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Essential Oil of *Origanum vulgare* ssp. *vulgare* L. and *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart from Moldova: Content and Chemical Composition

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***Abstract* – Different genotypes of *Origanum vulgare* ssp. *vulgare* L. and *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart have been evaluated. The quantitative characters vary in dependence of genotypes and subspecies. The plants height is 65-80 cm that is composed from 100-115 floral stems for ssp. *vulgare* and 120-240 for ssp. *hirtum*. Inflorescences of *O.vulgare* ssp. *vulgare* have 15-18 ramifications, *O.vulgare* ssp. *hirtum* - 20-30. Flowers are white (ssp. *hirtum*) or pink (ssp. *vulgare*). The content of essential oil separated by hydrodistillation in case of *O.vulgare* ssp. *vulgare* varies between 0.108% and 0.249% and *O.vulgare* ssp. *hirtum* 2.409% - 5.422%. GC GC-MS analysis attested 41 mono- and sesquiterpenes for *O.vulgare* ssp. *vulgare*, which constitutes 97.78% of the oil. The *O. vulgare* ssp. *hirtum* essential oils is composed from 24 to 31 components depending on the genotype. The range of identification is 99.07-99.90%. Germacrene D (17.01%); β-caryophyllene (13.05%); carvacrol (11.65%); sabinene (9.78%); *trans*- ocimene (9.38%); *cis* -ocimene (6.03%), and γ-elemene (4.10%) are the major components of *O.vulgare* ssp. *vulgare* oil. Essential oil of all genotypes of *O.vulgare* ssp. *vulgare* represents one chemotype-germacrene D/β-caryophyllene/carvacrol/trans-& cis-ocimene/γ-elemene. The major components of the essential oil of the *O.vulgare* ssp. *hirtum* genotypes include carvacrol (67.67-85.85%), p-cymene (3.64-9.33%) or γ-terpinene (8.22%). Thus, the *O.vulgare* ssp. *hirtum* genotypes are divided into three chemotypes: carvacrol/p-cymene; carvacrol/p-cymene/γ-terpinene; carvacrol/γ-terpinene/p-cymene.**

***Keywords* – Chemical Composition, Essential Oil, Genotype, *Origanum vulgare ssp. hirtum*, *Origanum vulgare ssp. vulgare*.**

I. Introduction

Origanum vulgare L., native to the Mediterranean, is an herbaceous perennial species in the family of *Lamiaceae* known and used for ages. Along with other *Origanum* species, *O.vulgare* represents an important part of the European natural biodiversity. Ample investigations have been carried out in different countries [14], [27], [15] [22] to successfully use and put in value the species in different areas. Multiple biotypes, genotypes, forms, and taxons of Oregano have a strictly local distribution and are distinguished through accentuated morphological and biochemical diversity [13],[10], which is confirmed by the studies on the species, subspecies in the definite areal of prevalence. The relevance of the studies on Oregano is also determined by the importance of the species as a medicinal, aromatic, culinary, spicy, ornamental, and meliferous plant, supported by the chemical composition – flavonoides, vitamins, bitter substances, and essential oil, synthesized and accumulated in the aerial part of the plant. This is essential oil [26], [6] and, especially, its major components – carvacrol and thymol, that is responsible for the antimicrobial, antifungal, and antioxidant action, as well as the capacity to inhibit bacterium growth [9], [7], [28] [16]. Oregano essential oil is also successfully employed for its antispastic and antiseptic action [7], [31]. Oregano is known to possess sedative, carminative, emanogogous, diuretical and other actions. It is also utilized as an aromatizer, preservative in food products [11]. The antimicrobial and antifungal properties of *Origanum vulgare* essential oil are successfully employed for flavouring and preservation, storage of food products [26], [9], [7], [8], [3]. It is known that the decoction of O.vulgare possesses antioxidant activity, while its hydroalcoholic extract demonstrates antimicrobial effect [17].

This work is dedicated to the studies on the content, qualitative and quantitative analysis of the essential oil extracted from the different genotypes *Origanum vulgare* L. subsp. *vulgare* and *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart cultivated in the Republic of Moldova.

II. Materials and Methods

Our study was carried out during the 2013 growing period and included six genotypes of *Origanum vulgare* ssp. *vulgare* L. and six genotypes of *O.vulgare* ssp. *hirtum* (Link) Ietswaart. In order to determine the content of essential oil, the samples of fresh herbs, aerial part of the plant, were harvested in the morning hours at the flowering stage. The essential oil was isolated by hydrodistillation for 60 minutes, using the Ginsberg apparatus: 100 g of fresh aerial part per 200 ml of water. The content of essential oil was recalculated per dry matter. Following distillation, the essential oil was dried over anhydrous sodium sulphate and stored at 4-6 ^{0}C. Qualitative and quantitative analysis of the essential oil was conducted using GC coupled with Mass Spectrometry (GC-MS): gas chromatograph - Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μм) with HP-5ms non-polar stationary phase. The injector and detector temperature were 250°C and 280°C respectively, using a temperature gradient from T_1 = 70°C (2 min), T_2 = 200°C (5°C/min) to T_3 = 300°C (20°C/min, 5 min). Mobile phase: helium 1ml/min, injected volume of essential oil - 0.03 μl, split rate - 1:100. The identification of chromatographic peaks was performed using the software package AMDIS ™, coupled with NIST database.

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III. Results and Discussion

The species *Origanum vulgare* ssp. *vulgare* L. is recorded in the spontaneous flora of the Republic of Moldova, but both species *O. vulgare* ssp. *vulgare* L. and *O.vulgare* ssp. *hirtum* (Link) Ietswaart (syn. *O.heracleoticum*) are cultivated in the Republic of Moldova.

Our studies have demonstrated that *O.vulgare* ssp. *vulgare* and *O.vulgare* ssp. *hirtum* plants are characterized by the formation of shrubs 65-80 cm tall and a large number of floral stems: 100-115 in ssp. *vulgare* and 120-240 in *O.vulgare* ssp. *hirtum*. The inflorescences of the genotypes belonging to the species *O.vulgare* ssp. *hirtum* develop 20-30 ramifications, while those of the species *O.vulgare* ssp. *vulgare* - 15-18. These two species also differ in the inflorescence ramification length - *O.vulgare* ssp. *hirtum* has shorter ramifications, thus the inflorescence being more compact. Flowers have are white flowers in *O.vulgare* ssp. *hirtum* as opposed to a pink flowers in *O. vulgare* ssp. *vulgare*.

The content of essential oil ranges between 0.108% and 0.249% in different *O. vulgare* ssp. *vulgare* genotypes. The *O.vulgare* ssp. *hirtum* genotypes studied differ in the oil content that also varies but is higher making 2.409-5.422% depending on the genotype.

The similar difference in the content of essential oil of these two genotypes was recorded in Hungary [12]. The variability of this character was also attested in the species *O.vulgare* ssp. *glandulosum* (Desf.) Ietswaart (2.5-4.6 %) in Tunisia [18]. Estimation of the essential oil content in *O. vulgare* (ssp. *vulgare* and ssp. *hirtum*) from the spontaneous flora of Albania also confirms the variability of this character [10]. It has been also demonstrated that breeding programs have resulted in the development of *O.vulgare* ssp. *hirtum* genotypes characterized by a very high content of essential oil (7-8.6%) [25].

Qualitative and quantitative analysis of *O. vulgare ssp. vulgare* essential oil through GC GC-MS techniques has demonstrated that the number of the components identified is actually similar in all the genotypes studied. The concentration of the components differs but not within wide limits. For this reason, the findings of the qualitative and quantitative analysis are presented only for the genotype VG-4 with a higher content of essential oil (0.249%). Forty one components have been identified in the essential oil of this genotype, the identification rate making 97.78% (table 1).

The similar results (42 components) have been found on the *O. vulgare* ssp. *vulgare* population from Lithuania [20] and Germany [2]. In Austria though, a higher number (53) of components have been identified in this species, all of them being mono- and sesquiterpenes [15], just like in the essential oil isolated from the genotypes studied by us.

The major components of *O. vulgare* ssp. *vulgare* essential oil (genotype VG-4) are as follows: germacrene D, 17.01%; β-caryophyllene, 13.05%; carvacrol, 11.65%; sabinene, 9.78%; *trans*- ocimene 9.38%; *cis* – ocimene, 6.03%; and γ-elemene, 4.10% as opposed to *O.vulgare* ssp *hirtum*, with very high concentrations of carvacrol.

The same major components, but at the concentrations differing from those estimated by us, are contained in the essential oil of this species from the wild flora of Lithuania. β-ocimene (14.9-21.6%), followed by germacrene D (10.0-16.2%), β-caryophyllene (10.8-15.7%), and sabinene (6.6-4.2%) are the major components with the highest concentration estimated there [20]. In other researches it was observed the most abundant contents of caryophyllene oxide (34.44%), β-caryophyllene (20.40%) and α-cadinol (7.02%)[4].

In the oil isolated from the *O. vulgare ssp. vulgare* genotype described by us, sabinene has higher concentrations, while ocimene (15.41%) is represented by *trans*- (9.38%) and *cis*- (6.03%) isomers. In the other species, *O. vulgare* L. ssp. *viride* (Boiss.) Hayek, the ocimene is the major component (35.1%) with the highest concentration [3]. Of the components with relatively elevated concentrations identified in the VG-4 genotype, we could mention □-elemene (4.10%). Thymol though constitutes only 0.33% in the oil of this genotype, being at a similar concentration (0.34%) to that attested in this species in Hungary [32], the major components being different - *p*-cymene (22.3%) and caryophyllene oxide (10.2%).

Thus, we can state that *O. vulgare subspecies* varies in both the content of essential oil and its chemical qualitative and quantitative composition.

The content of essential oil is much higher on *O. vulgare* ssp. *hirtum* (Link) Ietswaart than in *O. vulgare* ssp. *vulgare* L., but the variation in the genotype is within quite wide limits (2.409-5.422%). Carvacrol is the component with the highest concentration. The concentration of the major components in the essential oil differs with the genotypes. Thus, a qualitative and quantitative analysis of the essential oil extracted from six *O. vulgare* ssp. *hirtum* evaluated has demonstrated a different number of components, they varying between 24 in the HG-34/1 and 31 in the HG-64/2 genotype, the identification rate being 99.07-99.90% (Table 1). Other researchers have detected from 19 [14] to 56 [23], 81 [29] and 104 compounds [16] on *O. vulgare* ssp. *hirtum.*

The major components of the essential oil in all the *O. vulgare* ssp. *hirtum* genotypes evaluated are carvacrol (77.61-85.88%), followed by p-cymene (3.64-9.33%) and γ-terpinene (6.62-2.06%). The HG-64/1 genotype where the second major component is γ-terpinene (8.22%), followed by *p*-cymene (5.30%) is an exception. The quantitative and qualitative differences of the essential oil isolated from our *O. vulgare* ssp. *hirtum* genotypes are also accentuated by the concentration of another component – γ-terpinene that varies significantly in different genotypes. Thus, this component constitutes 2.10% and 2.46% in the essential oil of the HG-34/6 and HG-34/1 genotypes, respectively, the concentrations that are close to those attested in the VG-4 genotype of *O. vulgare ssp. vulgare* (2.06%). It reaches 6.62% (genotype HG-34/5) and 8.22% (genotype HG-64/1) on other *O. vulgare* ssp. *hirtum* genotypes.

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Table 1: The qualitative and quantitative composition of essential oil of the Origanum vulgare ssp. vulgare L. and O. vulgare ssp. hirtum (Link) Ietswaart genotypes

Nr. Pic	Component	Rt	Area, %						
		Sample	ssp. *vulgare*	ssp. *hirtum*					
			VG-4	HG-34/1	HG-34/5	HG-34/6	HG-53/1	HG-64/2	HG-64/1
1	p-xylene	3,52	0.12	0.08	0.08	0.08	0.36	0.08	0.06
2	Origanene	4,48	0.11	0.52	2.07	0.54	0.36	1.23	0.42
3	α-Pinene	4,64	0.13	0.22	0.84	0.22	0.17	0.50	0.18
4	Camphene	4,95	-	-	0.16	0.05	0.09	0.13	0.05
5	Sabinene	5,42	**9.78**	0.59	1.34	0.47	0.18	0.50	0.59
6	β-Pinene	5,52	0.15	0.08	0.28	0.09	0.07	0.16	0.07
7	3-Octanone	5,64	0.08	-	0.04	-	0.06	-	-
8	β-Mircene	5,74	1.07	0.87	2.97	0.93	0.67	1.72	0.87
9	3-Octanol	5,82	0.16	-	0.33	-	-	-	-
10	α-fellandrene	6,10	0.04	-	-	0.13	0.06	0.21	0.10
11	α-Terpinene	6,37	0.49	0.54	1.46	0.59	0.46	0.82	0.99
12	p-Cymene	6,56	0.73	**4.05**	**9.33**	**3.64**	**4.27**	**5.08**	**5.30**
13	Limonene	6,67	0.59	0.23	0.64	0.23	0.20	0.38	0.24
14	Eucalyptol	6,74	0.30	-	0.05	-	0.11	0.04	-
15	*trans*-ocimene	6,82	**9.38**	-	-	-	-	0.05	-
16	*cis*-Ocimene	7,08	**6.03**	0.05	0.09	-	0.05	0.07	0.04
17	γ-terpinene	7,38	2.06	**2.46**	**6.62**	**2.10**	**3.20**	**4.00**	**8.22**
18	4-terpinenyl acetate	7,60	0.09	0.44	0.44	0.36	0.78	0.28	0.50
19	α-Terpinolene	8,13	0.13	0.06	0.15	0.07	-	0.07	0.04
20	Linalool	8,38	0.82	0.32	0.24	0.31	0.23	0.28	0.40
21	Camphor	9,46	0.06	-	-	-	-	-	-
22	Borneol	10,18	0.05	0.22	0.10	0.09	0.54	0.22	0.25
23	4-Terpineol	10,48	1.53	0.59	0.51	0.40	0.28	0.58	0.33
24	α-Terpineol	10,82	0.57	0.13	0.09	0.08	0.10	0.12	0.16
25	Thymol metil ester	12,22	-	-	0.08	0.05	0.02	0.05	-
26	(+)Carvone	12,29	-	-	-	0.07	-	0.10	0.13
28	Thymol	13,45	0.33	0.23	0.19	0.23	0.19	0.22	0.23
29	Carvacrol	13,72	**11.65**	**83.75**	**67.67**	**85.77**	**85.85**	**79.93**	**77.61**
30	O-Acetyl thymol	15,59	-	-	-	0.14	0.10	0.24	0.06
31	β-Bourbonene	15,97	0.75	-	-	-	-	-	-
32	β-Caryophyllene	16,86	**13.05**	**2.77**	**3.16**	**2.37**	0.91	1.99	1.42
33	β-Cubebene	17,08	0.16	-	-	-	-	-	-
34	α-Cubebene	17,50	0.12	-	-	-	-	-	-
35	α-Caryophyllene	17,70	1.69	-	0.11	0.09	0.12	0.18	0.12
36	(+)Aromadendrene	17,88	0.66	-	-	-	-	-	-
37	α-Amorfene	18,24	0.10	-	-	-	-	-	-
38	D-germacrene	18,38	**17.01**	0.32	0.26	0.28	-	0.07	0.03
39	γ-Elemene	18,75	**4.10**	-	-	-	-	-	-
40	(+)β-Bisabolene	18,98	3.97	0.36	0.27	0.28	0.18	0.55	0.65
41	γ-Cadinene	19,36	1.91	0.15	0.09	0.10	-	0.05	0.06
42	γ-Muurolene	20,61	2.47	-	-	-	-	-	-
43	Caryophyllene oxid	20,81	1.56	0.11	-	0.10	0.10	-	0.10
44	β-Guaiene	22,10	1.39	-	-	-	-	-	-
45	δ-Cadinole	22,39	2.04	-	-	-	-	-	-
46	α-Muurolene	23,23	0.26	-	-	-	-	-	-
No. identified components			41	24	29	29	28	31	29
Total, identified components, %			97.78	99.07	99.57	99.86	99.71	99.90	99.22

Elevated concentrations of carvacrol (70-93%**),** that is the major component in the essential oil, have been found by other authors [25], [21], [32], [16] in *O. vulgare* ssp. *hirtum* and in O. vulgare ssp. *scabrum* [1]. The major components of the essential oil extracted from *O. vulgaris* ssp. *hirtum* in Sicilia included thymol (24.0-54.4%), γ-terpinene (9.8-30.5%), p-cimene (5.2%) [30]. According to the other researches one of the major components is thymol along with carvacrol [23], [9], [10], [16], is a minor component (0.19-0.23%) in the oil isolated from our *O. vulgare* ssp. *hirtum* genotypes just like in the oil isolated from *O. vulgare ssp. vulgare* (0.33%). Linalool is also a minor component, which makes the genotypes (chemotypes) evaluated by us differ from some chemotypes of *O. vulgare* ssp. *hirtum* in Italy [9].

It has been found that the content and composition of the essential oil are stable in both *O. vulgare ssp. vulgare* and *O. vulgare ssp. hirtum* during the whole flowering period, which is the time of harvesting [32]. According to the findings published by some authors, the content and composition of the essential oil are not dependent on the cultivation conditions of *O. vulgare* (ssp. *hirtum,* *creticum, samothrake*), [2]. Other researchers claim that the concentration of the major components (sabinene and ocimene) is dependent on the cultivation conditions [8] (. Some studies have concluded that the productivity and content of the essential oil in *O. vulgare* depend on the techniques of planting material production [5].

IV. Conclusion

1. The genotypes *Origanum vulgare ssp. vulgare* L. and *Origanum vulgare* L. *ssp. hirtum* (Link) Ietswaart are distinguished by the morphological characters (tall of plant, number of floral stems and ramifications of inflorescences, the color of flowers).
2. The content of *O.vulgare* ssp. *vulgare* essential oil (0.108% - 0.249%) is lower than *O.vulgare* ssp. *hirtum* (2.409%-5.422%).
3. The essential oil of *O.vulgare* ssp. *vulgare* content 41 components, but in case of *O. vulgare* ssp. *hirtum* genotypes the number of component varies from 24 to 31.
4. The main components in *O.vulgare* ssp. *vulgare* essential oil are D-germacrene (17.01%); β-caryophyllene (13.05%); carvacrol (11.65%); sabinene (9.78%); *trans*- ocimene (9.38%); *cis*-ocimene (6.03%), and γ-elemene (4.10%).
5. The essential oil of the *O.vulgare* ssp. *hirtum* genotypes contains four major compounds: carvacrol (77.61-85.88%), p-cymene (3.64-9.33%) or γ-terpinene (8.22%); *p*-cymene (5.30%) and are divided into three chemotypes: 1-carvacrol/p-cymene; 2-carvacrol/*p*-cymene/γ-terpinene; 3-carvacrol/γ-terpinene/p-cymene.

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