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Composition and variability of phenolic compounds in *Origanum vulgare* from Lithuania

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The study has contributed to the primary knowledge on *Origanum vulgare* phenolic compounds from Lithuania. The composition of ethanolic extracts from flowers, leaves and stems of 18 field accessions of *O. vulgare* was analysed by the HPLC method. Fourteen phenolic compounds, namely rosemarinic acid, chlorogenic acid, caffeic acid, hyperozide, naringin + rutin, luteolin, astragalin, vitexin, isovitexin, eriodictol, quercetin, naringenin were identified in the plant material. Rosemarinic acid was the dominant compound; its content in flower extracts ranged within 0.99–9.65 mg/g, in leaves 1.11–7.42 mg/g and in stems 0.53–0.77 mg/g dry weight. Analysis of variance illustrated differences in the content of compounds among flowers, leaves and stems. The most distinct differences were observed in respect of the content of hyperozide, rosemarinic acid, chlorogenic acid, luteolin and quercetin. In order to study the infraspecific variability, the results of chemical analysis were submitted to a hierarchical cluster analysis. The flower extracts were allotted to five groups on the basis of phenolics quantitatives, whereas leaf extracts showed differences among four clusters. The chemical variability of the analysed accessions seems likely to result from the genetic variability, since the influence of environmental factors has been eliminated.

Key words: *Origanum vulgare,* field accessions, phenolic compounds, HPLC method, chemical polymorphism

INTRODUCTION

The genus *Origanum* comprises about 38 species, most of which are indigenous to the Mediterranean region. *Origanum vulgare* L. is the widest spread among all the species within the genus which distributed all over Europe, West and Central Asia up to Taiwan [1]. *O. vulgare* plays a primary role among culinary herbs in world trade [2]. Despite its economic importance it is often referred to as an under-utilized species in the sense that its genetic resources and variability have not yet been fully explored [3]. Populations of *O. vulgare* in Lithuania are characterized by a limited distribution and by low sources of raw material. Traditionally, leaves and flowers of oregano are used in Lithuania mostly for their beneficial properties to cure cough and sore throats and relieve digestive complaints. *O. vulgare* is a source of essential oils and phenolic metabolites. Our previous research provided information on *O. vulgare* morphological identification and essential oil composition which indicated the existence of infraspecific variation and chemical polymorphism in local populations of oregano [4, 5]. Much of the literature data on *O. vulgare* chemical research consider its essential oils, and information concerning its phenolic compounds is very poor. The initial investigations on the phenolics composition of *O. vulgare* were carried out in Makedonia [6] and Poland [7, 8]. However, phenolic compounds such as flavonoids and phenolcarbonic acids constitute one of the most important groups of pharmacologically active substances acting as anti-oxidant, anti-microbial and anti-inflammation tools in cells [9–11]. Polyphenols have numerous favourable effects on human health such as inhibiting the oxidation of low-density lipo-proteins [12].

The aim of the present research was to provide information on the composition of *O. vulgare* phenolic compounds and its variability among local populations in order to contribute to the conservation and exploitation of the genetic resources of oregano.

MATERIALS AND METHODS

Plant material. The study was carried out in 2007. The plant material was four-year-old 18 field accessions of *O. vulgare*. The plants were multiplied from an initial mother plant originated from wild populations and presently grown in a field collection. The raw material was harvested at the full blooming stage, then dissected into floral, leaf and stem tissues and dried at 35 °C. Samples of 0.5–1.0 g of air-dried plant material with a moisture content of 10.0% were mechanically ground with a laboratory mill to obtain a homogenous drug powder and extracted with 96% EtOH (50 ml) for 72 h at room temperature and then assayed for chemical contents by HPLC.

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High perfomance liquid chromatography (HPLC) analysis. HPLC analysis with UV/PDA detection was performed using a model Waters 2690 chromatography system (Waters, Milford, USA) equipped with Waters 2487 UV/Vis and Waters 996 PDA detectors. For separation, a XTerra RP 18 3.5 µm column (150 × 3.9 mm) and SupelguardTM Ascentis™ RP-Amide 20 × 4.00 mm guard-precolumn were used.

The binary solvent system of the mobile phase consists of solvent A (5% of water containing 0.1% of trifluoracetic acid (TFA) and solvent B (95% of acetonitrile containing 0.1% of TFA). The gradient elution program for screening the extracts was the following: 0–45 min 95–55% A, 5–45% B; 45–50 min 55% A, 45% B; 50–55 min 55–95% A, 45–5% B. The flow rate: 0.4 ml/min; injection volume: 10 µl. The column temperature was at 20 °C. The elution was monitored at 290 nm [13]. The separated compounds were identified by comparing eluting retention times (R_t) and UV spectra of samples with those of authentic standards. The quantity of a compound was calculated from an external calibration curve established with five dilutions of each standard. Each sample was analysed twice, and the mean value in dry weight was presented. The quantitative identification of naringin and rutin was complicated due to the analogous eluting time from the column; therefore, the amount of corresponding compounds was presented as a total sum.

All solvents and authentic standards of reference compounds: caffeic acid, chlorogenic acid, rosemarinic acid, astragalin, eriodictol, diosmetin, hyperozide, luteolin, rutin, naringenin, naringin, quercetin, vitexin, and isovitexin were of HPLC grade and purchased from Fluka (Buchs, Switzerland) and Roth (Karlsruhe, Germany).

Statistical data analysis. The results were statistically analysed using the SPSS program (SPSS Chicago, IL, USA). Differences in the chemical composition of plant parts were tested by one-way analysis of variance (ANOVA) at the $\alpha = 0.05$ level. The accessions were grouped by hierarchical cluster analysis using the amounts of phenolic compounds as clustering variables. The dendrogram was constructed on the basis of agglomerative grouping and the average linkage between the groups, employing the clustering method based on squared Euclidean distances.

RESULTS AND DISCUSSION

The results of HPLC analysis of *O. vulgare* accessions for the contents of phenolic compounds separately in flowers, leaves and stems are presented in Table 1. Fourteen phenolic compounds – three phenolcarbonic acids and eleven flavonoids – were identified in *O. vulgare* plant material (Fig. 1). Analysis of variance illustrated differences in the amount of the compounds in flowers, leaves and stems. Rosemarinic acid was the dominant compound in flowers (0.99–9.65 mg/g), leaves (1.11–7.42 mg/g) and stems (0.53–0.77 mg/g) of all accessions (Table). No significant difference was found in the mean concentration of rosemarinic acid between flowers and leaves (4.19 and 4.33 mg/g, respectively), whereas the content of this compound in stems was considerably lower (0.65 mg/g) ($F = 22.52$). The mean content of chlorogenic acid significantly differed ($F = 18.24$) in flowers, leaves and stems (1.23 mg/g, 0.77 mg/g and 0.1 mg/g, respectively), while no significant differences ($F = 3.16$) were found in the mean values of caffeic acid among plant parts.

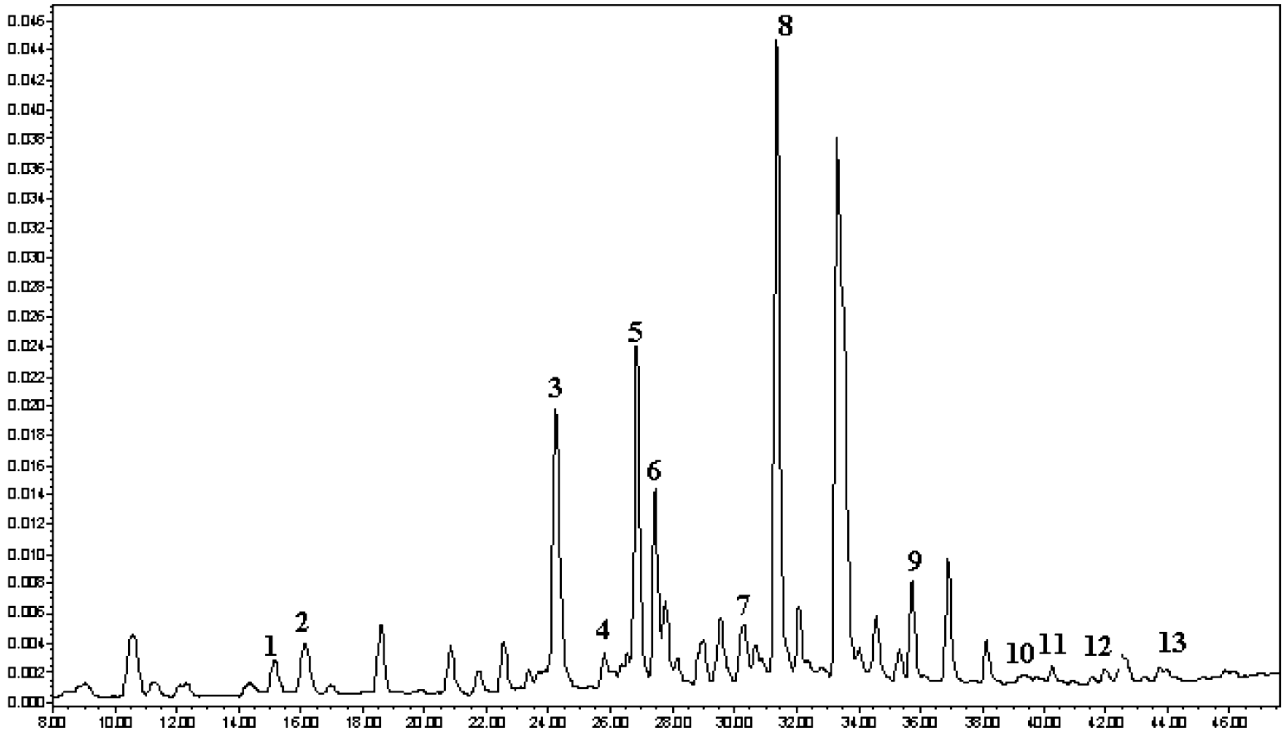

Fig. 1. Typical chromatogram of *Origanum vulgare* flower extract obtained by HPLC separation at 290 nm. Peaks identified: *1* – chlorogenic acid, *2* – caffeic acid, *3* – vitexin, *4* – isovitexin, *5* – naringin + rutin, *6* – hyperozide, *7* – astragalin, *8* – rosemarinic acid, *9* – eriodictol, *10* – luteolin, *11* – quercetin, *12* – naringenin, *13* – diosmetin

Regarding the composition of the flavonoid complex, the patterns of distribution in oregano accessions were characterized by a high variability and the dominance of hyperozide, naringin + rutin and astragalin. The contents of other compounds were rather low. One-way analysis of variance (ANOVA) revealed highly significant differences ($p < 0.05$) among plant parts in respect of the mean contents of hyperozide (1.7 mg/g / flowers, 0.8 mg/g / leaves and 0.24 mg/g / stems) ($F = 31.28$), luteolin (0.15 mg/g / flowers, 0.12 mg/g / leaves and 0.02 mg/g / stems) ($F = 11.27$), quercetin (0.16 mg/g / flowers, 0.06 mg/g / leaves and 0.09 mg/g / stems) ($F = 11.90$), isovitexin (0.22 mg/g / flowers, 0.08 mg/g / leaves and 0.05 mg/g / stems) ($F = 6.83$), and naringin + rutin (1.94 mg/g / flowers, 0.79 mg/g / leaves and 0.7 mg/g / stems) ($F = 5.35$) found in considerably lager quantities in flowers than those in leaves and stems (Table).

The results were submitted to a cluster analysis in order to reveal their infraspecific variability. The application of hierarchal cluster analysis the using amounts of phenolic components as clustering variables grouped flower extracts into five main clusters. The composition of extracted groups is presented in Fig. 2 as diagrams of the mean contents of flower phenolics, in order to better recognize the similarities and differences of each group. The major cluster (A_f), bringing together fourteen accessions, was characterized by the average amounts of identified components. The dendrogram manifested a distinct character of the other four clusters (B_f, C_f, D_f and E_f) represented by individual accessions. The cluster B_f, was formed by an accession characterized by comparatively high values of naringin (4.7 mg/g) and caffeic acid (0.35 mg/g) and least amounts of astragalin, luteolin, quercetin and diosmetin (0.16, 0.06, 0.06 and 0.04 mg/g, respectively). The cluster C_f dominated by rosemarinic and chlorogenic acids (9.7 and 2.0 mg/g, respectively), whereas the accession of cluster D_f contained the highest amounts of naringin (8.4 mg/g) and isovitexin (0.9 mg/g). The cluster E_f was characterized by the dominant contents of astragalin, eriodyctol and diosmetin (6.7, 2.2 and 1.9 mg/g, respectively) against the former clusters.

The dendrogram of leaf extracts grouped the accessions into four clusters. The first cluster (A_l) includes accessions that are characterized by their high values of rosemarinic acid (5.48 ± 0.28 mg/g) (Fig. 3). The cluster B_l, formed of one separate accession that differs from the others by a higher content of chlorogenic acid (2.69 mg/g) and hyperozide (1.4 mg/g). The third cluster (C_l) was characterized by a greater importance of eriodyctol (1.42 ± 0.47). The dendrogram showed the most distinct composition of accession distinguished as cluster D_l which produced the highest amount of naringin (2.73 mg/g) and the lowest contents of astragalin, rosemarinic acid, eriodyctol and luteolin. The results of phenolic compound composition in stems were not submitted to cluster analysis as their levels were rather low.

As the published data on phenolic compounds in *O. vulgare* are scarce, a comparison of our results with published data is problematic. In view of the previous reports, some compounds, as rosemarinic acid, caffeic acid, luteolin, diosmetin, rutin and quercetin [6, 8, 14] agreed with our results. To our knowledge, astragalin, hyperozide, naringin, naringenin, vitexin, and isovitexinin are reported for the first time in *O. vulgare*. Of late, one report from Poland [8] has appeared on the infraspecific variation of individual phenolics in herb of *O. vulgare*. Comparing the results obtained from our plant material of *O. vulgare* with those reported by the above references, considerable differences were found in the content of rosemarinic acid. The Polish populations produced much lager amounts of this acid (16.5–26.5 mg/g) than did our accessions – with the maximum (9.7 mg/g) of this compound in flowers, 7.4 mg/g in leaves and 0.8 mg/g in stems.

Table. **Phenolic compound content (mg/g dry weight) in flowers, leaves and stems of *Origanum vulgare* field accessions (n = 18) and their differences from each compound by ANOVA Fisher's F criterion**

Compounds	Flowers		Leaves		Stems		F
	m ± SE	Range	m ± SE	Range	m ± SE	Range	
Rosemarinic acid	4.19 ± 0.45	0.99–9.65	4.33 ± 0.42	1.11–7.42	0.65 ± 0.05	0.53–0.77	22.52*
Chlorogenic acid	1.23 ± 0.14	0.05–2.03	0.77 ± 0.13	0.04–2.6	0.1 ± 0.01	0.02–0.15	18.24*
Caffeic acid	0.37 ± 0.13	0.04–2.25	0.21 ± 0.05	0.06–1.11	0.04 ± 0.01	0.02–0.07	3.16
Isovitexin	0.22 ± 0.06	0.02–0.88	0.08 ± 0.01	0.01–0.18	0.05 ± 0.01	0–0.09	6.83*
Vitexin	0.01 ± 0.00	0.01–0.02	0.01 ± 0.00	0.001–0.17	0.01 ± 0.00	0.003–0.02	5.84*
Naringin+rutin	1.94 ± 0.45	0.08–8.4	0.79 ± 0.13	0.08–2.73	0.7 ± 0.13	0.08–1.56	5.35*
Hyperozide	1.7 ± 0.2	0.27–3.56	0.8 ± 0.07	0.8–1.45	0.24 ± 0.04	0.06–0.44	31.28*
Astragalin	0.89 ± 0.35	0.09–6.71	0.23 ± 0.03	0.01–0.46	0.04 ± 0.01	0.01–0.1	3.87
Eriodictol	0.5 ± 0.11	0.08–2.2	1.0 ± 0.25	0.08–2.2	0.21 ± 0.02	0.36–1.08	4.55*
Luteolin	0.15 ± 0.03	0.02–0.48	0.12 ± 0.01	0.03–0.24	0.02 ± 0.002	0.07–0.36	11.27*
Quercetin	0.16 ± 0.03	0.04–0.55	0.06 ± 0.01	0.02–0.1	0.09 ± 0.02	0.06–0.12	11.90*
Naringenin	0.18 ± 0.03	0.06–0.51	0.14 ± 0.03	0.01–0.4	0.06 ± 0.02	0.01–0.12	4.02
Diosmetin	0.18 ± 0.1	0.02–1.9	0.04 ± 0.01	0.02–0.09	0.01 ± 0.00	0.01–0.02	1.81

* Significant differences at $p < 0.05$.

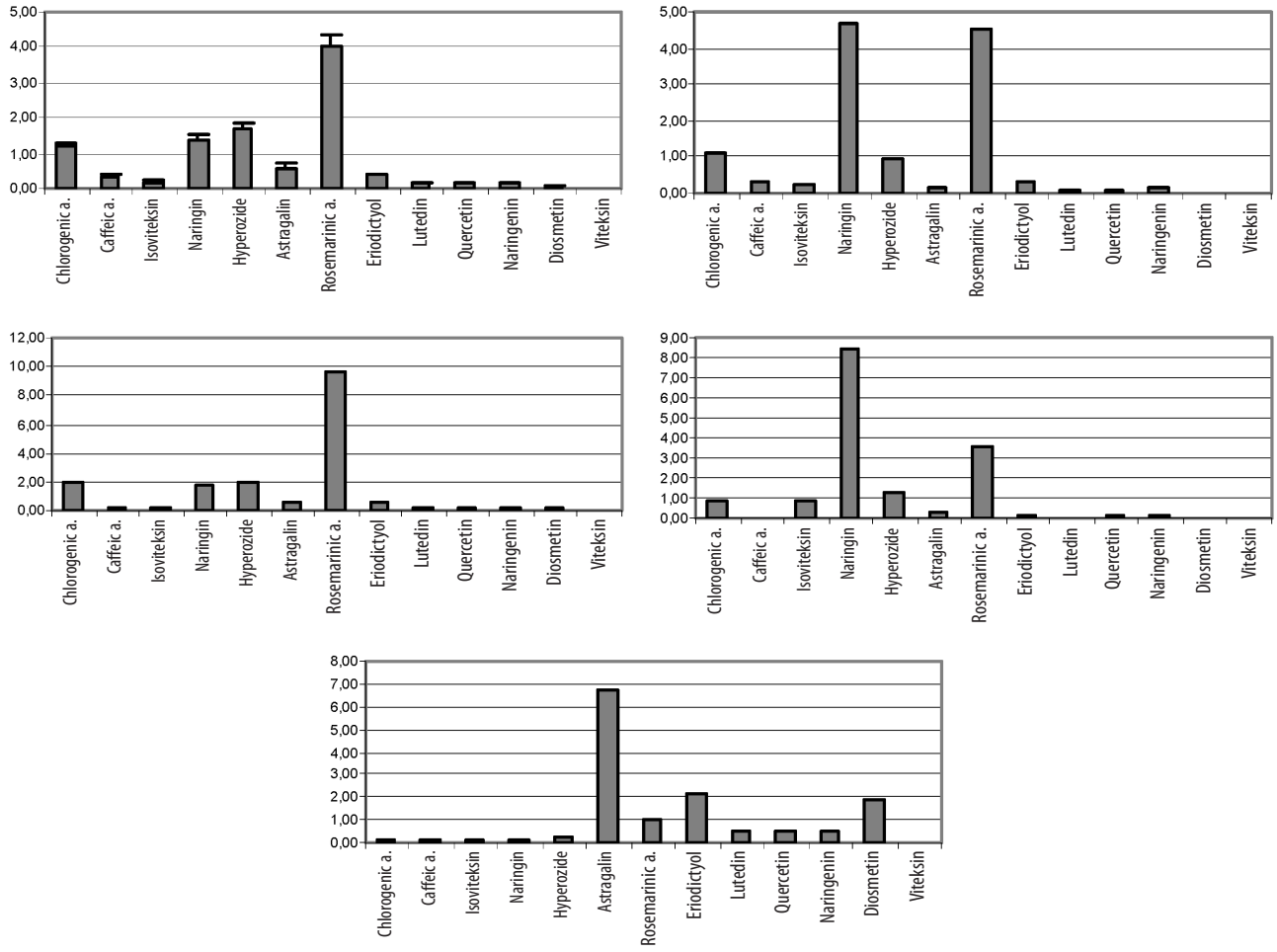

Fig. 2. Mean composition of phenolic compounds of A_f–E_f clusters separated according to the results of hierarchical cluster analysis of *Origanum vulgare* flower extracts. The heights of bars (*y*–axes) correspond to the mean content (mg/g dry weight) of compounds

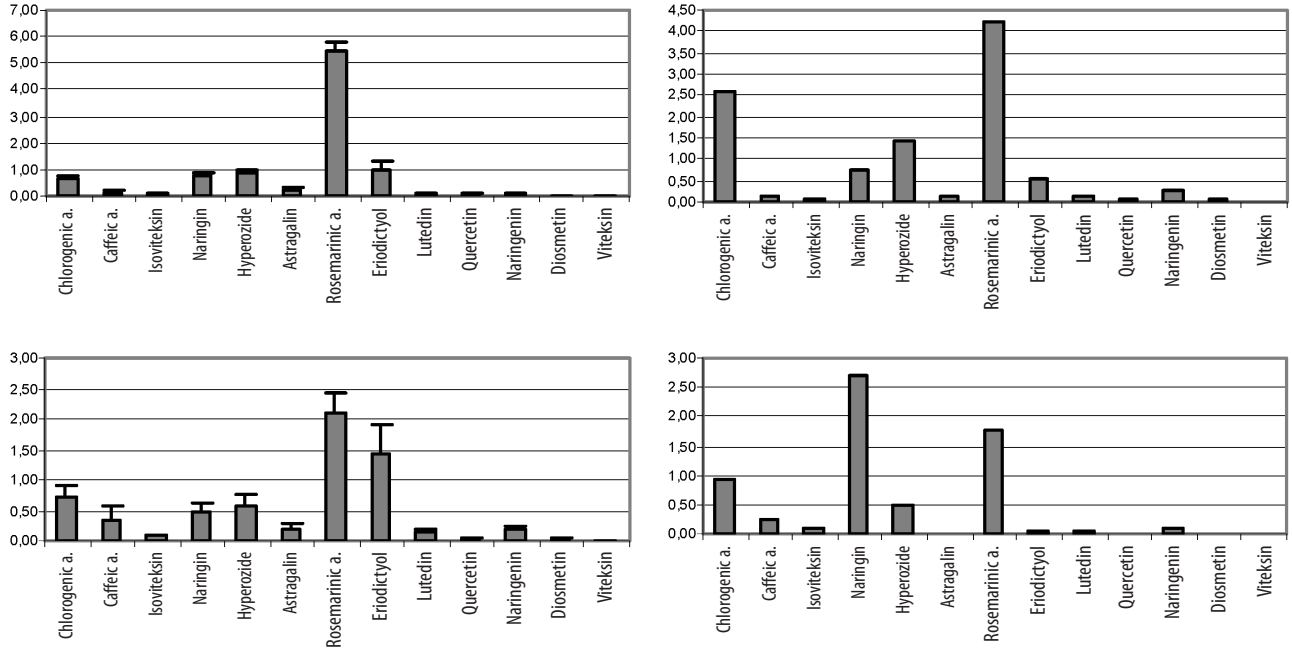

Fig. 3. Mean composition of phenolic compounds of A_l–D_l clusters separated according to the results of hierarchical cluster analysis of *Origanum vulgare* leaf extracts. The height of bars (*y*–axes) corresponds to the mean content (mg/g dry weight) of compounds

However, marginal concentrations of caffeic acid (0.13–0.28 mg/g), luteolin (0.16–0.34 mg/g), rutin (0.48–2.17 mg/g), and quercetin (0.02–0.06 mg/g) in *O. vulgare* herb from Poland were in partial agreement with our results.

In view of the previous reports, although common metabolites can be observed, a disparity regarding the quantitative composition of phenolics can be attributed to different methods adopted for chemical analysis. On the other hand, the chemical variations in the content of phenolic compounds may be attributed to the influence of different environmental conditions of sampling locations.

The results from phytochemical assay have revealed that there is a variation in the accumulation of phenolic metabolites among O. *vulgare* accessions, indicating the chemical polymorphism that might arise on the genetic background, since the influence of different environmental factors in our research has been eliminated by growing accessions in uniform field conditions. It is not surprising, because due to natural cross-pollination oregano shows a considerable genetic inconsistency in the morphological type and the level of phytochemicals produced. The research revealed that there are some oregano accessions which are a potentially important source of phenolic compounds. Therefore *O. vulgare* is a viable plant as a health-promoting material in nutraceutical applications. The study has contributed to the primary knowledge on the phenolic compounds from Lithuanian *O. vulgare*.

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ORIGANUM VULGARE FENOLINIŲ JUNGINIŲ SUDĖTIES IR ĮVAIROVĖS TYRIMAS LIETUVOJE

S a n t r a u k a

Tirta *Origanum vulgare* aštuoniolikos kolekcinių pavyzdžių žiedų, lapų ir stiebų etanolio ekstraktų fenolinių junginių sudėtis. Cheminė analizė atlikta efektyviosios skysčių chromatografijos metodu. Buvo identifikuota ir kiekybiškai įvertinta 14 fenolinių junginių, būtent: rozmarino, chlorogeno ir kavos rūgštys, hiperozidas, naringinas + rutinas, liuteolinas, astragalinas, viteksinas, izoviteksinas, eriodiktolis, kvercetinas ir naringeninas. Rozmarino rūgštis buvo pagrindinis junginys, kurio kiekis žiedų ekstraktuose kito 0,99–9,65 mg/g, lapų – 1,11–7,42 mg/g, o stiebų – 0,53–0,77 mg/g ribose nuo sauso svorio. Dispersine analize nustatyti skirtumai tarp identifikuotų junginių kiekio žieduose, lapuose ir stiebuose. Didžiausi statistiniai skirtumai rasti hiperozido, rozmarino ir chlorogeno rūgščių, liuteolino ir kvercetino atveju. Hierarchinės klasterinės analizės pagrindu tirti pavyzdžiai pagal fenolinių junginių kiekius sugrupuoti į penkis žiedų ir keturis lapų klasterius. Paprastojo raudonėlio vidurrūšinė cheminė įvairovė, matyt, turi genetinį pagrindą: aplinkos sąlygos negalėjo nulemti skirtumų, nes buvo tirti tos pačios lauko kolekcijos augalai.