phenol content, rosmarinic acid content and total antioxidant capacity in both species,
- making an assay on defining the most desirable phenological stage characterised by the highest total phenol and rosmarinic acid content as well as the strongest antioxidant activity.

Material and Method

<u>Wild populations of self-heal:</u> In our research the flowering sprouts of seven naturally occurred Hungarian self-heal populations – Börzsönyliget (B), Katalinpuszta (K), Királyrét (KR) (Börzsöny Hills), Recsk (R) (Mátra Hill), Gödöllő (G) (Grassalkovich Castle Park), Botanical Garden of Vácrátót (V), Botanical Garden of Soroksár (SB) – were collected during the summer of 2005, 2006 and 2007. In 2007, parallel with the Hungarian sample collection, natural Italian habitats were also involved in our experiment by collecting plant material from the Botanical Garden of Pisa (P), the Botanical Garden of Lucca (L) and from the Monte Pisani mountains (MP).

<u>Cultivated self-heal and garden thyme populations</u>: In the case of the cultivated populations the research work was carried out in Soroksár, in the experimental field of the Department of Medicinal and Aromatic Plants, Faculty of Horticultural Sciences, Corvinus University of Budapest. The cultivated self-heal populations are summarized in Table 1, while the garden thyme cultivars are presented by Table 2.

Table 1: Cultivated self-heal populations

Origin of the seed	Year of sowing and its parameters	Sample collection
Börzsönyliget (Bterm)	2006, seedling planting in May, spacing 20×20 cm	June, 2007
Katalinpuszta (Kterm)	2006, seedling planting in May, spacing 20×20 cm	June, 2007
Recsk (Rterm)	2006, seedling planting in May, spacing 20×20 cm	June, 2007
Gödöllő (Gterm)	2006, seedling planting in May, spacing 20×20 cm	June, 2007
Vácrátót (Vterm)	2006, seedling planting in May, spacing 20×20 cm	June, 2007
Germany-	2005, 2006, seedling planting in May,	September, 2005
purchased seed	spacing 20×20 cm	May, 2006
		June, 2007

Table 2: Cultivated garden thyme populations

Name of the culture	Year of sowing and its parameters	Sample collection
'Deutscher Winter' (DW)	2005 and 2006, seedling planting in	September – 2005, May and
German cultivar	May, spacing 50×50 cm	September – 2006 and 2007
Kalocsai (KA)	2005, seedling planting in May,	September – 2005: May and
Hungarian cultivated	spacing 50×50 cm	September – 2006 and 2007
population		
'Varico I.' (Var I)	2006, seedling planting in May,	September – 2006, May and
Swiss hybrid cultivar	spacing 50×50 cm	September – 2007
'Varico II.' (Var II)	2006, seedling planting in May,	September – 2006, May and
Swiss hybrid cultivar	spacing 50×50 cm	September – 2007

In the case of garden thyme the populations were not flowering at the autumn collection time, therefore only the green, not wooden sprouts were cut. In spring the flowering stems were collected in every year. The following characteristics were evaluated:

1. Morphological features of the wild and cultivated populations of self-heal:

- *length of the flowering stems* (cm)
- *length of the flowers* (cm)
- *number of the nodes*

2. Laboratory analysis:

- *Preparation of aqueous and alcoholic extracts*: the dried and powdered drug (1g) was infused with 100 °C distilled water (100 ml) and was allowed to stand for 24 hours. Then the extracts were filtered, and until the analysis were stored in freezer. In the case of alcoholic extracts the drug (1g) was extracted by ethanol (20%) and was allowed to stand for 72 hours. Then the extracts were filtered and were stored in freezer until the chemical experiments. All measurements were done in three replications.

- *Determination of total phenol content:* the total phenol content (TPC) was determined by the modified method of Singleton and Rossi (1965) by applying hot water bath (temperature 50 °C) to accelerate the colour reaction. The absorbance was measured at 760 nm by spectrophotometer (Scanning Spectrophotometer UV-VIS DUAL BEAM; Labomed. Inc.). Gallic acid (0.3 M) was used as chemical standard for calibration. The TPC content of the sample was expressed as mg of gallic acid equivalents per ml of sample (mg GAE/ml).

- *Investigation of total antioxidant capacity*: the total antioxidant capacity (TAC) was measured by the modified FRAP method (Benzie and Strain, 1996). The antioxidant capacity of the sample solution was calculated with reference to the standard curve given by the ascorbic acid of known concentrations. The TAC value of the samples was expressed in mg ascorbic acid equivalent per ml of sample (mg AAE/ml).

- *Essential oil content*: essential oil content was analysed by Clevenger type hydrodistillation according to the PhHg VII, its amount was given in ml/100 g dry material.

- *Essential oil composition:* The composition of the essential oil was evaluated by GC-MS method (6890 N Gaschromatograph equipped with 5975 inert mass selective detector, Agilent Technologies; injector temperature was 230 °C; split ratio: 30:1; transfer line: 240 °C; colonna – HP-5MS (5 % phenyl methyl siloxane) length: 30 m, id: 250 μ m, film thickness: 0.25 μ m; carrier gas: helium (constant speed of 1 ml/min); temperature programme: 60-240 °C by 3 °C/min; ionization energy was 70 eV). Detection of the compounds was done by comparing their mass spectra to librarian references (NIST, Wiley) and by calculating their linear retention indexes (LRI).

- Analysis of rosmarinic acid content: for the evaluation of rosmarinic acid content (RAC) thin layer chromatographic method of Janicsák & Máthé (1997) was applied in two replications. For the quantitative analysis densitograms were obtained by using a Shimadzu CS-9301 PC (Japan) densitometer. The fluorescence of RA was measured at 325 nm, the amounts were expressed in mg rosmarinic acid/g dry material.

3. Statistical analysis

The results were summarized by Microsoft Office Excel 2003. The statistical analysis was carried out by Statistica 7.0. The means were compared by one-way variance analysis. Levene-test was done to investigate the homogeneity of the standard deviations. When the t-test indicated significant differences Brown-Forsythe test was carried out (Brown and Forsythe, 1974; Vargha, 2000). The significance level was 95 % in all cases (p<0.05).

Results

Prunella vulgaris

With only one exception (K) the <u>different way of extraction</u> had a <u>significant effect</u> on the total phenol content and total antioxidant capacity of self-heal samples. Similar to other plant species from the *Lamiaceae* family (garden thyme, rosemary) the *water extracts* were characterised by <u>higher TPC and TAC values</u>.

The lenght of the flowering stems in the wild populations was found to be connected strongly to the natural habitats. During the three years of investigation significant differences were observed in every year. Populations collected in underwood (B, K, KR, R) developed longer flowering sprouts (average of 23.38 cm) than those coming from cut and maintained grasses (G, V, SB) (average of 12.26 cm). The variability of the results seems to confirm that <u>this plant species</u> probably has a great adaptability. In the case of the underwood plant populations the length of the flowers was also higher (average of 2.15 cm); in cut grasses the flowers were shorter (average of 1.44 cm). The same tendency was experienced in the number of the nodes, because of the permanent cutting the shortened flowering stems developed less pairs of leaves (average of 2.86) than those plants growing in half shade position, under the trees (average of 3.43).

<u>During the cultivation of self-heal</u>, because of the altered climatic conditions (direct sun instead of half shadow, higher temperature), the <u>length of the flowering stems</u>, and the <u>number of the nodes decreased significantly</u> in those populations originated from underwood habitats (B, K). However, the <u>length of the flowers increased</u>. In all cases the cultivated populations showed less variability than the wild ones, which was indicated by much lower CV% values.

In accordance with the result of Bomme et al. (2006) we ascertained that, the flowering stems of self-heal should be collected at the end of spring in the second year, when all of the measured morphological features showed the highest values (length of the flowering stems: 32.28 ± 6.86 cm, length of the flowers: 2.40 ± 0.88 cm, number of the nodes: 4.04 ± 0.68). In the first year of cultivation the occurrence of the flowers was rare, while in the third year, due to several diseases as well as to the drought and pests, many individuals shrivelled, and the rest bore significantly shorter stems (11.28 ± 3.01 cm).

In the case of wild-growing self-heal populations the TPC, RAC and TAC values were measured during three years. In 2007 we had the possibility to involve in our study Italian self-heal populations as well in three natural habitats (Botanical Garden of Pisa, Botanical Garden of Lucca, Monte Pisani Mountains) characterised by much warmer and sunnier climatic conditions than that of Hungary. The results were compared in every case to garden thyme populations (DW, KA). In 2005, in the most rainy and cold yearly weather conditions, all of the three investigated features

were characterised by <u>lower values</u>. Between the observed wild growing populations significant differences were found. Among the Hungarian populations plants growing in the Botanical Garden of Vácrátót accumulated the highest level of TPC (2005: 0.41 mg GAE/ml; 2006: 0.55 mg GAE/ml; 2007: 0.46 mg GAE/ml) and RAC (2005: 15.80 mg/g; 2006: 21.70 mg/g; 2007: 23.30 mg/g). In the case of TAC the results were less obvious. In 2005 the population of Recsk (R) (0.35±0.04 mg AAE/ml) and plants from the Botanical Garden of Soroksár (SB) (0.33±0.01 mg AAE/ml) showed significantly the highest results, in 2006 the two botanical gardens (SB: 1.35±0.28 mg AAE/ml, V: 0.91±0.33 mg AAE/ml) were characterised by outstanding values. In 2007 significant difference was not detected among the Hungarian populations.

In two Italian natural habitats (Botanical Garden of Lucca and the Monte Pisani Mountains), having much warmer and sunnier weather conditions than the Hungarian places of collection, the TPC (0.70 ± 0.01 mg GAE/ml; 0.60 ± 0.05 mg GAE/ml) and TAC (1.14 ± 0.06 mg AAE/ml; 0.91 ± 0.19 mg AAE/ml) values were significantly higher than that of the Hungarian populations. In the case of TAC the results also exceeded those values measured in the garden thyme.

Summarizing the results it was clear that the TPC, RAC and TAC values of self-heal could reach the level of the investigated garden thyme populations, moreover, in some cases, even exceeded them.

As a <u>result of cultivation</u>, owing to the changed climatic conditions, in two underwood originated self-heal populations (B, K) the level of <u>TPC and RAC</u> was <u>doubled</u>, the <u>TAC</u> values also <u>increased significantly</u>.

The possible effect of the <u>different cultivation years</u> and the <u>plant age</u> on the TPC, RAC and TAC was investigated on a 2 and 3 year old standing self-heal culture made by using German purchased seed. In the case of the <u>TPC</u> an <u>increasing tendency</u> was observed, the lowest results were measured in 2005 (0.43 ± 0.01 mg GAE/ml), while the year 2007 was characterised by the highest values (0.50 ± 0.01 mg GAE/ml). Similar to the TPC, in <u>2007</u>, also the <u>RAC was outstanding</u> (21.80±1.60 mg/g). However, from the TAC point of view the best results were given by 2006 (0.97 ± 0.12 mg AAE/ml). In 2007 the 2 and 3 years old cultures were analysed at the same time. The <u>older plants accumulated more phenolic compounds</u> (2 years old population: 0.39 ± 0.02 mg GAE/ml; 3 years old population: 0.50 ± 0.01 mg GAE/ml), as well as <u>higher TAC</u> (2 years old population: 0.36 ± 0.02 mg AAE/ml; 3 years old population: 0.67 ± 0.04 mg AAE/ml). In the case of the <u>RAC</u> the <u>different plant age did not influence significantly</u> the results.

Sample collection was done in four different phenological phases (leafy stems, flowering stems holding buds, stems in full flowering, stems holding overblown flowers) for the optimization of the harvesting time. As a result we proved, that in accordance with previous results (del Baño et

al., 2003), the <u>leafy stems</u> were characterised by significantly the <u>highest TPC, RAC and TAC</u> values.

Thymus vulgaris

The investigated <u>garden thyme cultivars</u> ('Deutscher Winter', 'Varico I', 'Varico II') could <u>not</u> be <u>differentiated by their essential oil content</u> either in spring or the autumn collection period. However, according to the cultivars' official descriptions, the two <u>Varico</u> contained <u>higher</u> amount of <u>thymol</u> in the essential oil (73.13 ± 5.53 % and 73.87 ± 6.45 %), than the German cultivar (53.69 ± 14.19 %). The hot, dry and sunny weather during the spring of 2007 had different effect on the garden thyme populations. Probably, the above mentioned extraordinary climatic conditions caused a more significant stress on the <u>German cultivar</u>, resulting in a <u>high level of TPC</u> (1.58 ± 1.02 mg GAE/ml), twice bigger than those of the Varico cultivars (0.62 ± 0.08 and 0.82 ± 0.11 mg GAE/ml). In the case of the <u>TAC the results were similar</u>, however, the difference was not confirmed statistically. On the contrary of the TPC and TAC values the <u>RAC</u> was significantly <u>higher in the Varico cultivars</u> reaching the avegare amount of 33.5 mg/g at the spring collection time.

The possible effect of the <u>different cultivation years</u> and the <u>plant age</u> on the accumulation of the active compounds as well as on the total antioxidant capacity was investigated also in the case of garden thyme. Referring to previous literature data these two effects are difficult to be separated as unique factors (Pluhár et al., 2003). In our study the cultivar 'Deutscher Winter' was used as a standing culture investigating a 2 and 3 years old population at the same time in 2007.

Comparing the two harvesting times (May and September) the higher <u>TPC</u> and the <u>TAC</u> were <u>connected</u> to the <u>warmer and sunnier weather</u>. In the case of the spring cuttings the year 2007 was characterised by the above mentioned conditions resulted in a higher level of TPC (0.95 ± 0.11 mg GAE/ml) and TAC (0.95 ± 0.17 mg AAE/ml); while among the autumn cuttings the best values (0.94 ± 0.11 mg GAE/ml and 0.66 ± 0.16 mg AAE/ml) were measured in 2006, when the weather was less rainy and more sunny. The amount of the <u>essential oil decreased</u> in both cutting times, while in the case of the <u>thymol</u> the different <u>cultivation years did not influence</u> significantly the results. The <u>RAC</u>, however, <u>increased</u> by the years (independently from the weather conditions), its highest amount was measured in 2007.

According to the literature data the amount of the essential oil decreases in the older plants, however, the ratio of thymol can increase significantly (Pluhár et al., 2003; Pank and Krüger, 2003). Our results were accordance with these observations. However, in the case of TPC and TAC the results were less obvious. The <u>TPC</u> in correlation with the <u>TAC</u> cannot be related to the plant age

because of the contradictory results. In both cases the values were more influenced by the weather conditions. Therefore, the higher amounts were measured during the autumn of 2006, and the spring of 2007. The changes of <u>RAC</u> were less explainable, too. In the spring of 2007, when the weather conditions were dry, sunny and quite warm, the older plants contained higher amounts, while during autumn, when the weather was more rainy and cold, no significant difference was observed between the 2 and 3 years old populations.

Similar to self-heal, the leafy stems accumulated higher amounts of TPC and TAC; however, in this plant species the difference was significant only in the case of TPC. The different phenological phases did not influence the RAC content of garden thyme.

New scientific results

Based on the 3 years long (2005-2007) experimental work the following scientific results were gained:

- In the case of self-heal, similar to other plant species from the *Lamiaceae* family (rosemary

 Engel, 2005; garden thyme Stefanovits, 2008), the <u>water extracts</u> can be characterised by <u>significantly higher amounts of TPC and TAC</u>.
- 2. The morphological features as well as the active compounds of self-heal were investigated for the first time in Hungarian natural habitats. According to our results the following statements were given:
 - The great <u>morphological variability</u> of the Hungarian self-heal populations confirms that this plant species has a <u>good adaptability</u>. This characteristic makes possible the cultivation as well.
 - The <u>TPC, RAC and TAC</u> of the analysed self-heal populations showed <u>significant</u> <u>differences</u>, too. Plants from underwood natural habitats (Börzsönyliget and Katalinpuszta) had lower amounts in every year. On the contrary, in two botanical gardens, populations coming from the cut, maintained grasses (V, SB) were characterised by high values in the case of TPC and TAC during the whole period of the investigations.
- 3. For the evaluation of the different ecological and climatic conditions on the chemical characteristics of self-heal, in 2007 three Italian natural habitats were involved in our study. Our findings were the following:

- <u>The TPC, RAC and TAC of self-heal can be significantly influenced by the weather conditions.</u> The lowest values were measured in the coldest, most rainy year of the investigations, in 2005. <u>The connection between the accumulation of phenolic compounds and the sunnier and warmer weather conditions</u> was also confirmed by those results were given by two Italian populations. In the case of TAC the values even exceeded significantly the results of garden thyme.
- 4. The effect of the cultivation on the morphological and chemical features on self-heal was also investigated for the first time:
 - During the cultivation of self-heal <u>considerable changes</u> can be observed referring to the <u>morphological and chemical features</u> of the plants:
 - the length of the flowering stems decreases,
 - the length of the flowers increases,
 - the number of the nodes decreases,
 - the TPC, RAC and TAC values of the samples increase.
 - According to our results we ascertained that the standing culture of self-heal was recommended to be maintained <u>under cultivation not more than 2 years</u>.
- 5. Investigations were done also for the evaluation of the possible effect of the different cultivation years and the plant age on the TPC, RAC and TAC of self-heal and garden thyme:
 - The meshing effects of the weather conditions of the cultivation years and the different plant ages had a significant effect on the results in both plant species:
 - the <u>TPC and TAC</u> of *self-heal* increased by the aging of the plants, while the RAC was in stronger connection with the cultivation year,
 - the <u>TPC and TAC</u> of *garden thyme* were <u>more influenced by the weather</u> conditions of the different cultivation years, while the <u>RAC</u> was <u>higher in</u> <u>the older</u> plants.
- 6. For the first time an assay was carried out on defining the most desirable phenological stage in both plant species characterised by the highest TPC, RAC and TAC values:
 - The <u>leafy stems</u> had <u>significantly the highest level of TPC</u> in both plant species. In the case of self-heal this phenological phase was also characterised by the highest RAC and TAC values.

Conclusions, recommendations

In our study the volatile and non-volatile phenolic compounds were analysed in two plant species from the *Lamiaceae* family in a 3 years long investigation. Our conclusions and recommendations are given separately to the self-heal and the garden thyme.

The morphological features as well as the active compounds of **self-heal**, commonly distributed in Hungary, were investigated for the first time in Hungarian natural habitats. The plant populations were significantly different referring to their morphological (length of the flowering stems, length of the flowers, number of the nodes) and chemical (total phenol content, rosmarinic acid content, total antioxidant capacity) features. Because of the great variability of the above mentioned characteristics as well as the dispersed occurrence of the individuals and the differences between the flowering phases (stems holding buds, flowering stems, stems having overblown flowers) during the collection period it would be <u>advisable</u> to <u>elaborate the cultivation process</u> of this plant species.

According to our results <u>the cultivation of self-heal is possible also in Hungary</u>. In a previous study, in Germany, an experimental work had been already made on cultivated self-heal populations (Bomme et al., 2006). In accordance with the German study the standing culture of self-heal also in Hungary is recommended to be maintained <u>under cultivation not more than 2 years</u>. In the third year of the cultivation different kinds of diseases occurred causing leaf-blight, and the plants could not recover from being cut in the second year. Self-heal can be propagated by seed sowing; it can grow even in sunny exposure by assuring occasional watering depending on the weather conditions. To preserve the flowers on the stems as well as to achieve a high level of phenolic compounds, the stems holding flower-buds are advised to be collected in the second year of the cultivation, in June.

The <u>different weather conditions under cultivation</u> (basically the self-heal populations occur in half-shade, while during the cultivation they grow in full sunny exposure) can <u>increase</u> <u>significantly the TPC, RAC and TAC values</u>, therefore a high quality raw material can be produced having the same or more phenolic compounds than the reference plant species, the garden thyme.

In Hungary, an officially registered Hungarian **garden thyme** cultivar is still missing from the market. Most of the time selected, cultivated populations can be found having a great heterogeneity either in the case of the morphological or the chemical characteristics. Two hybrid garden thyme cultivars - <u>'Varico I' and 'Varico II'</u> – were analysed for the first time in Hungary testing their cultivation ability in our country. During the breeding process of the Varico cultivars the main target was to establish a great homogeneity of the plants, high drug yields, outstanding essential oil content, and high ratio of thymol. The selection work was carried out in Switzerland,

by using German and Italian originated parent lines. The standing cultures in our experimental field were homogeneous indeed; according to the official descriptions the cultivar 'Varico I' developed only female flowers. The sunnier and warmer climatic conditions compared to the Swiss habitats, however, caused a significant variability in the <u>essential oil</u> content. Although the standard deviations were rather big, the <u>average amounts met the requirements of the Ph.Hg. VIII</u>.; while the ratio of <u>thymol was outstanding</u>. In the case of the non-volatile compounds especially the <u>RAC</u> was <u>continuously high</u> in both cultivars. According to our results the <u>cultivation of 'Varico I' and 'Varico II'</u> in Hungary is recommended unambiguously.

To acquire <u>higher amounts of TPC and TAC</u> the <u>leafy stems should be collected</u> also in the case of garden thyme. The phenol content can increase significantly if the weather is warmer and sunnier, therefore higher amounts were measured in that collection time characterised by the above mentioned conditions. In Hungary these weather parameters are more typical in the first collection time, in June.

For <u>therapeutic usage teas made in a common way are advised to be prepared</u> instead of the alcoholic extracts. This statement has already been proven by previous results in the case of garden thyme (Stefanovits, 2008), however, it was the first time to confirm that the water extracts of self-heal can be characterised by significantly higher amount of phenols and stronger antioxidant effect than the alcoholic extracts.

Publications in relation to the PhD Thesis

Publications with IF:

1. Sárosi Sz., Bernáth J.(2008): The antioxidant properties of *Prunella vulgaris* L. Acta Alimentaria 37 (2): 293-300.

Publications without IF:

- 1. Sárosi Sz., Bernáth J. (2007): Plant of interest Prunella vulgaris L. International Journal of Horticultural Science, 13 (2): 73-77.
- Sárosi Sz., Bernáth J. (2007): A közönséges gyíkfű egy újra felfedezett gyógynövényfaj és élelmiszer-tartósító alapanyag. *Kertgazdaság*, 39 (2): 52-56.

Conference papers (Hungarian, abstracts):

- Sárosi Sz., Bernáth J. (2005): A közönséges gyíkfű (*Prunella vulgaris* L.) antioxidáns hatásának vizsgálata. *Lippay János-Ormos Imre-Vas Károly Tudományos Ülésszak*. 2005. október 19-21., Budapest. Book of Abstracts, 138-139.
- Sárosi Sz., Bernáth J. (2008): Az évjárat hatása a kerti kakukkfű (*Thymus vulgaris* L.) illó és nem illó komponenseinek mennyiségi és minőségi paramétereire. *Gyógynövény Szimpózium*. 2008. október 16-18. Pécs. Előadás-összefoglalók, Gyógyszerészet Supplementum: 5.

International conference papers (full paper):

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