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Biological and biochemical evaluation of wild marjoram subspecies O*riganum vulgare* L. and O*riganum vulgare* subs. *hirtum*

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Investigations of botanical and biochemical characters of wild marjoram subspecies *Origanum vulgare* L. and *Origanum vulgare* subs. *hirtum* were carried out at the Lithuanian Institute of Horticulture. It is observed that subspecies of wild marjoram *Origanum vulgare* subs. *hirtum*, which grows in the South Europe, differs from *Origanum vulgare* L native in Lithuania by aroma and especially large amount of essential oils. According to the data of investigations the productivity of wild marjoram subspecies (grass yield) was similar in various growth stages. It was established that in various growth stages the grass of wild marjoram subspecies *Origanum vulgare* L. and *Origanum vulgare* subs. *hirtum* had the similar chemical properties. The most significant differences were established when the amount of essential oils in wild marjoram raw material was analyzed. It was found from 0.04 to 0.16% of essential oils in the raw material of *Origanum vulgare* L. during various stages of growth. From 0.56 to 1.78% of essential oils was found in the raw material of subspecies *Origanum vulgare* subs. *hirtum*. Consequently, amount of essential oils was ten times as large. *Origanum vulgare* subs. *hirtum* is a valuable subscpecies for use as aromatic and medicinal raw material.

Key words: wild marjoram, *Origanum vulgare* L., O*riganum vulgare* subs. h*irtum*, chemical composition, essential oils, chlorophyll, carotenoids, yield

INTRODUCTION

Wild marjoram (*Origanum vulgare* L.) is valuable for its aromatic and medicinal properties, strong antimicrobic and antioxidative effect [1-4]. This species is native in Lithuania and is being cultivated also. There are rather abundant genetic resources of marjoram in Lithuania, but this plant synthesizes only a small amount of essential oils [5, 6]. Numerous investigations of essential oils of wild marjoram showed that their amount and quality mostly depends on habitats of raw material and genetic properties of the plant [7, 8]. Subspecies of wild marjoram (*Origanum vulgare* subs. *hirtum*), which grows in the South Europe, have especially large amount of essential oils [9]. According to the investigations, which are being conducted in the world nowadays, it is possible to state that the analysis of the chemical composition of wild marjoram is important when producing high quality aromatic and medicinal raw material [10-12]. The aim of our investigation is to compare and to evaluate biological and chemical properties of *Origanum vulgare* L. and *Origanum* vulgare subs. *hirtum* in various growth stages in Lithuania.

MATERIALS AND METHODS

Investigations were carried out at the Lithuanian Institute of Horticulture. The seedlings of the investigated plants were planted in the field at the end of May in rows, each 70 cm. Distance between seedlings in a row – 30 cm. Yield and chemical analyses were carried out in various growth stages: 1 – regrowth (at the beginning of vegetation and when plants grow again after winter); 2 – growth (intensive vegetative growth); 3 – butonization (formation of inflorescence); 4 – flowering (at the time of mass flowering); 5 – after flowering (when seeds ripen). Fresh grass of wild marjoram was used for investigations. The yield of the grass of wild marjoram subspecies *Origanum vulgare* L. and *Origanum vulgare* subs. *hirtum* and the amounts of pigments (carotenoids, chlorophylls *a* and *b*), essential oils, ascorbic acid, nitrates and dry soluble solids were evaluated during various stages of growth. Chemical analyses were carried out at LIH, at the Laboratory of Biochemistry and Technology: essential oils were analysed by the method of hydro distillation with an apparatus of Clevenger type; dry soluble solids – by refractometer; ascorbic acid – by titration with the solution of sodium chloride of 2.6-dichlorphenolindophenol; nitrates – by potentiometric method with yon selective electrode; the amount of chlorophylls and carotenoids – by spectra-photometrical method [13-17]. Results are mean for 2003-2006. The three sets of data were analyzed by one-way and two-way

analysis of variance (ANOVA, vers. 3.43, 2002). Significance of difference was estimated at 5 % level.

RESULTS

Two forms of wild marjoram, which belong to the same *Origanum vulgare* species and which were used in our investigations, differ by several morphological features. The species *Origanum vulgare* L. naturally grown or cultivated in Lithuania distinguishes itself by light or dark red blooms and dark green leaves. During flowering these plants reach the height of 60 cm. *Origanum vulgare* subs. *hirtum* native in the South Europe grows up on the average up to 50 cm in Lithuania. Its flowers are white and leaves – light green (Table 1).

The yield of fresh material depended on the growing year and growth stage of wild marjoram. Most abundantly yielded flowering marjoram: then the yield of their fresh weight on the average reached 18.03-18.06 t ha^{-1}. At the beginning of vegetation the yield of marjoram, which grew up after winter, reached 8 t ha^{-1}, during growth – 12 t ha^{-1}, during butonization – 15 t ha^{-1}, and after flowering – only 10 t ha^{-1} (Table 2). It was observed that *Origanum vulgare* subs. *hirtum* formed more stems, therefore their two-year-old and older shrubs were denser and slightly more productive.

According to the data of the investigation, the yield of their fresh weight of subspecies *Origanum vulgare* subs. *hirtum* was the same or slightly bigger in comparison with *Origanum vulgare* L. grown in Lithuania. The output of dry weight of the investigated marjoram during vegetation and plant ripening varied from 19% up to 49% (Table 3). The output of dry weight from the yield of fresh grass is defined by fourth degree regression equation: $y = -0.1466x^4 + 7.8419x^3 - 152.88x^2 + 1282.7x - 3863.1$ with determination coefficient $R^2 = 0.999$.

The amount of the pigment substances – chlorophylls and carotenoids was established in the investigated objects. The total chlorophyll amount varied from 0.69 up to 1.30 mg g^{-1} in fresh grass of *Origanum vulgare* subs. *hirtum* and it was larger and varied from 0.92 up to 1.18 mg g^{-1} in the grass of *Origanum vulgare* L. (Fig. 1). The total amount of chlorophylls increased in the raw matter up to the flowering of the plants, and then suddenly decreased because of the changed biochemical composition of the plants, more abundant carotenoids synthesis during flowering and the changed plant physiology during seed ripening.

Origanum vulgare L. preserves more stabile ration of chlorophylls *a* and *b* – from 1.45 up to 1.73; Ratio of chlorophylls *a* and *b* varies from 1.15 up to 1.91 for *Origanum vulgare* subs. *hirtum* (Fig. 2). Larger carotenoids amount at the beginning of vegetation is characteristic of *Origanum vulgare*

L. The plants of *Origanum vulgare* subs. *hirtum* synthesize more of them at the second half of vegetation (Fig. 3).

It was established that *Origanum vulgare* L. during all growth stages distinguished itself by more abundant (12.7-19.7%) amount of dry soluble solids in comparison to the raw material of *Origanum vulgare* subs. *hirtum* (4.9-17.8%). Small amount of ascorbic acid (from 3.8-4.6 mg 100 g^{-1}), which was at the beginning of vegetation in the grass of wild marjoram, increases during flowering (up to 11.4-14.0 mg 100 g^{-1}). *Origanum vulgare* L.synthesizes larger amount of this substance. Both investigated plants synthesize equable amounts of nitrates during various growth stages. Their amount fluctuates from 680 to 970 mg kg^{-1} (Table 4).

Analysis of the amount of essential oils showed the largest differences between the investigated forms of wild marjoram. It was established from 0.04 to 0.16% of essential oils in fresh raw material of *Origanum vulgare* L. during various growth stages and from 0.56 to 1.78% of essential oils was found in raw material of *Origanum vulgare* subs. *hirtum* (Fig. 4). This amount is ten times as large.

DISCUSSION

Origanum vulgare L. and *Origanum vulgare* subs. *hirtum* are similar by biological but different by several chemical features. The results showed differences among essential oil amount and composition of wild marjoram. Valuable features of *Origanum vulgare* subs. *hirtum* give reasons for introduction and cultivation of this aromatic and medicinal plant in Lithuania.

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E. Dambrauskienė, P. Viškelis, M. Rubinskienė

PAPRASTOJO RAUDONĖLIO *ORIGANUM VULGARE* L. IR *ORIGANUM VULGARE* SUBS. *HIRTUM* PORŪŠIO BIOLOGINIS IR BIOCHEMINIS ĮVERTINIMAS

Santrauka

Lietuvos sodininkystės ir daržininkystės institute atlikti paprastojo raudonėlio *Origanum vulgare* L. ir *Origanum vulgare* subs. *hirtum* porūšio botaninių ir biocheminių rodiklių tyrimai. Pastebėta, kad Pietų Europoje augantis paprastojo raudonėlio *Origanum vulgare* subs. hirtum porūšis skiriasi nuo Lietuvoje auginamo *Origanum vulgare* L. aromatu ir ypač dideliu eterinių aliejų kiekiu. Tyrimų duomenimis, paprastųjų raudonėlių produktyvumas (žolės derlius) įvairiais augimo etapais buvo panašus. Nustatyta, kad įvairiais augimo etapais paprastojo raudonėlio *Origanum vulgare* L. ir *Origanum vulgare* subs. *hirtum* porūšio žolė pasižymi panašiomis cheminėmis savybėmis. Didžiausi skirtumai nustatyti ištyrus eterinių aliejų kiekį raudonėlio žaliavoje. Įvairiais augimo etapais *Origanum vulgare* L. žaliavoje nustatyta nuo 0,04 iki 0,16 % eterinių aliejų, o *Origanum vulgare* subs. hirtum porūšio žaliavoje rasta nuo 0,56 iki 1,78 % eterinių aliejų. Skirtumas buvo dešimt kartų didesnis. Atsižvelgiant į šią *Origanum vulgare* subs. *hirtum* porūšio savybę, verta šį augalą auginti ir naudoti kaip aromatinę ir vaistinę žaliavą.

Table 1. **Differences of morphological features of wild marjoram.**

Wild marjoram	Average plant height, cm	Colour of flowers	Colour of leaves
Origanum vulgare subs. *hirtum*	50	white	light green
Origanum vulgare L.	60	rose	dark green

Table 2. **The yield of fresh marjoram grass (t ha^{-1}) in various growth stages.**

Wild marjoram	Growth stage				
	1	2	3	4	5
Origanum vulgare subs. *hirtum*	8.01	12.09	14.99	18.06	10.06
Origanum vulgare L.	7.99	12.03	15.04	18.03	10.04
LSD_{05}	0.428	1.230	0.541	0.277	0.155

Table 3. **The output (%) of dry weight of marjoram grass in various growth stages.**

Wild marjoram	Growth stage				
	1	2	3	4	5
Origanum vulgare subs. *hirtum*	19.4	23.0	27.2	31.3	49.3
Origanum vulgare L.	19.3	22.9	26.8	31.1	49.1
LSD_{05}	0.89	0.91	1.30	1.42	1.66

Fig. 1. The amount of chlorophylls (mg g^{-1}) in fresh marjoram raw material in various growth stages.

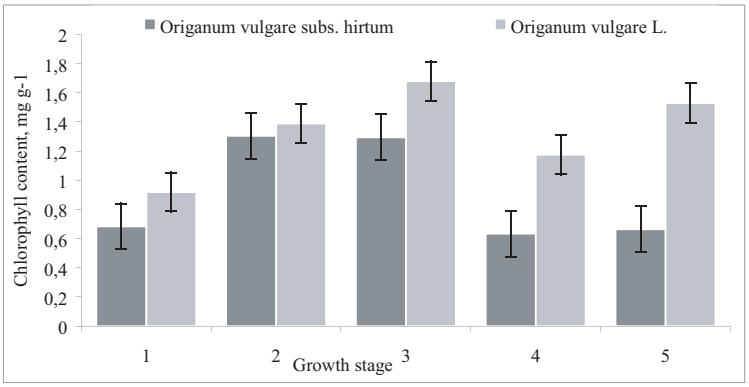

Fig. 2. The ratio of chlorophylls *a* and *b* in fresh marjoram raw material in various growth stages.

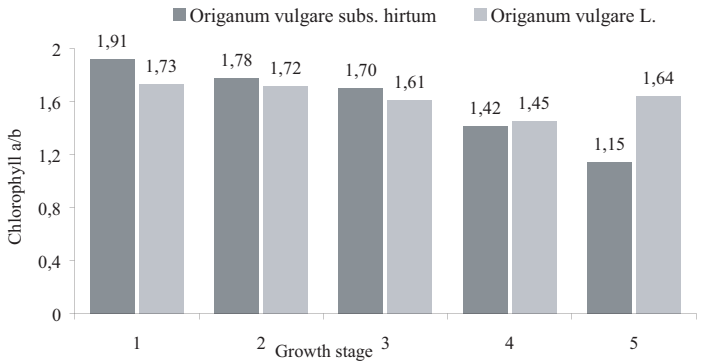

Fig. 3. The amount of carotenoids in fresh marjoram raw material in various growth stages, mg 100 g^{-1}, A-forms of marjoram; B-growth stages.

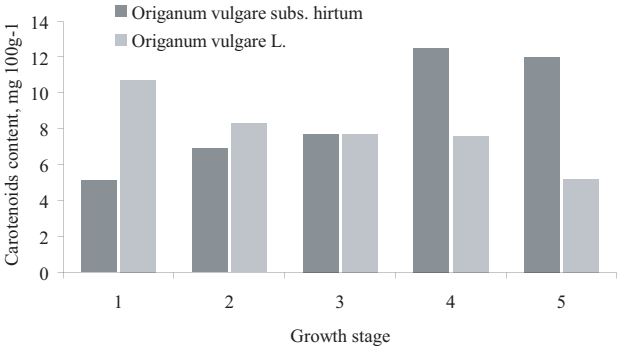

Table 4. **Chemical composition of fresh marjoram raw material.**

Wild marjoram	Growth stage				
	1	2	3	4	5
Dry soluble solids, %					
Origanum vulgare subs. *hirtum*	4.9	5.1	10.0	14.9	17.8
Origanum vulgare L.	12.7	14.2	16.5	17.7	19.7
LSD_{05}	0.92	1.11	1.81	1.59	1.64
Ascorbic acid, mg 100 g^{-1}					
Origanum vulgare subs. *hirtum*	3.8	5.6	10.0	11.4	6.8
Origanum vulgare L.	4.6	6.0	10.3	14.0	8.4
LSD_{05}	0.27	0.39	1.02	1.18	0.75
Nitrates, mg kg^{-1}					
Origanum vulgare subs. *hirtum*	960	970	850	810	790
Origanum vulgare L.	940	870	820	730	680
LSD_{05}	75.2	79.4	67.5	59.1	64.9

Fig. 4. The amount of essential oils (%) in fresh marjoram raw material in various growth stages.