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EVALUATION OF PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY OF HERBS

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Key words: herbs, antioxidant activity, phenolics, $ABTS^{\bullet+}$, TEAC, GAE

Research material constituted fresh herbs available in retail trade, *i.e.*: sweet basil *(Ocimum basilicum L.)*, lemon balm *(Melissa officinalis L.)*, marjoram – oregano *(Origanum vulgare L.)*, rosemary *(Rosmarinus officinalis L.)* and thyme *(Thymus vulgaris L.)*. Both whole herbs and their particular morphological parts (leaf, stalk, stem) were subjected to analyses. Polyphenolic compounds content was determined with the method of Singleton & Rossi and results were presented as GAE/g (Gallic Acid Equivalent). Antioxidant properties of the examined herbs were assayed with the spectrophotometric method with the use of ABTS cation radicals and expressed as TEAC/g (Trolox Equivalent Antioxidant Capacity). Total polyphenolics content and antioxidant capacity were determined with the extraction method using alcohol and water. Alcohol extraction influenced a considerably higher concentration of polyphenolic compounds and higher antioxidant capacity to a more favorable extent than water extraction.

Antioxidant capacity of particular herbs and their morphological parts was closely correlated with the content of polyphenolic compounds. The highest concentration of polyphenols and simultaneously the highest antioxidant capacity were observed in herb leaves and whole plants, whilst the lowest – in stems and stalks. Among the examined fresh herbs, rosemary was characterised by the highest concentration of polyphenols, *i.e.* 35-45% of polyphenol compounds more than oregano and thyme, by about 58% more than lemon balm and about 80% more than sweet basil. When converted into dry matter, a significantly higher concentration of polyphenolic compounds and simultaneously the highest antioxidant capacity were typical of oregano and rosemary and then thyme and lemon balm, whereas the lowest one – of sweet basil.

INTRODUCTION

Herbs are a valuable source of active substances beneficially influencing human body. These substances include among others: flavonoids, phenolic acids and their derivates, tannins, essential oils, vitamins or minerals [Strzelecka & Kowalski, 2000; Kohlmünzer, 2003]. Particular attention is paid to antioxidant substances included in herbs, thanks to which, some of them can be used in food as natural preservatives, preventing disadvantageous oxidative processes, and therefore, prolonging their shelf life [Chipault *et al*., 1952; Tang *et al*., 2001; Ahn *et al*., 2002; Jamora *et al*., 2002; Almeida-Doria & Regitano-D'arce, 2000]. The content of active substances in herbs can vary among others due to plant species variability, its growth phase, the country of origin, and seasonal environmental variability (biotic factors – vermins, alleopathy, diseases; and abiotic factors – climate, soil, fertilization) [Young *et al*., 2005].

Available literature lacks research characterising the antioxidant capacity of particular morphological parts of plant, therefore, this work was aimed comparing the antioxidant activity of whole plants and their particular morphological parts (leaf, stalk, stem) of selected fresh herbs. Moreover, total polyphenol compound content using two methods of extraction: alcohol and water has also been marked.

MATERIALS AND METHODS

The research material constituted fresh pot herbs of Swedoponic Ltd., available in retail trade, *i.e.* sweet basil *(Ocimum basilicum L.)*, lemon balm *(Melissa officinalis L.)*, oregano *(Origanum vulgare L.)*, rosemary *(Rosmarinus officinalis L.)* and thyme *(Thymus vulgaris L.)*. Determinations were conducted on the material from 3 different batches and in at least 3 repetitions. Both whole herbs and their particular morphological parts (leaf, leafstalk, stem) were examined. Antioxidant properties of the examined herbs were assayed with a spectrophotometric method with the use of ABTS synthetic cationradicals. The results were presented as TEAC (Trolox Equivalent Antioxidant Capacity), *i.e.* as μmol of Trolox per 1 g of fresh herb. The calculations were carried out for the solutions showing the ability to "scavenge" ABTS radicals within the range of 20-80% based on curve calibration equation [Re *et al*., 1999]. Total polyphenol compound content was determined with Singleton & Rossi method [1965], and the results were presented as GAE (Gallic Acid Equivalent), *i.e.* mg of gallic acid per 1 g of fresh herb. Antioxidant capacity of fresh herbs and polyphenol compound content was assayed for alcohol and water extraction.

The content of dry matter of fresh herb, indispensable for calculation, was determined with the use of gravimetric method according to Polish Standard [PN-90/A-75101.03].

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Statistical analysis of the results was conducted with the use of Statgraphic software, ver 5.1. for Windows. The results were subjected to the analysis of variance at a significance level of p=0.05.

RESULTS

Table 1 presents antioxidant capacity of fresh herbs taking into consideration their particular morphological parts, determined with two methods, *i.e.* with the use of alcohol and water extract. Figure 2 presents this data as expressed per dry matter of a particular morphological part. In almost all cases significantly higher results were obtained with the use of alcohol extraction method. These values were higher by a few to over 100% in comparison with the values obtained with the water extraction method (multifactor ANOVA, p<0.001), (Figure 1). No significant differences were observed between the methods only in the case of some morphological parts (stalks and/or leaves) of quite low polyphenol compound concentration.

TABLE 1. Antioxidant capacity of various morphological parts of fresh herbal plants, determined with the method of water and alcohol extraction.

Herb	Morphological part of herb	TEAC (μmol of Trolox /1 g of fresh herbs)		Difference (M–A) (%)
		Water extraction (A)	Alcohol extraction (M)	
Sweet basil (*Ocimum basilicum L.*)	whole herb	9.7±0.41	13.6±0.61	40.2
	leaf	17.1±0.30	19.4±0.61	13.5
	leafstalk	7.5±0.19	7.9±0.35	5.3*
	stem	4.8±0.27	5.0±1.07	4.2*
Lemon balm (*Melissa officinalis L.*)	whole herb	17.6±1.22	31.8±1.69	80.7
	leaf	31.4±1.92	66.4±11.16	111.5
	leafstalk	8.7±0.75	12.5±0.68	43.7
	stem	5.7±0.19	9.3±1.90	63.2
Oregano (*Origanum vulgare L.*)	whole herb	50.7±3.30	73.5±2.30	45.0
	leaf	69.0±1.92	102.9±5.94	49.1
	leafstalk	33.5±2.51	41.9±4.19	25.1
	stem	16.0±1.04	17.1±0.31	6.9*
Thyme (*Thymus vulgaris L.*)	whole herb	32.2±1.40	37.6±2.74	16.8
	leaf	37.1±1.79	52.2±3.10	40.7
	leafstalk	37.0±1.19	36.2±3.38	2.8*
	stem	25.9±0.66	33.9±0.87	30.9
Rosemary (*Rosmarinus officinalis L.*)	whole herb	40.9±2.93	81.0±3.15	98.0
	leaf	60.4±3.79	86.3±1.16	42.9
	stem	30.1±1.61	34.2±2.94	13.6

*lack of significant differences between the used methods

Antioxidant capacity of particular morphological parts of herbs varied greatly. The highest antioxidant capacity obtained with both methods of extraction was observed in fresh oregano leaves (from 69.0 μmol of Trolox/1 g – water extraction to 102.9 μmol of Trolox /1 g – alcohol extraction). Quite high values were characteristic of fresh rosemary leaves (respectively 60.4-86.3 μmol of Trolox/1 g) and lemon balm (31.4-66.4 μmol of Trolox/1 g). The lowest antioxidant capacity was characteristic of sweet basil (17.1 μmol of Trolox/1 g of leaves and 4.8-5.0 μmol of Trolox/1 g of stems) as well as stalks and stems of lemon balm (respectively 8.7and 5.7 μmol of Trolox/1 g).

Among the examined herbs, antioxidant capacity of fresh rosemary and oregano was considerably higher than that of the remaining herbs, *i.e.* by about 27-30% higher than that of thyme, by 55-57% than that of lemon balm and by 76-78% than that of sweet basil (Figure 1, Table 1).

The conducted analysis of variance showed that irrespective of the herb kind and assay method, the highest antioxidant capacity was observed in leaves (by about 25% higher than the activity characteristic of the whole plant), whilst the lowest in stems. Leafstalks, in comparison with leaves, showed on average by 48% lower antioxidant activity, whilst stems in relation to leaves by over 60% (Figure 1, Table 1). Similar interdependences were observed in the case of particular morphological parts of herbs once results were expressed per dry matter content (Figure 2).

Antioxidant capacity of herbs was connected with the content of polyphenol substances. The amount of extracted polyphenol compounds from the examined herb plants depended on the kind of solvent. In all plants over twice higher concentration of these compounds was obtained using alcohol extraction as compared to water extraction (Table 2, Figure 3).

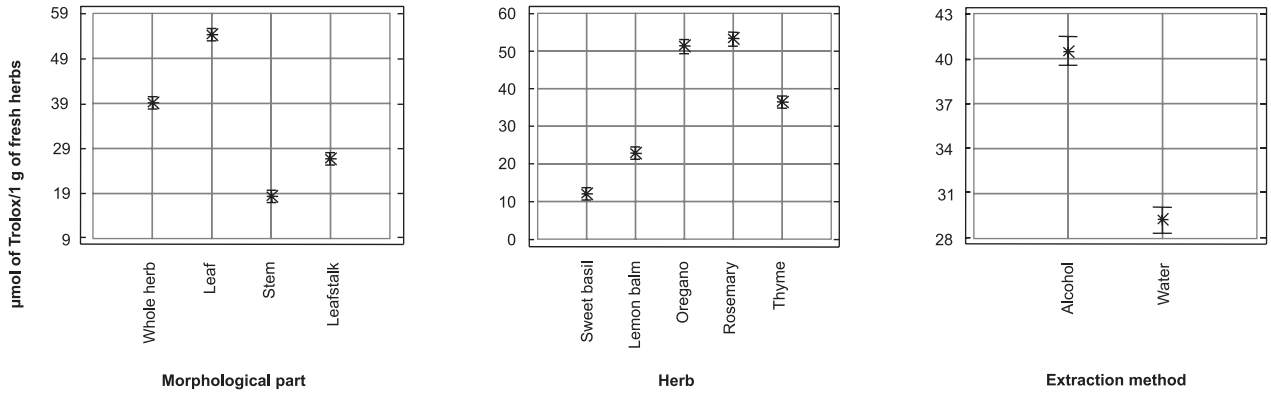

FIGURE 1. The comparison of antioxidant capacity (TEAC) of herbal plants, depending on the plant kind, their morphological parts and the extraction method (Multifactor ANOVA, p<0.001).

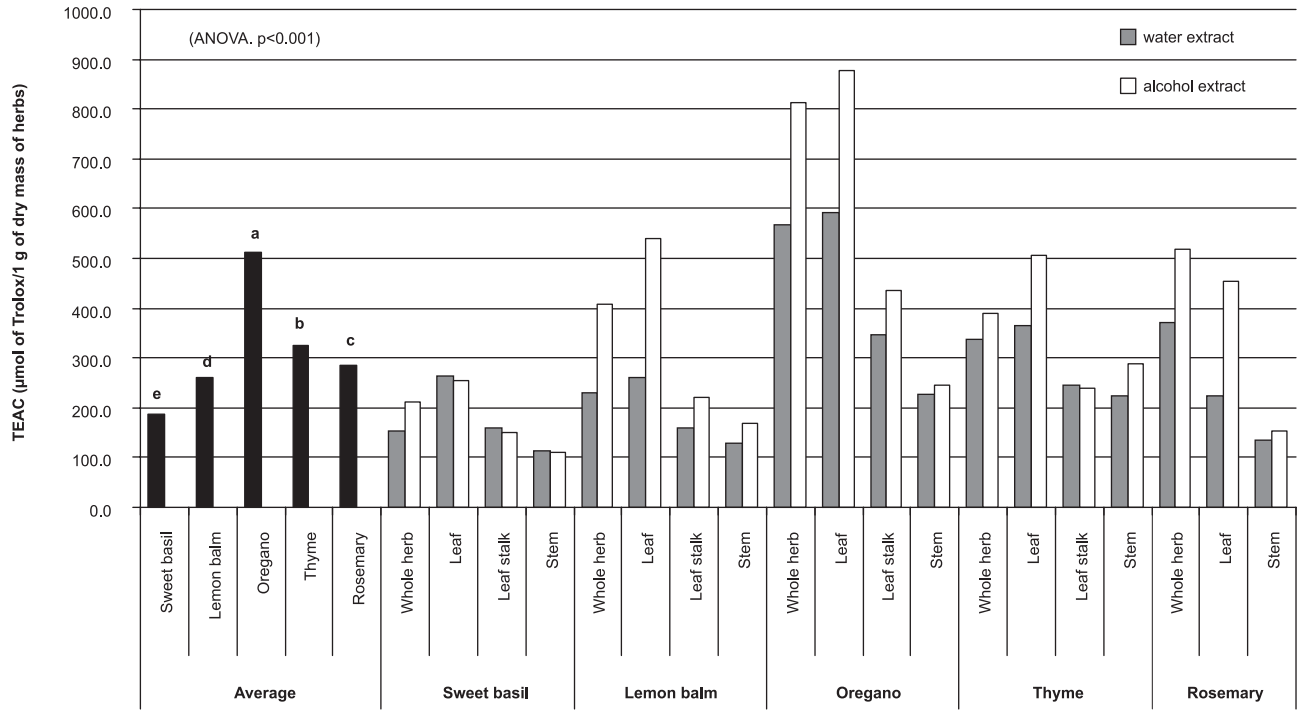

FIGURE 2. Antioxidant capacity (TEAC) of particular morphological parts of herbal plants converted into dry matter of herbs, analysis of variance (Multifactor ANOVA, $p<0.001$, the same letters denote a lack of significant differences between compared means).

TABLE 2. Polyphenol compounds content in various morphological parts of fresh herbal plants, marked with the method of water and alcohol extraction.

Herb	Morphological part of herb	GAE (mg/1 g of fresh herbs)		Difference (M–A) (%)
		Water extraction (A)	Alcohol extraction (M)	
Sweet basil (*Ocimum basilicum* L.)	whole herb	0.07±0.008	0.11±0.020	57.1
	leaf	0.09±0.006	0.14±0.022	55.6
	leafstalk	0.07±0.001	0.07±0.002	0.0*
	stem	0.06±0.007	0.06±0.006	0.0*
Lemon balm (*Melissa officinalis* L.)	whole herb	0.10±0.011	0.31±0.013	210.0
	leaf	0.19±0.014	0.51±0.007	168.4
	leafstalk	0.04±0.004	0.10±0.010	150.0
	stem	0.03±0.003	0.08±0.004	166.7
Oregano (*Origanum vulgare* L.)	whole herb	0.31±0.023	0.35±0.013	12.9
	leaf	0.29±0.005	0.57±0.008	96.6
	leafstalk	0.17±0.012	0.19±0.003	11.8
	stem	0.08±0.008	0.10±0.002	25.0
Thyme (*Thymus vulgaris* L.)	whole herb	0.20±0.020	0.35±0.010	75.0
	leaf	0.18±0.012	0.27±0.034	50.0
	leafstalk	0.18±0.014	0.28±0.007	55.6
	stem	0.11±0.001	0.15±0.003	36.4
Rosemary (*Rosmarinus officinalis* L	whole herb	0.28±0.006	0.57±0.015	103.6
	leaf	0.31±0.015	0.69±0.005	122.6
	stem	0.24±0.014	0.38±0.010	58.3

* lack of significant differences between the used methods

High variety was observed of polyphenols compound content in particular morphological parts of the examined herbs (Table 2, Figure 4). The highest concentration of polyphenol compounds was observed in leaves, then in the whole plant, leafstalks and stems. The greatest differences in the content of polyphenol compounds in leaves, leafstalks and stems were found in the case of lemon balm and oregano (Table 2, Figure 3, 4).

Analysis of variance allowed concluding that irrespectively of the kind of herb and assay method, the highest contents of polyphenol compounds were found in leaves (by about 17--18% more than in the whole plant), whilst the least in stems. Leafstalks in relation to leaves, contained by about 43% fewer compounds, whilst stems in relation to leaves by about 60% (Figure 1). Similar interdependences were obtained for the antioxidant capacity of particular morphological parts of herbs (Figure 3).

Analysis of variance also enabled concluding that among the examined fresh herbs, rosemary contained most polyphenol compounds. This spice contained by 35-45% more polyphenol compounds than oregano and thyme, by about 58% more than lemon balm and by about 80% more than basil (Figure 3, Table 2).

Statistical analysis of the results obtained in this work (ANOVA, $p<0.05$) showed that the total content of polyphenol compounds in the examined herbs was positively correlated with antioxidant capacity, in both applied methods of extraction of active compounds in the examined herbs (Figure 5).

DISCUSSION

Available literature lacks uniform methodology of presenting research results concerning both antioxidant capacity and

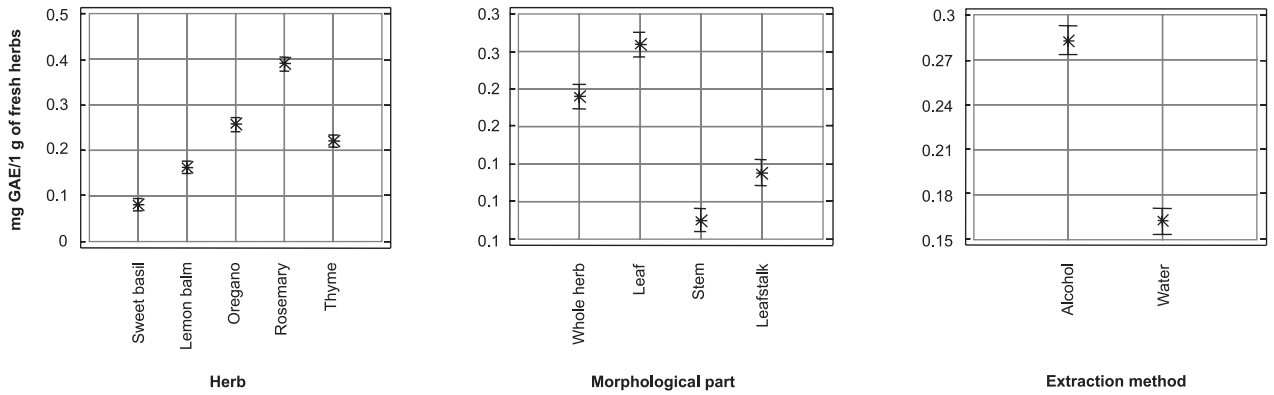

FIGURE 3. The comparison of polyphenol compounds content in fresh herbal plants, expressed as gallic acid (GAE), depending on the morphological plant part and the used extraction method (Multifactor ANOVA, $p<0.001$).

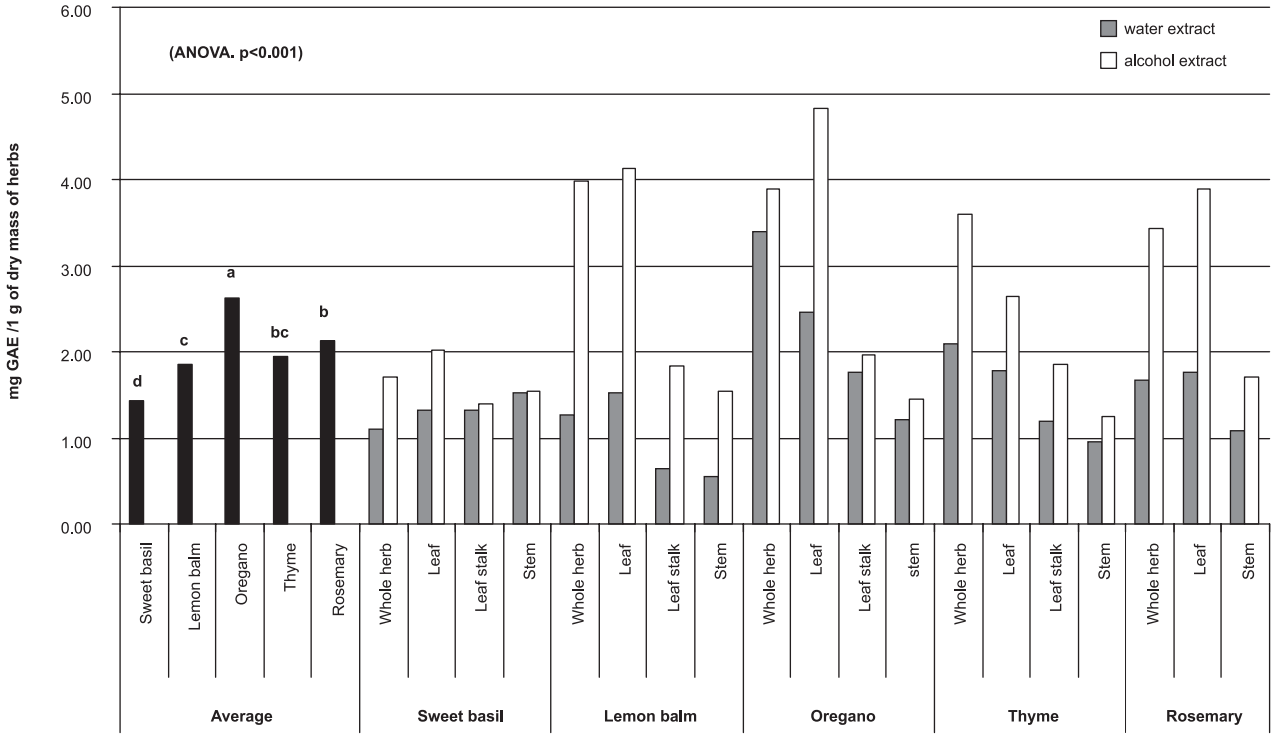

FIGURE 4. Total polyphenol compounds content (GAE) in particular morphological herbal plants converted into dry matter of herbs, analysis of variance (Multifactor ANOVA, $p<0.001$, the same letters denote a lack of significant differences between compared means).

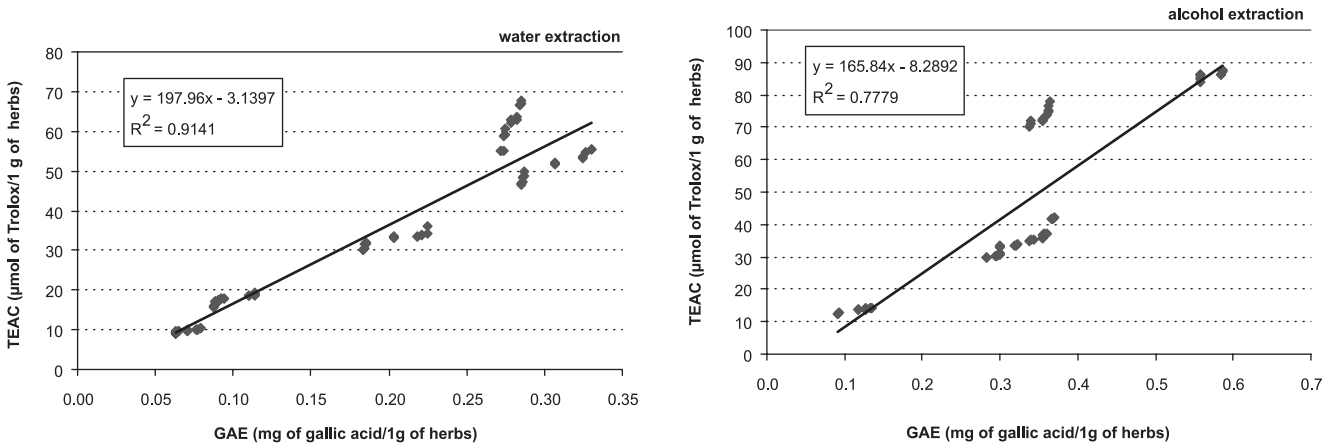

FIGURE 5. Regression equations expressing a correlation between the polyphenols content in herbs and their antioxidant capacity.

content of total polyphenols. Authors use various methods of extraction (temperature, time, solvent) while preparing solutions for research and express final results in various conversion units [Kulišić *et al*., 2006; Ivanova *et al*., 2005; Aoshima *et al*., 2007; Chanwitheesuk *et al*., 2005; Wong & Kitts, 2006], which makes it difficult to compare results obtained in the research with research results by other authors. In spite of this, great convergence was observed between the obtained results and literature data.

Kulišić *et al*. [2006] determined the content of polyphenol compounds and antioxidant capacity of water infusions of oregano *(Origanum vulgare L.)* and thyme *(Thymus vulgaris L.)*. using air-dried herbs (flowered tops and stalks). The authors showed that oregano was characterised by over 6 times higher content of polyphenols than thyme. Moreover, they stated that the antioxidant capacity of oregano infusion is higher than that of thyme and depends on the marking method. This work also pointed out higher by about 30% polyphenol compounds content in oregano than thyme, but it should be emphasized that these were fresh plants that underwent the research, not dried ones. The difference in the antioxidant capacity of these herbs remained at a similar level.

Ivanova *et al*. [2005] while examining antioxidant capacity of water extracts of medicinal plants, also proved a very high antioxidant capacity and simultaneously, a very high content of polyphenol compounds in oregano. Moreover, those authors also proved quite high antioxidant capacity of lemon balm, which in this work was characterised by both quite low content of polyphenol compounds and quite low antioxidant capacity.

Aoshima *et al*. [2007] in determining the content of polyphenols and antioxidant capacity (DPPH radical scavenging method) of herbal water infusion stated that rosemary and thyme contained high, similar contents of polyphenol compounds (2.29 and 2.08 μmol of Gallic Acid Equivalents, respectively) and were characterised by strong antioxidant capacity (23.0 and 21.5% radical scavenging DPPH). The obtained results concerning polyphenol compounds content are similar to those obtained in this work.

Dorman *et al*. [2003] showed in their research that the content of polyphenols in water extracts of rosemary, oregano and thyme amounted to 185, 149 and 95.6 mg GAE/g of extract respectively, whilst antioxidant capacity of oregano and rosemary did not differ statistically and was about twice higher than that of thyme activity. However, the authors did not provide the extract's concentration, which makes comparing the results with this work more difficult.

Cai *et al*. [2004] showed that the content of polyphenol compounds and antioxidant capacity depend on the extraction method. Having examined 112 medicinal plants, they observed that the influence of the solvent (water, methanol) on the efficiency of extraction closely depended on the kind of plant.

This work has proved that in the case of every examined herbal plant, alcohol extraction advantageously influenced obtaining a higher content of polyphenol compounds and, therefore, higher antioxidant capacity of these herbs. It suggests a higher efficiency of the extraction from these herbal plant compounds of antioxidant character with the use of alcohol.

CONCLUSIONS

1. The content of polyphenol compounds and antioxidant capacity of herbal plants differs depending on the extraction method. Using alcohol extraction enables achieving a considerably higher concentration of polyphenol compounds and, consequently, antioxidant capacity, in comparison with the values obtained in the case of water extraction.

2. Antioxidant capacity of particular morphological parts of herbal plants is closely correlated with the content of polyphenol compounds. The highest antioxidant capacity and simultaneously the highest concentration of polyphenol compounds was recorded in the leaves of herbs and whole plants, whilst the lowest on in stalks and leafstalks.

3. Comparing the antioxidant capacity and polyphenol compounds content in fresh herbs, the highest values were found for rosemary then oregano and thyme. The lowest antioxidant capacity and simultaneously the lowest content of polyphenol compound is characteristic of fresh basil.

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ZAWARTOŚĆ ZWIĄZKÓW POLIFENOLOWYCH I AKTYWNOŚĆ PRZECIWUTLENIAJĄCA ZIÓŁ PRZYPRAWOWYCH

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Materiał badawczy stanowiły świeże rośliny przyprawowe dostępne w handlu detalicznym, tj.: bazylia pospolita *(Ocimum basilicum L.)*, melisa lekarska *(Melissa officinalis L.)*, lebiodka pospolita – oregano *(Origanum vulgare L.)*, rozmaryn lekarski *(Rosmarinus officinalis L.)* i tymianek pospolity *(Thymus vulgaris L.)*. Badaniom poddano zarówno całe rośliny przyprawowe, jak i ich poszczególne części morfologiczne (liść, ogonek liściowy, łodyga). Zawartość związków polifenolowych oznaczono metodą wg Singleton i Rossi, przedstawiając wyniki jako GAE/g (Gallic Acid Equivalent). Właściwości przeciwutleniające badanych ziół oznaczono metodą spektrofotometryczną z wykorzystaniem kationorodników ABTS i wyrażano jako TEAC/g (Trolox Equivalent Antioxidant Capacity). Ogólną zawartość związków polifenolowych oraz aktywność przeciwutleniającą, oznaczono przy zastosowaniu ekstrakcji alkoholowej i wodnej. Wykazano, że zawartość związków polifenolowych i aktywność przeciwutleniająca była zróżnicowana w zależności od rodzaju zioła oraz zastosowanej ekstrakcji (wodna, alkoholowa). Ekstrakcja alkoholowa sprzyjała uzyskiwaniu istotnie wyższej koncentracji związków polifenolowych oraz wyższej aktywności przeciwutleniającej, niż ekstakcja wodna. Potencjał przeciwutleniający poszczególnych ziół i ich części morfologicznych był ściśle skorelowany z zawartymi w nich związkami polifenolowymi.

Najwyższą koncentrację związków polifenolowych i równocześnie najwyższą zdolność przeciwutleniającą wykazywały liście ziół oraz całe rośliny, natomiast najniższą – łodyżki i ogonki. Spośród badanych, świeżych ziół przyprawowych, najwięcej związków polifenolowych ogółem zawierał rozmaryn, tj. o 35-45% więcej związków polifenolowych niż oregano i tymianek, o ok. 58% więcej niż melisa i o ok. 80% więcej niż bazylia. W przeliczeniu na suchą masę, istotnie najwyższą koncentracją związków polifenolowych i równocześnie najwyższą aktywnością przeciwutleniającą charakteryzowało się oregano i rozmaryn, następnie tymianek i melisa a najniższą bazylia.