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YIELDS AND BIOLOGICAL VALUE OF THREE HERBAL SPECIES FROM THE LAMIACEAE FAMILY

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Abstract

Lemon balm, garden sage and common thyme are herbal species possessing versatile medicinal properties. They are also used as spices for culinary purposes. In the Polish climate, despite being perennial, these herbs usually grow on plantations for a year. The reason is their tendency to develop more lignified lower parts of stems, which in consequence depreciates the quality of yield and make the plants more sensitive to spring frost when there is no snow cover. The study aimed at evaluating the yielding of three herbal species (lemon balm, garden sage and common thyme) grown from seedlings in south-eastern Poland. Another objective was to determine the content of essential oils and minerals in raw material against the backdrop of the weather conditions during the growing season. The plant height was determined before harvest. Herbs were cut at the beginning of flowering, i.e. mid-July (lemon balm) and mid-August (common thyme); garden sage does not flower in the first year, hence the herb was cut at the end of August. Weight of fresh herbage was determined after the harvest, while air-dried herbage weight, ground herbage weight (for common thyme) and air-dried leaf weight (for lemon balm and garden sage) were assessed after drying under natural conditions. The content of essential oils was evaluated in dried material according to the applicable method descibed in Pharmacopoeia. Quantities of total nitrogen, ammonia, nitrates, phosphorus, potassium, calcium, magnesium, and sulfur were also determined in the harvested material. The height of lemon balm, garden sage and common thyme plants depended on the rainfall sum. Herbs grew higher in the years characterized by larger sums of atmospheric precipitations from May till July. Rainfalls also significantly affected the yields of fresh lemon balm, garden sage and common thyme herbs. In 2006, a year with less atmospheric precipitation, considerably more total nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur accumulated in lemon balm leaves. More total nitrogen in garden sage and common thyme, ammonia in garden sage leaves as well as calcium in common thyme herb were found as well.

Key words: *Melissa officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., yield, essential oil, macroelements.

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INTRODUCTION

Herbs are helpful in combating various health ailments and diseases. They are added into meals as natural spices and also serve for making refreshments and herbal infusions. The antimicrobial properties of many species from the Lamiaceae family were proven earlier by (Sarac, Ugur 2007). Many herbal plants from this family show versatile effects. Lemon balm can be used as a tranquilizer, although it is also applied during chronic and mild gastritis and intestinal catarrh. Common thyme, beside its expectorate, bacteriostatic and fungistatic properties, is recommended as an auxiliary agent for dyspepsia and disturbances in the secretion of gastric acids. Garden sage manifests bacteriostatic and anti-inflammatory features, and is used to relieve diarrhoea. Moreover, garden sage and common thyme are plant species applied during dental treatment (Szyszkowska et al. 2010). Herbal plants originate from different climatic zones, with a particularly high number grown in the Mediterranean climate, where they are harvested from natural habitats as well as from plantations. In the Polish climate, these herbs usually grow on plantations for a year, even though many are perennial plants. The reason is their tendency for developing more lignified lower parts of stems, which depreciates the quality of yields. According to Kołodziej (2010), another reason is the lesions these plants sufer due to spring frosts, which can depress the yields to remarkably.

Many herbal plant species are grown in Poland from seeds sown directly into soil. Although the cultivation from a seedling is more labour-consuming, it is advisable in regions with crusty soils, where seed sowing into field fails.

The yield structure along with its quality – which is determined by the content of biologically active substances and minerals – are important aspects of herbal production. The content of active substances determines the medicinal properties of herbs, whereas the presence of minerals in plants contributes to a wider spectrum of action. The yielding of herbal species as well as the content of biologically active substances and minerals depend on numerous factors, e.g. the weather conditions during the growing season.

The aim of present study was to evaluate three herbal plant species (lemon balm, garden sage, and common thyme) grown from seedlings in south-eastern Poland as well as to assess the content of essential oils and minerals in raw plant material. The quantitative and qualitative composition of essential oils was determined as well.

MATERIAL AND METHODS

The experiment was carried out in 2005-2006. The following herbal species were studied: lemon balm, garden sage and common thyme. The field

research was conducted at the Experimental Farm of the University of Life Sciences in Lublin (51°14′ N 22°34′ E). Seedlings were produced in a greenhouse. Seeds were sown on March 20th to boxes filled with peat substrate. Seedlings grown from these seeds were transferred to plug trays. They were planted in the field after May 15th, at 30 x 40 cm spacing. The substrate soil was prepared in accordance with the agrotechnical recommendations. Mineral fertilization was applied: N 60 kg ha^{-1}, P_2O_5 60 kg ha^{-1}, and K_2O 100 kg ha^{-1}. Phosphorus and potassium fertilizer was applied in a full rate while preparing the field for planting plug plants. Nitrogen fertilizer was split into two doses: first half of the full dose was incorporated during the field preparation, while the other half was applied to the plants in one dose after the seedlings had become established. Each plot covered 2.4 m^2. The experiment was set up in randomized blocks with four replicates. The whole plantation was manually weeded twice and the inter-row gaps were loosened during the season. The height of plants was determined before harvest using 20 randomly selected plants. Herbs were cut at the beginning of flowering, i.e. mid-July (lemon balm) and mid- August (common thyme); garden sage does not bloom in the first year, hence that herb was cut at the end of August. The plants were cut 5 cm above the ground. The weight of fresh herbage was determined after the harvest, while air-dried herbage weight, ground herbage weight (for common thyme) and air-dried leaf weight (for lemon balm and garden sage) were measured after drying under natural conditions. The content of essential oils was determined in dried material according to a Pharmacopoeia method (2002).

The qualitative and quantitative composition of essential oils was determined by means of gas chromatography combined with mass spectrometry technique (GC/MS). An ITS-40 device was used (GC/ITMS system by Finnigan MAT, USA) with a DB-5 column (J&W, USA) of 30 m length, 0.25 mm diameter and 0.25 mm stationary phase film thickness. The injector temperature was 280°C, whereas the temperature gradient was 35°C for 2 minutes, and then increased by 4°C up to 280°C.

The qualitative analysis was done on the basis of MS spectra by comparing them with the NIST library (62 thousand spectra) and LIBR terpene library (TR) provided by Finnigan MAT. Identities of recorded compounds were confirmed by retention indices from literature data (Adams 2001).

Chemical analyses of ground herbage were performed in 2% acetic acid extracts by means of the Nowosielski versatile method (1988). The mineral nitrogen was determined by the Bremner method with modifications by Starck, total nitrogen – the Kjeldahl method, phosphorus by using ammonium metavanadate, sulfur – colorimetrically (a spectrophotometer Nicolet Evolution 300) using $BaCl_2$. Potassium, calcium and magnesium were determined after digesting the herbage at 550°C and dissolving the ash in diluted hydrochloric acid (1:2, v/v) by the AAS technique (Analyst 300 Perkin Elmer).

The results were statistically processed applying a variance analysis for single classification at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Herbs are very abundant source of many biologically active substances as well as minerals, and their content is closely dependent on a species.

The meteorological data indicate that the mean air temperatures in May, June and August 2005 and 2006 were similar to the multiannual average (Table 1). In July 2005 and 2006, the temperatures were slightly higher than the long-term mean. Thus, the thermal conditions favored the growth and development of herbal plant. The total precipitation sum from May to July was higher in 2005 than in 2006, being above the multi-year average. Thus, lemon balm, garden sage and common thyme grew taller in 2005, when there was more rainfall (Figure 1). The average height of lemon balm plants in 2005 was 31.35 cm, 6 cm more than in 2006. The difference in the height of plants in 2005 and 2006 was 3.43 cm for garden sage, and 2.81 cm for common thyme. This can be explained by the low sensitivity to drought of these herb species, which results from their place of origin. The height of lemon balm was 28.25 cm, on average. Garden sage plants reached a similar height, 27.12 cm. Martyniak-Przybyszewska (2005) as well as Zawiślak (2006) reported the height of garden sage to be over 30 cm. Among the herbal species analyzed in this study, common thyme was the shortest. Its mean

Table 1

Air temperature and total precipitation in 2005 and 2006 years against the background of multi-annual averages

Temperature (°C)									
Month	2005				2006				1951-2005
	decade			mean	decade			mean	
	I	II	III		I	II	III		
May	10.8	10.5	18.0	13.1	13.5	14.6	12.8	13.6	13.0
June	13.4	17.2	17.4	16.0	11.6	17.9	21.1	16.9	16.5
July	18.9	19.9	20.4	19.7	21.2	20.8	23.5	21.9	17.8
August	16.5	16.4	17.8	16.9	18.4	18.3	15.6	17.3	17.1
Precipitation (mm)									
Month	decade			Σ	decade			Σ	1951-2005
	I	II	III		I	II	III		
May	32.8	65.0	0.2	98.0	9.0	18.4	32.1	59.5	57.7
June	47.1	7.4	1.4	55.9	28.4	0.0	9.5	37.9	65.7
July	0.0	22.4	87.4	109.8	0.0	6.8	0.0	6.8	83.5
August	103.9	3.2	1.6	108.7	73.0	79.7	45.6	198.3	68.6

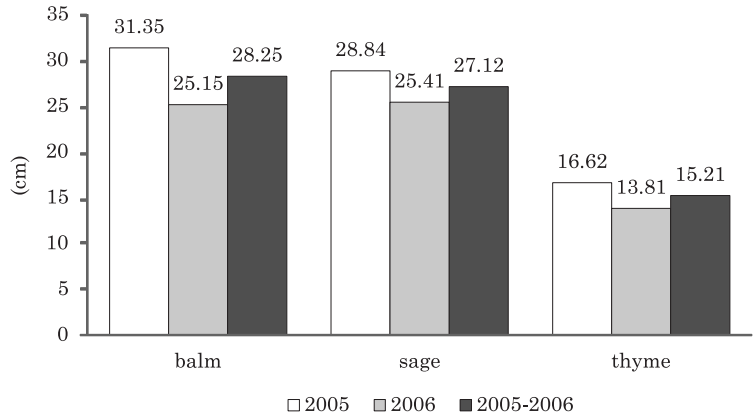

Fig. 1. Plant height of *Melissa officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L.

height was just 15.21 cm. In contrast, it reached up to 19.0 cm in a study by Zawiślak (2006) and 21.7 cm in an experiment performed by Martyniak-Przybyszewska and Wojciechowski (2004).

This study revealed substantially higher yields of herbage from fresh lemon balm, garden sage and common thyme in 2005 (Table 2), which most

Table 2

Yield and essential oil content of *Melissa officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L.

Plant species	Year	Yield of fresh herbage (kg m^{-2})	Yield of air dry herbage (kg m^{-2})	Yield of air dry leaves (kg m^{-2})	Yield of air dry herbage (kg m^{-2})	Share of herbage without stems/ leaves in dry herbage (%)	Essential oil (%)
Balm	2005	0.93	0.21	0.16	-	76.19	0.20
	2006	0.73	0.20	0.14	-	71.00	0.21
	mean	0.83	0.20	0.15	-	73.59	0.20
	$LSD_{0.05}$	0.043	n.s.	n.s.	-	-	n.s.
Sage	2005	0.73	0.27	0.19	-	70.37	1.35
	2006	0.66	0.23	0.15	-	65.22	1.60
	mean	0.69	0.25	0.17	-	67.79	1.47
	$LSD_{0.05}$	0.033	n.s.	0.029	-	-	0.048
Thyme	2005	0.69	0.23	-	0.09	39.13	2.04
	2006	0.54	0.16	-	0.07	43.75	1.45
	mean	0.61	0.19	-	0.08	41.44	1.74
	$LSD_{0.05}$	0.027	0.024	-	n.s.	-	0.027

n.s. – not significant differences

probably resulted from more rainfall from May through July in 2005 than in 2006 (Table 1). A significantly higher yield of air-drier herbage from common thyme was also obtained in 2005; no such findings occurred for lemon balm and garden sage (Table 2). Likewise, there were not any considerable differences between air-dried yields of lemon balm leaves and common thyme ground herbage. Yields of fresh and ground herbage of common thyme were comparable to those found by other authors (Seidler-Łożykowska et al. 2008).

The share of ground plant matter in air-dried herbage of common thyme equalled 41.44%, i.e. stems made up almost 60% of dry material. According to Kołodziej (2009), essential oils are accumulated in oil glands on the leaf surface; because they are absent from the surface of stems, the latter use useless as herbal material. The lowest share of stems in raw material was found in samples of lemon balm; the percentage of leaves in air-dried lemon balm herbage reached up to 73.59%.

The experiment unveiled remarkable differences between the two particular years pertaining to the content of essential oil in garden sage and common thyme herbage (Table 2). Significantly more oils were found in common thyme ground herbage in 2005 (2.04%). Seidler-Łożykowska (2007) reported a considerable influence of the weather conditions on the concentration of volatile oil in the cultivar of common thyme called Słoneczko. An average content of thyme ethereal oils in the current research reached 1.74%, which is comparable to that found by Zawiślak (2006) in cv. Słoneczko common thyme. On the other hand, Seidler-Łożykowska et al. (2008) the percentage of volatile oils in common thyme herbage was nearly twice as high, in which it resembled the results cited by Dzida (2007). The mean oil content in garden sage leaves was 1.47% (Table 2). The concentration of essential oils in leaves of the garden sage of cv. Bona grown on annual plantation appeared to be similar (Zawiślak 2000, 2006). The amount of oils in garden sage grown in Serbia oscillated from 1.7 to 2.% (Velicovic et al. 2002). According to Zawiślak (2003), the harvest date determines the quantity of essential oils in garden sage plant material. The lemon balm leaves appeared to have the lowest content of essential oils (0.2%) – Table 2. Experiments performed in Slovakia revealed that concentrations of essential oils in lemon balm leaves ranged from 0.13 to 0.27% (Mrlianova et al. 2002), while the lemon balm originating from Turkey contained only 0.067–0.036% of these compounds (Sari, Ceylan 2002).

The following substances dominated in the volatile essential oils from lemon balm: geranial, neral, β-citronellal, and β-caryophyllene (Table 3), which is consistent with other literature reports (Mimica-Dukic et al. 2004, Basta et al. 2005). The content of geranial was 25%. Studies performed by Sari and Ceylan (2002) revealed that the percentage of geranial was higher, ranging from 38.13% up to 53.68%. According to Klimek et al. (2000), the concentration of this compound can be from 6.27% to 37.17%, while Sharafzadeh et al. (2011) determined it at 38.1-45.3%. Geranial did not dominate in the compo-

Table 3

Major compounds of the essential oils of *Melissa officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L.

Plant species	Compounds
Balm	geranial (25.0%), neral (18.6%), *β*-citronellal (13.4%), *β*-caryophyllene (7.3%)
Sage	*α*- thujone (19.4%), *β*-thujone (12.7%), camphor (15.9%), 1,8-cineole (8.5%), viridiflorol (6.1%)
Thyme*	thymol (49.9%), *p*-cymene (13.5%), *γ*-terpinene (12.1%), carvacrol (6.3%)

* Zawiślak (2007)

sition of essential oils achieved from lemon balm in Iran, as its content made up about 5-5.7% (Askari, Sefidkon 2004). Neral, the second most abundant ethereal oil in annual lemon balm plants, was recorded at the level of 18.6%. Other authors report that neral makes up 29.2-34.1% (Sharafzadeh et al. 2011), 14.7% (Anicic et al. 2005), 12.22% (Sari, Ceylan 2002), 4.59-27.39% (Klimek et al. 2000) or 5.3% of essential oils in these plants (Askari, Sefidkon 2004).

The essential oil from garden sage contained *α*- and *β*-thujone, camphor and 1,8-cineole as the main components (Table 3), which agrees with earlier studies by Zawiślak (2000) as well as Zawiślak and Dyduch (2006).

The experiment on common thyme revealed that thymol was the dominating component of essential oils (49.9%). Its similar percentage was reported by Zawiślak (2007). Moreover, it also dominated in essential oils from common thyme grown in Albania and Hungary (Asllani, Toska 2003, Horváth et al. 2006). Raal et al. (2005) found that thyme oils from different European countries differ in their chemical composition. Thymol was the main component of essential oil achieved from common thyme originating from the Netherlands (65.5%) and Estonia (75.7%), while carvacrol dominated in essential oils made from common thyme in Greece.

Concentrations of macronutrients varied between the experimental years (Table 4). Significantly more total nitrogen was determined at lemon balm, garden sage and common thyme in 2006. Lemon balm harvested in the middle of July 2006 also contained considerably more phosphorus, potassium, magnesium and sulfur than in the previous year. The time period prior to the harvest of lemon balm (since mid-June till mid-July 2006) was characterized by low atmospheric precipitations, hence the different weather conditions could have caused the differences in the concentrations of these components in the two years.

Ammonia was present only in the plant material produced of garden sage. Its remarkably higher level was recorded in 2005 (1.2 g kg^{-1} d.m.). The garden sage leaves also contained small amounts of nitrates (0.3-0.9 g kg^{-1} d.m.). However, this form of nitrogen was not found in lemon balm nor in common thyme leaves.

Table 4

The content of macroelements in herbage of *Melissa officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L. (g kg^{-1} d.m.)

Plant species	Year	N-total	N-NH_4	N-NO_3	P	K	Ca	Mg	S
Balm	2005	17.9	-	-	1.10	14.5	5.50	1.30	0.20
	2006	32.1	-	-	2.30	17.9	12.0	3.80	1.00
	mean	25.0	-	-	1.70	16.2	8.75	2.50	0.60
	$LSD_{0.05}$	2.92	-	-	0.60	2.02	0.91	0.30	0.30
Sage	2005	23.3	1.20	0.30	1.00	21.2	10.9	2.50	1.50
	2006	28.2	0.80	0.90	0.70	15.0	11.7	2.80	1.90
	mean	25.7	1.00	0.60	0.80	18.1	11.3	2.60	1.70
	$LSD_{0.05}$	2.99	0.22	0.07	n.s.	1.09	n.s.	n.s.	0.21
Thyme	2005	24.0	-	-	1.50	18.1	5.40	1.20	0.50
	2006	27.5	-	-	1.50	18.1	7.40	1.20	0.70
	mean	25.7	-	-	1.50	18.1	6.40	1.20	0.60
	$LSD_{0.05}$	2.32	-	-	n.s.	n.s.	1.25	n.s.	n.s.

n.s.- not significant differences

Phosphorus is an element whose content in garden sage leaves and common thyme herbage did not depend on the weather conditions. Its quantities in garden sage ranged from 0.7 to 1 g kg^{-1} d.m., while in common thyme, it amounted to 1.5 g kg^{-1} d.m. Seidler-Łożykowska et al. (2006) reported twice as much phosphorus. Özcan (2004) found that phosphorus concentrations in common thyme and lemon balm from Turkey were 0.08% and 1.35%, respectively.

The potassium quantity in garden sage leaves was significantly higher in 2005, when the sum of atmospheric precipitations during the growing season was higher than in 2006. The content of this element in garden sage oscillated from 15 g kg^{-1} d.m. (2006) to 21.2 g kg^{-1} (2005). For common thyme herbage, the potassium levels in both years reached 18.1 g kg^{-1} d.m. Özcan and Akbulut (2007) reported similar potassium concentrations in common thyme herbage, while lower values were recorded by Seidler-Łożykowska et al. (2006).

A similar content of calcium was determined both in lemon balm and common thyme herbs. The latter contained more calcium in 2006 (7.4 g kg^{-1} d.m.), when the sum of rainfall was lower than in 2005. Garden sage leaves also proved to contain more calcium in 2006, although the differences between the experimental years were not statistically significant.

Chemical analyses revealed similar quantities of magnesium in lemon balm and garden sage (2.3 and 2.6 g kg^{-1} d.m.), while distinctly less of the element was found in common thyme (1.2 g kg^{-1} d.m.). When analyzing *Me-*

lissa officinalis, Özcan et al. (2008), determined 0.32% of magnesium. Dzida (2007) reported that the magnesium content in common thyme depends on the nitrogen fertilizer dose, and can range from 0.18% to 0.29% d.m. According to Seidler-Łożykowska et al. (2006), the concentration of magnesium was higher: 0.39% on average. The sulfur content in garden sage leaves was 1.7 g kg^{-1} d.m., compared to 0.6 g kg^{-1} d.m. in common thyme herb.

Quantities of total nitrogen and potassium in the plant material produced from the three herbal plants were lower than reported by Zawiślak and Dzida (2010), or by Dzida and Jarosz (2006) for marjoram herb. The phosphorus content in lemon balm leaves and common thyme herbage, calcium in garden sage leaves as well as magnesium in common thyme herbage oscillated around similar levels as in marjoram herbage (Zawiślak, Dyduch 2006).

CONCLUSION

1. The highest yield of fresh herbage was produced by lemon balm. The share of balm leaves in air-dry herbage was the largest, i.e. 73.59%.

2. The raw material possessing the highest amount of essential oil originated from thyme (1.74%), while the least oil was accumulated by the annual plants of lemon balm (0.2%).

3. The concentration of N-total in leaves of balm, sage and thyme in the herbage was approximately 25 g kg^{-1} d.m.; sage contained most of calcium, magnesium and sulfur contained, while the potassium content remained on a similar level in the raw plant material from sage and thyme.

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