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DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND FRAP IN CULINARY HERBS IN RELATION TO HARVEST TIME

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Abstract

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In this study there were evaluated total phenolic and flavonoid contents, and ferric reducing antioxidant power (FRAP) of 3 herbs species, that are commonly used in fresh stage (summer savory – *Satureja hortensis* L., marjoram – *Majorana hortensis* M. and thyme – *Thymus vulgaris* L.) in dependence on time of harvest. The total flavonoid content ranged from 2.36 to 4.10 g of catechin equivalents (CE).100g^{-1} of dry weight (dw) of plant material. The highest average total flavonoids content was ascertained in aerial part of summer savory colected in first harvest (4.10 g CE.100g^{-1} dw) and the lowest in aerial part of summer savory collected in third harvest (2.36 g CE.100g^{-1} dw). The highest total flavonoid content was measured by all tested species in plant material harvested in first time of harvest. The highest total phenolic content was estimated in plant material of marjoram harvested in the second time of harvest (6.74 g gallic acid equivalents (GAE).100g^{-1} dw) and the lowest in aerial part of summer savory harvested in the third time of harvest (4.16 g GAE .100g^{-1} dw). Ferric reducing antioxidant power (FRAP) ranged from 1,13 (summer savory, harvest No. 3) to 1.91 g GAE .100g^{-1} dw (thyme, harvest No. 1). The best results of total flavonoid content, total phenolic content and FRAP were obtained by marjoram. Among harvest times there were the highest contents of measured compound mainly by the samples collected in the first time of harvest.

total phenolic content, total flavonoid content, FRAP, summer savory, marjoram, thyme

Human body constantly creates free radicals, culminating in an "oxidative stress" when their elimination by antioxidant defence mechanisms is not sufficient. Oxidative stress contributes to the pathogenesis of many human diseases; therefore the intake of antioxidative agents is important for the prevention of chronic diseases. (Stahl-Biskup and Veskuntonis, 2004). Antioxidants play an important role in preserving of food too. In food industry widely used synthetic antioxidants as butylated hydroxitoluen (BHT) and butylated hydroxianisole (BHA) are very effective, but they are instable and they can play role as promoters of carcinogenesis. Due to these reasons, there is a growing interest in the study of natural additives as potential antioxidants. The presence of antioxidants in many spices gives them food-preserving properties too, especially in preventing oxidation of lipids (Potty and Krishna Kumar, 2001; Stahl-Biskup and Veskutonis, 2004).

Lamiaceae family is a group of about 210 genera and some 3500 species. Many of them are commonly used as culinary herbs. They are often cultivated because of their aromatic qualities and also of their easy cultivation. Many species of the family are reported with high phenolic contents and antioxidant capacities. (Yi and Wetzstein, 2010).

Phenols are among the largest group of secondary metabolites. They range from simple structures with one aromatic ring to complex polymers such as tannins and lignins. The role of flavonoids and, in general, polyphenolic compounds in plants is not

completely established. Flavonoids have pigmentary function. They are responsible for the colour or flowers, fruits and sometimes leaves. They play a role in pollination and dispersion by attracting animals by their colours. Other functions as antioxidants, antimutagenics, on plant growth regulation and on resistance to plant diseases have also been attributed to this group of natural polyphenols. Flavonoids protect the plant from UV-damaging effects too. Among *Lamiaceae* the presence of flavonoids is well known (Gurib-Fakim, 2006; Vila, 2002).

Herbs contain many further phytochemicals such as nitrogen compounds, carotenoids and ascorbic acid. Many of these phytochemicals possess significant antioxidant capacities (Wang, 2003).

Chemical and biological diversity of medicinal and culinary plants depends on many factors, e.g. cultivation area, harvesting time, vegetative phase, storage conditions and environmentally growth factors such as temperature, soil, light and nutrients (Yi and Wetzstein, 2010).

Summer savory (*Satureja hortensis* L.), marjoram (*Majorana hortensis* M.) and thyme (*Thymus vulgaris* L.) are native to southern Europe. They are widely used as a flavouring agent, a culinary herb and as a herbal medicine (Habán, Černá, Dančák, 2001; Potty and Krishna Kumar, 2001; Stahl-Biskup and Veskutonis, 2004). Historically, herbs and spices have enjoyed a rich tradition of use for their flavour enhancement characteristics and for their medicinal properties (Kaefer and Milner, 2008).

MATERIAL AND METHODS

Plants

For the experiment 3 *Lamiaceae* species were used: summer savory – *Satureja hortensis* L., marjoram – *Majorana hortensis* M. 'Marietta' and thyme – *Thymus vulgaris* L. Seeds were obtained from Czech seed companies Seva Flora Valtice and Semo Smržice. Sowing was done on the 27th March 2009 in greenhouse of Mendel University, Faculty of Horticulture in Lednice. The seedlings were planted out on the 15th May 2009. The plants were grown in the open field of Faculty of Horticulture in conditions of Lednice. Aerial parts were harvested on the day of analysis: first time on the 9th July 2009, second time on the 7th August 2009 and third time on the 15th September 2009.

Extraction

Fresh matter was extracted using 75% methanol (Shan *et al.*, 2005). The mixture was filtered after 24 hours of extraction in room temperature through filter paper. The extract was stored in the refrigerator until analysis.

Evaluation of total flavonoid content

The total flavonoid content was measured using a modified colorimetric method (You *et al.*, 2007). The appropriate amount of extract was added to a test-tube together with distilled water. Then was added 5% $NaNO_2$, after 5 minutes 10% $AlCl_3$ and after another 5 minutes 1M NaOH followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 minutes. The standard curve was prepared using different concentration of catechin. The flavonoid content was expressed as g catechin equivalents (CE) per 100 g of dry weight (dw).

Evaluation of total phenolic content

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method (You *et al.*, 2007). The appropriate amount of filtered methanol extracts were oxidized with Folin-Ciocalteu reagents and after 5 minutes was the reaction neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 minutes of incubation at room temperature against the blank. As the standard was used Gallic acid. The total phenolic content is here expressed as g Gallic acid equivalents (GAE) per 100 g of dry weight (dw).

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma – FRAP assay by Benzie and Strain (1996) as a measure of antioxidant power with slight modification. The FRAP reagent was prepared from sodium acetate buffer (300 $mM.l^{-1}$, pH 3,6), 10 $mM.l^{-1}$ 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 $mM.l^{-1}$ HCl and 20 $mM.l^{-1}$ $FeCl_3$ solution in proportions of 10:1:1. 50 µl of sample were added into testing tube with 4 ml of FRAP reagent. After 10 minutes of incubation was measured the absorbance at 593 nm. The standard curve was prepared using different concentration of Gallic acid. The results were expressed as g Gallic acid equivalents (GAE) per 100 g of dry weight (dw) of plant material.

Statistical analysis

Significance was evaluated by analysis of variance (Anova) followed by Tukey's HSD (Honestly Significant Difference) test using the PC software Statistica Cz v. 8 (Stat Soft). Probability value of p 0.05 was used as the criteria for significance differences.

RESULTS AND DISCUSION

Total flavonoid content

The total flavonoids content was measured in methanolic extract of fresh plant material. The results are shown in Tab. I and Fig. 1. The highest level of total flavonoid content was determined by summer savory from first harvest (4.10 g CE.100 g^{-1}dw). In the case of summer savory there was found significant difference between

the plant material obtained from the first harvest and from the second (2.66 g CE.100 g^{-1}dw) and the third (2.36 g CE.100 g^{-1}dw) harvests. Marjoram contained 3.72 g CE.100 g^{-1}dw on the first time of harvest, 3.24 g CE.100 g^{-1}dw on the second time of harvest and 3.14 g CE.100 g^{-1}dw on the third time of harvest. No significant difference in total flavonoid content wasn't found among harvest times. In the case of aerial part of thyme, the highest level of total flavonoid content was found in samples of the first harvest (3.71 g CE.100 g^{-1}dw). Thyme of the second harvest contained 2.40 g CE.100 g^{-1}dw and of the third harvest 2.96 g CE.100 g^{-1}dw. Plant material obtained from the first harvest contained significantly higher level of total flavonoid content than from the second harvest. In the case of all species, plant material obtained from first harvest contained the highest amount of total flavonoid content. Among species, there weren't found significant differences in total flavonoid content. Data to discuss about the total flavonoids content in fresh cut herbs were not found.

Total phenolic content

The results of evaluation of total phenolic compounds are shown in Tab. II and Fig. 2. The total phenolic content measured by summer savory ranged from 4.16 g GAE.100 g^{-1} dw (harvest No. 3) to 6.44 g GAE.100 g^{-1} dw (harvest No. 1). The significant differences in total phenolic content were found between plant material of the first harvest and of the second and the third harvests. In the case of marjoram was the highest total phenolic content measured by the plant material obtained from the second harvest 6.72 g GAE.100 g^{-1} dw. In aerial part harvested in the first time was ascertained the level of total phenolic content 6.52 g GAE.100 g^{-1} dw and in the third time 6.12 g GAE.100 g^{-1} dw. Thyme contained from 4.67 g GAE.100 g^{-1} dw (harvest No. 2) to 5.90 g GAE.100 g^{-1} dw (harvest No. 1) of total phenolic content. No significant differences in total phenolic content were found among times of harvest. Among species, there was found significantly higher average content of total phenols by marjoram (6.46 g GAE.100 g^{-1} dw).

Shan, Yizhong, Mei and Corke (2005) evaluated total phenolic content in 6 species of *Lamiaceae* family. They measured amounts that ranged from 3.64 g GAE.100 g^{-1} dw (*Ocimum basilicum* L.) to 10.17 g GAE.100 g^{-1} dw (*Origanum vulgare* L.). The amount measured by thyme was 4.52 g GAE.100 g^{-1} dw. In this study there were ascertained similar or little

I: *Total flavonoid content (g CE.100 g^{-1} dw)*

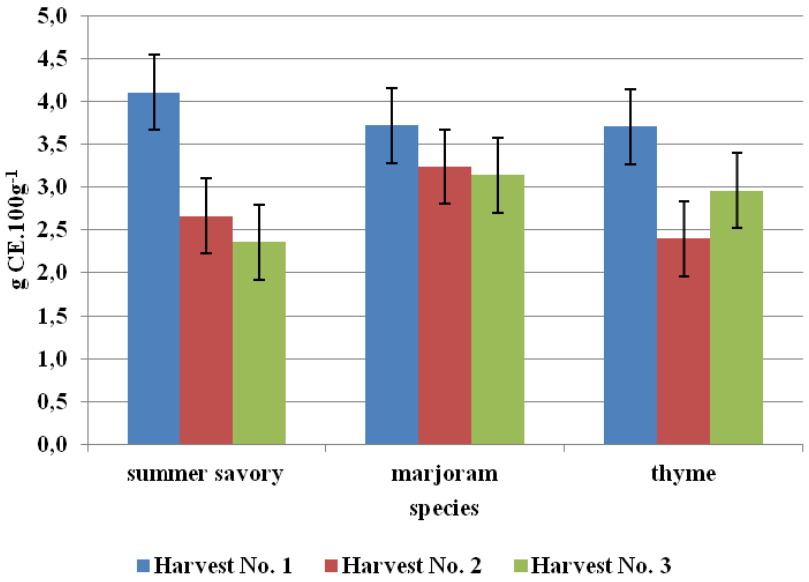
	species		
	summer savory	marjoram	thyme
harvest No. 1	4.10 a	3.72 a	3.71 a
harvest No. 2	2.66 b	3.24 a	2.40 b
harvest No. 3	2.36 b	3.14 a	2.96 ab
average	3.04 A	3.36 A	3.02 A

Means within the same column (row) followed by the same small (capital) letter are not significantly different $\alpha = 0.05$.

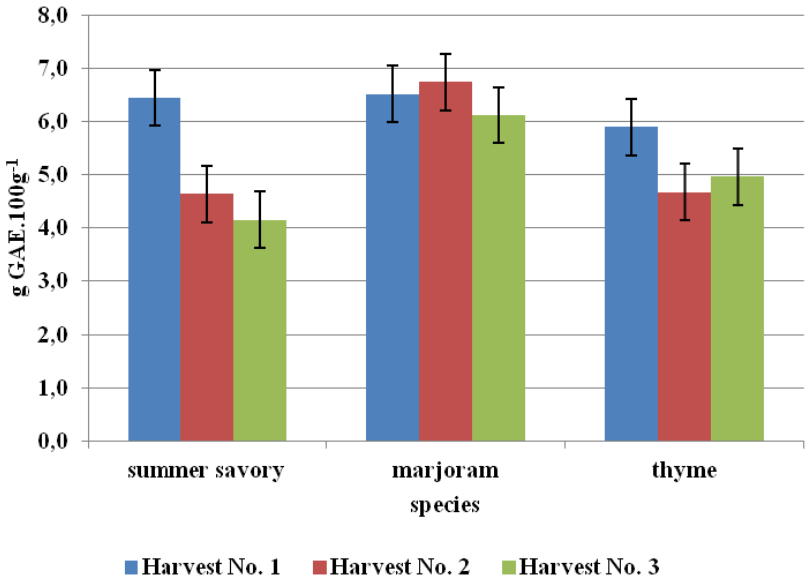
II: *Total phenolic content (g GAE.100 g^{-1} dw)*

	species		
	summer savory	marjoram	thyme
harvest No. 1	6.44 a	6.52 a	5.90 a
harvest No. 2	4.64 b	6.74 a	4.67 a
harvest No. 3	4.16 b	6.12 a	4.97 a
average	5.08 A	6.46 B	5.18 A

Means within the same column (row) followed by the same small (capital) letter are not significantly different $\alpha = 0.05$.



1: *Total flavonoid content (g CE. 100 g^{-1} dw)*



2: *Total phenolic content*

bit higher levels (4.67–5.90 g GAE.100 g^{-1} dw). In the study by Yi and Wetzstein (2010) was determined total phenolic content of some culinary and medicinal herbs grown under greenhouse and field conditions. Thyme contained 6.90 g GAE.100 g^{-1} dw (greenhouse conditions) and 4.49 g GAE.100 g^{-1} dw (field conditions). The amount measured in thyme grown in field conditions is similar to those reported by Yi and Wetzstein (2010).

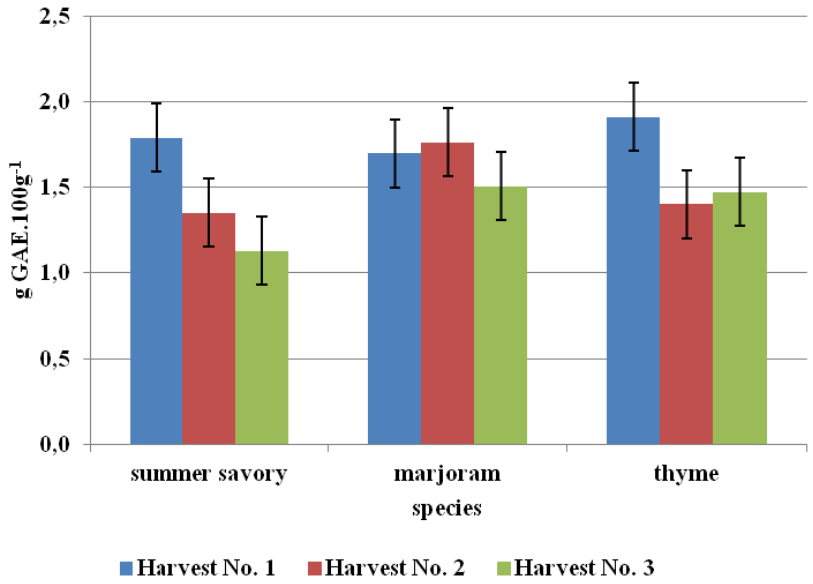
III: *FRAP (g GAE.100 g^{-1} dw)*

	species		
	summer savory	marjoram	thyme
harvest No. 1	1.79 a	1.70 a	1.91 a
harvest No. 2	1.35 a b	1.76 a	1.40 b
harvest No. 3	1.13 b	1.51 a	1.47 ab
average	1.42 A	1.66 B	1.60 AB

Means within the same column (row) followed by the same small (capital) letter are not significantly different $\alpha = 0.05$.

Ferric reducing antioxidant power (FRAP)

The results of evaluation of Ferric reducing antioxidant power (FRAP) are shown in Tab. III and Fig. 3. FRAP of summer savory ranged from 1.13 g GAE.100 g^{-1} dw (harvest No. 3) to 1.79 g GAE.100 g^{-1} dw (harvest No. 1). There was found significant difference in FRAP between summer savory harvested in the first time and in the third



3: *FRAP*

time. In the case of marjoram the highest FRAP was measured by the plant material ascertained on the second harvest time (1.76 g GAE.100 g^{-1} dw) and the lowest by the plant material ascertained on the third harvest time (1.51 g GAE.100 g^{-1} dw). No significant differences in FRAP were found by marjoram among harvest times. The level of FRAP ranged by thyme from 1.40 g GAE.100 g^{-1} dw (harvest No. 2) to 1.91 g GAE.100 g^{-1} dw (harvest No. 1). FRAP measured by thyme harvested on the first time of harvest is significantly higher than by thyme from the second harvest. Marjoram showed significantly higher FRAP than summer savory but not than thyme. There is very difficult to find out other studies to compare because of different result expression (different used standard or expressed on fresh weight). Different species of culinary herbs are often used in the studies too.

SUMMARY

In this study there were evaluated total phenolic content, total flavonoid content and ferric reducing antioxidant power (FRAP) in three species commonly used as culinary herbs (*Satureja hortensis* L., *Majorana hortensis* M. and *Thymus vulgaris* L.). These herbs were grown on the field in conditions of Lednice. Aerial parts were collected three times. On the day of harvest the samples (fresh stage) were extracted in 75% methanol. Total phenolic and flavonoid content and ferric reducing antioxidant power were measured in prepared extract. The results of different harvest times were statistically compared. In the case of total flavonoid content were the highest levels ascertained in the first harvest by all species. Among species there wasn't found significant differences in total flavonoid content. Total phenolic content and FRAP were similarly the highest in aerial parts harvested on first time of harvest by summer savory and thyme. Marjoram contained significantly higher amount of total phenolic content than summer savory and thyme and significantly higher FRAP than summer savory. The plant material harvested on the first time of harvest contained mostly the highest amounts of studied compounds. Among species were the best results measured by marjoram.

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REFERENCES

BENZIE, I. F. F. and STRAIN, J. J., 1996: The ferric reducing ability of plasma (FRAP) a s a measure of "antioxidant power"; The FRAP assay. *Analytical Biochemistry*, 239, 1: 70–76. ISSN 0003-2697.

GURIB-FAKIM, A., 2006: Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27, 1: 1–93. ISSN 0098-2997.

HABÁN, M., ČERNÁ, K. and DANČÁK, I., 2001: *Koreninové rastliny.* Nitra: Ústav vedecko-technických informacií pre pôdohospodárstvo v Nitre. ISBN 80-85330-95-4.

KAEFER, CH. M. and MILNER, J. A., 2008: The role of herbs and spices in cancer prebention. *Journal of Nutritional Biochemistry*, 19, 6: 347–361. ISSN 0955-2863.

POTTY, N. S. and KRISHNA KUMAR, V., 2001: Marjoram. In: Peter, K. V. (ed.) *Handbook of herbs and spices.* Boca Raton: CRC Press LLC, Vol. 1, ISBN 0-8493-1217-5.

SHAN, B., YIZHONG, Z. C., MEI, S. and CORKE, H., 2005: Antioxidant Capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem*, 53, 20: 7749–7759. ISSN 0021-8561.

STAHL-BISKUP, E. and VENSKUTONIS, R. P., 2004: Thyme. In: PETERS, K. V. (ed.) *Handbook of herbs and spices.* Boca Raton: CRC Press LLC, Vol. 2, ISBN 0-8493-2535-8.

Vila, R., 2002: Flavonoids and further polyphenols in the genus *Thymus.* In: Stahl-biskup, E. and Sáez, F. (ed.) *Thyme, The Genus Thymus*. London: Tailor and Francis, pp. 144–176. ISBN 0-415-28488-0.

WANG, S. Y., 2003: Antioxidant Capacity of Berry Crops, Culinary Herbs and Medicinal Herbs. *Acta Horticulturae*, 620: 461–472. ISSN 0567-7572.

Yi, W. and Wetzstein, H. Y., 2010: Biochemical, biological and histological evaluation of some culinary and medicinal herbs grown under greenhouse and field conditions. *Journal of the Science of Food and Agriculture,* 90, 6: 1063–1070. ISSN 1097-0010.

YOO, K. M., LEE, C. H., LEE, H., MOON, B. and LEE, C. Y., 2008: Relative antioxidant and cytoprotective activities of common herbs. *Food Chemistry,* 106, 3: 929–936. ISSN 0308-8146.

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