

# CHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF ESSENTIAL OIL FROM *Origanum vulgare* L. IN DIFFERENT HABITATS

M. Kryvtsova<sup>1</sup>

<sup>1</sup>Uzhhorod National University, Ukraine

M. Hrytsyna<sup>2</sup>

<sup>2</sup>Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine

I. Salamon<sup>3</sup>

<sup>3</sup>University of Prešov, Prešov, Slovakia

E-mail: [maryna.krivcova@gmail.com](mailto:maryna.krivcova@gmail.com)

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Essential oils are widely used in beauty therapy, medicine and food industry, and they are considered to be a valuable consumer product. At the same time, the biochemical composition and properties of essential oils, including their antimicrobial activity, varies depending on the habitat, climatic conditions and plant chemotype. The purpose of our work is to study the qualitative and quantitative composition of essential oils and their antimicrobial properties, from *Origanum vulgare* plants harvested in eastern Slovakia and Lviv region, Ukraine.

In the wild, *O. vulgare* L. were gathered in close vicinity of the village of Trostianets, Lviv region, Ukraine, in 2019. In Slovakia, the plants were grown by Agrokarpaty Company, Plavnica. Essential oils were extracted by hydrodistillation (2 hours) in a Clevenger apparatus, according to the European pharmacopoeia.

The analysis of the biochemical properties of essential oils extracted from plant populations from Lviv region, showed that the contents of essential oils were within  $0.35 \pm 0.05\%$ . The composition of essential oils shows that *O. vulgare* L. plants from the natural population grown in Lviv region, belongs to the monoterpene chemotype. Monoterpene hydrocarbons  $\alpha$ -terpinene and  $\alpha$ -terpineol together accounted for 29–33%, acyclic monoterpenes —  $\beta$ -myrcene — 7%, linalool — 4%, while the polyphenol compound p-cymene accounted for only 15%.

The *O. vulgare* plants from Slovakia were characterised by the essential oil content of 0.15 to 0.50%, and the composition which allowed us to refer them to the carvacrol chemotype, with phenols as its main ingredients — carvacrol and thymol (together 71%), and isopropyltoluene (4.0%). Monoterpene hydrocarbons terpinene (5.0%) and terpineol alcohol (6.0%) jointly accounted for 11%; acyclic monoterpene myrcene — 3%; and sesquiterpene  $\beta$ -caryophyllene — 4,5%.

Essential oil from *O. vulgare* harvested in Slovakia demonstrated high antimicrobial activity against reference and clinical isolates of opportunistic microorganisms. Essential oil from the samples gathered in Lviv region, showed low antimicrobial activity.

Thus, it has been shown that the reviewed plants referred to different chemotypes, which calls forth the prospect of the use of essential oils extracted from different plant chemotypes for different purposes, depending upon their biochemical composition and properties.

**Key words:** oregano essential oil, antimicrobial activity, biochemical properties.

Common oregano, or wild marjoram (*Origanum vulgare* L.), genus *Origanum* (*Lamiaceae* Martinov) has somewhat been ‘forgotten’ in Ukraine as a medicinal plant, though it remains widely spread in the Mediterranean where it is used as an essential-oil-bearing and aromatic plant. This herb

demonstrates antibacterial and antioxidant activity, and antifungal property. It owes its high biological activity to the presence of essential oils, flavonoids, and glycosides.

*O. vulgare* is a polymorphic species, and *Flora Europaea* (1972) distinguishes several subspecies of this species: *O. vulgare* ssp.

*vulgare*, *O. vulgare* ssp. *hirtum*, *O. vulgare* ssp. *viride*. The subspecies differ by the structure of their reproductive organs, location of the essential-oil trichomes, and composition of the respective essential oils [1].

Academic literature distinguishes chemotypes of populations that form “biochemical varieties” or “physiological forms” in botanic species. Say, Italian scholars have singled out four reasons affecting the population chemotype of *O. vulgare* and its subspecies. First of all, it is the environmental conditions, especially climate, that affect the biosynthetic way of phenol compounds; secondly, the sexual polymorphism and the genetic mechanism; thirdly, the state of the plants (fresh or dried); fourth, the phenophase of the plants’ development.

The phytochemical analysis of plants from 51 populations in 17 European countries has established that active cymyl and/or acyclic linalool/linalyl acetate synthesis of essential oils is peculiar for plants from the Mediterranean, whereas active sabinyl was peculiar for the plants growing in the continental [2]. In these plants, the contents of essential oils amounted to 0.03–4.6%, and their composition included sabinene, myrcene, p-cymene, 1.8-cyneol,  $\beta$ -ocimene,  $\gamma$ -terpinene, sabinene hydrate, linalool,  $\alpha$ -terpineol, calvacrol methyl ether, linalyl acetate, thymol and carvacrol,  $\beta$ -caryophyllene, germacrene D, germacrene D-4-ol, spathulenol, caryophyllene oxide, and oplopanone.

The chemotype with phenolic prevalence was typical for southern Italy. Four variants of thymol- and carvacrol-chemotype were distinguished in the *O. vulgare* ssp. *hirtum* populations from different parts of Sicily. The author connects such differences with the mixture of genetic and environmental factors affecting the biosynthesis of essential oils [3]. In the *O. vulgare* ssp. *hirtum* populations from Campagna [4], carvacrol/thymol chemotype prevailed along the sunny coast; another, thymol/ $\alpha$ -terpineol chemotype was peculiar for the populations located in the mainland part of the country characterised by lower air temperatures. The third chemotype, with prevalence of linalyl acetate and linalool (the lowest content of phenols) was spread along the mountainous coastline.

In Bulgaria, carvacrol, whose content reached as much as 73.4%, dominated in all samples [5]. The samples of *Origanum vulgare* ssp. from southern Croatia picked in different seasons belonged to the thymol/carvacrol type [6]. The plants of *O. vulgare* ssp. *hirtum*

collected from northern Greece were rich in thymol, and those collected from southern Greece — in carvacrol [7]. Studies of the contents of essential oils from *O. vulgare* grown in the south-eastern part of Spain showed the following results: (E)- $\beta$ -caryophyllene (0.5–4.9%), thymol (0.2–5.8%), p-cymene (3.8–8.2%),  $\gamma$ -terpinene (2.1–10.7%) and carvacrol (58.7–77.4%) [8].

Another chemotype characterised by prevalence of sesqui- or monoterpenes, was typical for the populations from the continental climate with significantly lower solar radiation and temperatures [9]. In the *O. vulgare* plants from China and Pakistan, from 11 to 46 components of essential oil were isolated, in which oxygenic monoterpenes prevailed, and, besides, two populations showed high contents of sesquiterpene hydrocarbons — 33.7% and 43.7%, respectively [10].

In the essential oil from *O. vulgare* grown in the Ukrainian Polissia region, 24 components were identified, of which the following ones prevailed:  $\alpha$ -cadinol (14.24%), germacrene D (13.76%),  $\beta$ -caryophyllene (12.23%), 1,6-germacradiene-5-ol (11.12%), epi- $\alpha$ -cadinol (8.56%),  $\alpha$ -farnesene (5.75%), terpinene-4-ol, thymol, cis-sabinenhydrate, linalool,  $\gamma$ -terpinene-trans-ocimene, geraniol, and neral [11].

Quite often, different chemotypes of a population are linked with different subspecies of *O. vulgare*. As seen from the literature analysis, thymol-chemotype is typical for *O. vulgare* ssp. *hirtum*, whereas linalool is the main volatile component of *O. vulgare* ssp. *viridens*, where the content of thymol is insignificant [12].

Thereby, both the composition and the properties of *O. vulgare* depend upon the natural climatic conditions as well as the genetic conditionality of the plants’ biochemical properties.

The purpose of our work is to study the qualitative and quantitative composition and antimicrobial properties of essential oils from *O. vulgare* plants collected in eastern Slovakia and Lviv oblast, Ukraine.

## Materials and Methods

### *Oregano herb growing and harvesting conditions*

The demand of oregano raw material is satisfied by large-scale cultivation by the Agrokarpaty Company, Plavnica, Eastern Slovakia (N 49° 16' 28", E 20° 46' 50", altitude above sea level: 530 m). The production of

oregano monocultures needs a light, rich, well-drained soil with pH of 6.0–8.0. Growth occurs between 4 and 32 °C, optimally at 23 °C. Oregano plants are propagated vegetatively to obtain plants with the desired flavour and aroma characteristics of their parents (the Krajova variety). The first harvest of leaves and tender tops occurs just as flowering commences. The plants are cut 60–100 mm from the ground. In the region 3–4 cuts can be made over a single year. Where commercially grown, oregano plantings are productive for 4–5 years. The leaves are thoroughly dried, cleaned, and stored as soon as possible. The area of cultivated parcels varied from 2 to 5 ha in the last years. Typically, yields of 1,500–3,000 kg per ha of dried herb are obtained.

Plant samples from the natural habitat of *Origanum vulgare* L. were taken in 2019 in the stow which is a geological nature sanctuary of local significance. It is located by the road in the western part of the village of Trostianets, Mykolaiv rayon, Lviv oblast (geographical coordinates: 49°33'03" north latitude and 24°00'28" east longitude; average altitude above sea level — 283 m). The site represents Tortonian sandstone — tightly pressed sand, white-coloured with a yellowish tint; with patches of hard rock with small grottoes, where leftovers of the Tortonian layer flora had been found.

January is the coldest month in Lviv oblast; its mean temperature is by 2–3 °C lower than in December. All winter months in the Opillia region are characterised by a big variability of air temperature (2.5–4.7 °C). The temperatures in July have been observed to drop insignificantly (to 17.0–17.5 °C) in the elevations of Roztochia and Opillia, whereas Little Polissia and Precarpathia have shown the highest monthly mean temperatures (18.0–18.5 °C). Lviv oblast is peculiar for rather significant yearly precipitation totals, varying between 579 and 1,070 mm. The largest amount of precipitation falls on June — July (90–140 mm a month); the lowest — on January — February (24–40 mm a month). Thus, the amount of precipitation in summer is by 2 to 3 times higher than in winter.

#### *Origanum oil isolation*

The essential oil from this raw-material was prepared by hydro-distillation (2 hours) in Clevenger-type apparatus according to the European Pharmacopoeia and a mixture of hexane was used as a collecting solvent. The essential oils stored under N<sub>2</sub> at + 4 °C in the dark space before their composition identification.

#### *GC-FID analyse*

The analysis of oregano essential oils was carried out using a Vega Series Carloerba Gas Chromatograph, connected to a Spectrophysics SP 4270 integrator. The following operating conditions were used: column: DB5, 30 m×0.32 mm inner diameter (i. d.), film thickness: 0.25 mm, carrier gas: nitrogen, adjusted to a flux of 1 mL per min, injection and FID-detector temperatures: 220 °C, respectively 250 °C. Components were identified by their GC retention times, and the resulting values were comparable to those of literature. Oil component standards for comparison were supplied by Extrasynthese Ltd. (France).

#### *GC/MS analyse*

GC/MS analyses were carried out on a Varian 450-GC connected with a Varian 220-MS. The separation was achieved using a Factor Four TM: Capillary Column VF 5 ms (30 m × 0.25 mm i. d., 0.25 µm film thickness). Injector type 1177 was heated on temperature 220 °C. Injection mode split less (1 µl of a 1:1,000 n-hexane/diethyl ether solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 mL per min. Column temperature was programmed: initial temperature 50 °C for 10 min, then to 100 °C at 3 °C per min; isothermal for 5 min and then continued to 150 °C at 10 °C per min. Total time for analysis of one sample took 54.97 min. Identification of components was done by comparison of their mass spectra with those stored in NIST 02 (software library) or with mass spectra from the literature [13] (Adams, 2007) and a home-made library, as well as on comparison of their retention indices with the standards.

#### *Antimicrobial assay*

Antimicrobial activity of Origanum EO was determined using agar diffusion test [14]. The bacterium inocula 100 µL in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37 ± 2 °C for 24 hours); yeasts — on SDA agar (incubated at 35 ± 2 °C for 48 hours). The extracts 20 µL were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. Each antimicrobial assay was performed at least three times.

As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922; *Enterococcus*

*faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615; reference *S. aureus* CCM 4223 biofilm-forming strain. We also used clinical strains of bacteria and yeasts (*S. aureus*, *E. coli*, *S. pyogenes*, *E. faecalis*, *C. albicans*) isolated from the oral cavities of patients suffering from inflammatory periodontium and pharynx. We chose the clinical strains with multiple resistance at least to two classes of antibiotics. As a positive control were used: gentamicin (10 mg/disk) for Gram-negative bacteria, ampicillin (10 mg/disk) for Gram-positive bacteria, and nystatin (100 UI) for *Candida*. As negative control, DMSO were used.

#### Statistical analysis

Values mentioned are the mean with standard deviations, obtained from three different observations. Values in the control and treatment groups for various molecules were compared using Student's *t*-test. A value of  $P < 0.05$  was considered as statistically significant.

## Results and Discussion

The State Pharmacopoeia of Ukraine [15] lists *O. onites* L. or *O. vulgare* L. subsp. *hirtus*, or mixture of the two species (Origanum herba, OREGANO). The contents of essential oil are no less than 25 ml/kg on anhydrous basis; the aggregate total of carvacrol and thymol in essential oil is no less than 60%. *Origanum*<sup>N</sup> — national description; dried *O. vulgare* grass harvested during the blossoming period. Contents: the total of flavonoids — at least 1.5% calculated with reference to luteolin 7-glucoside and on anhydrous basis; essential oil — at least 1 ml/kg on anhydrous basis.

As a result of the study, it was established that dried *O. vulgare* grass grown in Slovakia predominantly contained essential oils (0.15–0.50%), in which such phenols as carvacrol and thymol (together — 71.25%) and isopropyltoluene (4.0%) were the main ingredients. Such monoterpene hydrocarbons as terpinene and terpineol alcohol jointly made up 11%: acyclic monoterpene myrcene — 3%; and sesquiterpene  $\beta$ -caryophyllene — 4.5% (Table 1).

According to our study, *O. vulgare* grass harvested from a sunny hill close to the village of Trostianets, Lviv oblast, Ukraine contained in total  $1.14 \pm 0.04\%$  of flavonoids. According to the *Origanum*<sup>N</sup> national description, *Origanum vulgare* L. should contain the flavonoid total of at least 1.5% calculated with

reference to luteolin 7-glucoside ( $C_{23}H_{24}O_{10}$ ; M. m. 460) and on anhydrous basis. The population from Lviv oblast demonstrated high aggregate contents of essential oils —  $0.35 \pm 0.05\%$  hm (3,5 g/kg of dry weight), with a 10.5% humidity loss. In the reviewed *O. vulgare* isolated during blossoming phase, 16 essential oils were identified (Table 1).

Monoterpene hydrocarbons  $\alpha$ -terpinene and  $\alpha$ -terpineol together made up 29–33%, i. e. a third part of all essential oils, with  $\alpha$ -terpineol alcohol making two thirds of them. The survey also showed high contents of acyclic monoterpenes —  $\beta$ -myrcene (7%) and linalool — 4%, and only 15% fell on such polyphenol compound as *p*-cymene. Besides, the reviewed species was observed to synthesize quite a big amount of sesquiterpene  $\beta$ -caryophyllene — 7%. Linalool and  $\alpha$ -terpineol are odorous volatile alcohol monoterpenes. All other essential oils: geraniol, terpinolene, cineole, limonene, thujon, borneol, bornylacetate, fenchol, carvacrol, and thymol were observed to be contained from 2% down to trace amounts.

Accumulation of essential oils depends upon the growth environment — solar radiation, climate, and topographic conditions. The [16] ascertained that in the presence of phenol compounds plants synthesize essential oils by transforming  $\gamma$ -terpinene into *p*-cymene with subsequent hydroxylation of *p*-cymene to thymol or carvacrol, subject to solar radiation.

In the environmental conditions of Lviv oblast, Ukraine, due to the considerably lower summer air temperatures, higher amounts of precipitation, especially in summer, and on poor sandy soils, *O. vulgare* tended to form the monoterpene chemotype, where monoterpenes made up 41%, sesquiterpenes — 7%, and the phenol compound of *p*-cymene — only 15%; carvacrol and thymol together accounted for approx. 2%. In Slovakia, the reviewed plants belonged to the carvacrol chemotype, typical for the Mediterranean region with warm climate.

Plant phenophase was also observed to be affecting the contents and the character of essential oils. In spring, *p*-cymene prevailed over carvacrol in *O. vulgare* subsp. *hirtum*; but by the end of the growth season the ratio would reverse. Such regularity was observed within one plant, where the young leaves contained more cymene than the older ones [17]. Kokkini et al. [7] showed that the contents of essential oils ( $\gamma$ -terpinene, *p*-cymene, thymol and carvacrol) would change during the season: in autumn, the

**Table 1. Composition of essential oils from medicinal herbal material *Origanum vulgare* ( $n = 3, x \pm SD$ )**

Essential oil components	Essential oil content, %	
	Trostianets, Ukraine	Plavnica, Slovakia
Acyclic monoterpenes		
Geraniol	0.7 ± 0.1	n/a
β-Myrcene	7.0 ± 0.5	3.0 ± 0.5
Linalol	4.0 ± 0.5	n/a
Monocyclic monoterpenes		
α-Terpinene	11 ± 1	5.0 ± 1.0
α-Terpineol	18 ± 1	6.0 ± 2.5
Terpinolene	1.8 ± 0.2	n/a
Cyneol	1.8 ± 0.2	n/a
Limolene	traces	n/a
Bicyclic monoterpenes		
Thujone	1.2 ± 0.2	n/a
Borneol	1.0 ± 0.1	n/a
Bornyl acetate	0.6 ± 0.1	n/a
Fenchol	traces	n/a
Sesquiterpenes		
β-Caryophyllene	7.5 ± 0.5	4.5 ± 0.5
Aromatic (phenol) compounds		
p-Cymene	15 ± 1	n/a
Carvacrol	1.6 ± 0.2	55.21 ± 3.0
Thymol	0.3 ± 0.1	16.04 ± 1.5
Isopropyltoluene	n/a	4.0 ± 1.5

plants had more phenols compared with those harvested in midsummer. The number of oils in the populations of *O. vulgare* ssp. *virens*, (*O. vulgare* ssp. *viridulum*) in southern Italy achieved maximum values during full blossoming of the plants [18]. The contents of phenols is as a rule high during blossoming of plants of phenolic type [5].

The volatile constituents of *O. vulgare* L. ssp. *hirtum* grown in Croatia were established to be affected by both the time of harvesting and desiccation of the plants. The content of p-cymene reached its maximum in August. Upon drying of the plant material, all samples demonstrated insignificant decrease in the yield of essential oils compared with the fresh plants. Drying at room temperature did not affect the qualitative composition of oregano oil [6].

Among the phenolic essential oils extracted from the reviewed plants from Lviv oblast, Ukraine, p-cymen dominated (15%); the rest (1.9%) fell on carvacrol and thymol. Whereas the plants were in the blossoming phenophase, it would be reasonable to check their chemical composition by the end of this phenophase — at the beginning of fructification.

The antimicrobial properties of essential oils of *O. vulgare* collected in Lviv oblast, with domination of monoterpenes (nearly 50%) — monoterpene essential oils (α-terpinene and α-terpineol — 29–33%), acyclic monoterpenes (β-myrcene and linalool — 11%); and only 17% fell on polyphenol compounds — were very weak (Table 2).

The studies showed that essential oil from *O. vulgare* L. grown in Slovakia demonstrated antimicrobial activity upon all isolates taken into the experiment — both typical and clinical ones (Figure). High antimycotic activity of essential oil was established. It was also revealed that oil is active against methicillin-resistant and biofilm-forming isolates of *S. aureus*. At the same time, the antimicrobial activity of the oil received from the plants of the local population was low. Such pattern may have been caused by the low contents of carvacrol and thymol, which play a decisive role in the antimicrobial activity of plants of the given species.

The composition of essential oils affects the pharmacological properties of the raw materials. Say, the essential oil with maximum content of carvacrol was received from the plants *O. vulgare* ssp. *virens* from southern Italy [18] demonstrated the highest antibacterial activity. Somewhat lower antibacterial activity was shown by the oil received from the populations of *O. vulgare* ssp. *viridulum* from “Ricigliano”, which were characterised by a big amount of thymol, whereas the populations from “Acerno” had the lowest contents of phenols and demonstrated the least antimicrobial activity.

Extract of essential oils from *O. vulgare* with carvacrol, thymol and cymol being the main components, showed an expressed inhibiting activity against enteropathogenic bacteria of *E. coli* and *S. enteritica* var. *enteritidis*.

Owing to the presence of cinnamaldehyde, carvacrol, thymol and eugenol, essential oils of cinnamon, oregano, thyme and cloves showed strong antimicrobial activity against *L. monocytogenes*, *S. typhimurium*, *E. coli* O157: H7 and bacteria causing food spoilage (*B. thermosphacta* ra *P. fluorescens*)

Table 2. Antimicrobial activity of essential oil from *Origanum vulgare* ( $n = 3, x \pm SD$ )

Test culture	Plavnica, Slovakia	Trostianets, Ukraine
<i>S. aureus</i> ATCC 25923	26.7 ± 0.58	10.5 ± 0.58
<i>S. aureus</i> clinic biofilm creation	25.7 ± 0.58	9.50 ± 0.33
<i>S. aureus</i> MRSA clinic	25.5 ± 0.5	–
<i>E. coli</i> ATCC 25922	21.3 ± 0.58	9.0 ± 0.50
<i>E. coli</i> clinic	25.8 ± 0.29	8.50 ± 0.25
<i>E. faecalis</i> ATCC 29212	20.3 ± 0.58	10.5 ± 0.25
<i>E. faecalis</i> clinic	19.3 ± 0.58	9.8 ± 0.25
<i>S. pyogenes</i> ATCC 19615	31.3 ± 0.58	–
<i>S. pyogenes</i> clinic	29.7 ± 0.58	–
<i>C. albicans</i> ATCC 885-653	36.3 ± 0.58	12.0 ± 0.58
<i>C. albicans</i> clinic	35.3 ± 0.58	11.0 ± 0.30

Note: \* the data were statistically significant as compared with the control ( $P < 0.05$ ) as a control were used: ampicilin — for gram-positive bacteria; gentamicine — for gram-negative bacteria; nystatin — for microscopic fungi; control of antibiotic — no inhibition; control of methanol — no inhibition.



Antimicrobial activity of essential oil from *O. vulgare* against clinical isolates of *E. coli*

[19]. The mixture of essential oils from *O. vulgare* (carvacrol (66.9%)) and *Rosmarinus officinalis* (1.8-cineolom (32.2%)) used as spices, provided for the inhibition of the growth of bacteria located on food products (*L. monocytogenes*, *Y. enterocolitica* and *A. hydrophilla*, *P. fluorescens*) [20]. It was proved that *O. vulgare* can be used as condiment to inhibit the growth of *S. aureus*, and to inhibit the synthesis of staphylococcal enterotoxins [21].

In our previous studies, we showed that by their biochemical properties and antimicrobial activity, essential oils can be used to inhibit opportunistic and pathogenic microorganisms, and as natural preserving agents [22–24].

Aqueous extract of *O. vulgare* was revealed to exert a high antimicrobial activity upon ten bacteria: *E. coli* ATCC 25922, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* ATCC 27835 et al. Their activity was stronger against gram-

positive pathogens *S. epidermidis* (ATCC 12228) than against gram-negative *E. coli* [4].

Thus, owing to the presence of aromatic essential oils (carvacrol and thymol), flavonoids (rosmarinic acid) and derivatives of pyrocatechin acid, the essential oil received from *O. vulgare* grown in Slovakia has a high antimicrobial activity, compared with the monoterpene essential oil of the plants grown in Lviv oblast, Ukraine. The obtained data have proved the possibility of the use of essential oil from Slovakia as an antimicrobial medication. At the same time, it appears relevant to proceed with the studies of the properties of essential oils introduced in other climatic and paedological conditions, and use of plants from Slovakia as planting material.

## Conclusions

Accumulation of essential oils has been shown to depend upon the growth conditions — solar radiation, climate, and topographic conditions. In sunlit places and warm climate, phenol compounds were accumulated, whereas under continental conditions monoterpenes prevailed, which was typical for populations of *O. vulgare* L.

The populations growing in Lviv oblast, Ukraine, were characterised by the monoterpene chemotype, with the contents of essential oil amounting to  $0.35 \pm 0.05\%$ . The essential oil contained monoterpene hydrocarbons  $\alpha$ -terpinene and  $\alpha$ -terpineol (29–33%), acyclic monoterpenes —  $\beta$ -myrcene

(7%), linalool (4%); whereas the polyphenol compound p-cymene accounted for 15% only.

In the plants *O. vulgare* from Slovakia, essential oil accounted for 0.15–0.50% of their composition, with domination of the carvacrol chemotype where phenols are the main ingredients — carvacrol and thymol (71% together), and isopropyltoluene (4.0%). Monoterpene hydrocarbons terpinene (5.0%) and terpineol alcohol (6.0%) jointly accounted for 11%; acyclic monoterpene myrcene — 3%, and sesquiterpene  $\beta$ -caryophyllene — 4.5%.

The essential oil from *O. vulgare* collected

in Slovakia demonstrated high antimicrobial properties against reference and clinical isolates of opportunistic microorganisms. The essential oil from the samples taken in Lviv oblast, Ukraine showed low antimicrobial activity.

The study was conducted in the framework of bilateral cooperation between Ukraine and Slovakia.

## REFERENCES

1. Shafiee-Hajjabad M., Novak J., Honermeier B. Characterization of glandular trichomes in four *Origanum vulgare* L. accessions influenced by light reduction. *J. Appl. Botany and Food Quality*. 2015, V. 88, P. 300–307. <https://doi.org/10.5073/JABFQ.2015.088.043>
2. Lukas B., Schmiederer C., Novak J. Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). *Phytochem.* 2015, V. 119, P. 32–40. <https://doi.org/10.1016/j.phytochem.2015.09.008>
3. Tuttolomondo T., Leto C., Leone R., Licata M., Virga G., Ruberto G., Edoardo M. Napoli, Salvatore La Bella. Essential oil characteristics of wild Sicilian oregano populations in relation to environmental conditions. *J. Essential Oil Res.* 2014, 26 (3), 210–220. <https://doi.org/10.1080/10412905.2014.882278>
4. De Martino L., De Feo V., Formisano C., Mignola E., Senatore F. Chemical Composition and Antimicrobial Activity of the Essential Oils from Three Chemotypes of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart Growing Wild in Campania (Southern Italy). *Molecules*. 2009, V. 14, P. 2735–2746. <https://doi.org/10.3390/molecules14082735>
5. Putievsky E., Ravid U., Dudai N. Phenological and seasonal influences on essential oil of a cultivated clone of *Origanum vulgare* L. *J. Sci. Food Agriculture*. 1988, V. 43, P. 225–228. <https://doi.org/10.1002/jsfa.2740430304>
6. Jerković I., Mastelić J., Miloš M. The impact of both the season of collection and drying on the volatile constituents of *Origanum vulgare* L. ssp. *hirtum* grown wild in Croatia. *Inter. J. Food Sci. Technol.* 2001, V. 36, P. 649–654. <https://doi.org/10.1046/j.1365-2621.2001.00502.x>
7. Kokkini S., Karousou R., Dardioti A., Krigas N., Lanaras T. Autumn essential oils of greek oregano. *Phytochem.* 1997, V. 44, P. 883–886. <https://doi.org/10.3390/molecules14082735>
8. Carrasco A., Perez E., Cutillas A.-B., Martinez-Gutierrez R., Tomas V., Tudela J. Origanum Vulgare and Thymbra Capitata Essential Oils from Spain: Determination of Aromatic Profile and Bioactivities. *Natural Product Communications*. 2016, V. 11, P. 113–120. <https://doi.org/10.1177/1934578X1601100133>
9. Mockute D., Bernotiene G., Judzentiene A. The essential oil of *Origanum vulgare* L. ssp. *vulgare* growing wild in Vilnius district (Lithuania). *Phytochem.* 2001, 57 (1), 65–69. [https://doi.org/10.1016/s0031-9422\(00\)00474-x](https://doi.org/10.1016/s0031-9422(00)00474-x)
10. Xiao-LiZhang, Yu-ShanGuo, Chun-HuaWang, Guo-QiangLi, Jiao-JiaoXu, Hau YinChung, Wen-CaiYe, Yao-LanLi, Guo-CaiWang. Phenol compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food Chem.* 2016, 152 (1), 300–306. <https://doi.org/10.1016/j.foodchem.2013.11.153>
11. Kotyuk L. A., Rakhmeto D. B. Bioloichno aktyvni rehovyny *Origanum vulgare* L. *Plant physiol. genetics*. 2016, 48 (1), 20–25. (In Ukrainian).
12. Perez R. A., Navarro T., Lorenzo C. D. HS-SPME analysis of the volatile compounds from spices as a source of flavour in ‘Campo Real’ table olive preparations. *Flavour and fragrance j.* 2007, 22 (4), 265–273. <https://doi.org/10.1002/ffj.1791>
13. Adams R. P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. *Carol Stream, IL, USA: Allured Publishing Corporation*. 2007, 804 p. 2007 ISBN 13:978-1932633214
14. Rhos J. L., Recio M. C. Medicinal Plants and Antimicrobial Activity. *J. Ethnopharmacol.* 2005, 100 (1–2), 80–84. <https://doi.org/10.1016/j.jep.2005.04.025>
15. State Pharmacopoeia of Ukraine: in 3 volum — 2nd type. V. 3. *Kharkiv: State. Enterprise “Ukr. Sciences Pharmacopoeia*

- Centre for the Quality of Medicines". 2014, P. 385–389 (In Ukrainian).
16. Poulouse A. J., Croteau R. Biosynthesis of aromatic monoterpenes: conversion of gamma-terpinene to p-cymene and thymol in *Thymus vulgaris* L. *Arch. Biochem. Biophys.* 1978, 187 (2), 307–314. [https://doi.org/10.1016/0003-9861\(78\)90039-5](https://doi.org/10.1016/0003-9861(78)90039-5)
  17. Johnson C. B., Kazantzis A., Skoula M., Mitteregger U., Novak J. Seasonal, populational and ontogenic variation in the volatile oil content and composition of individuals of *Origanum vulgare* subsp. *Hirtum*, assessed by GC headspace analysis and by SPME sampling of individual oil glands. *Phytochem. Anal.* 2004, V. 15, P. 286–292. <https://doi.org/10.1002/pca.780>
  18. De Falco E., Roscigno G., Landolfi S., Scandolera E., Senatore F. Growth, essential oil characterization, and antimicrobial activity of three wild biotypes of oregano under cultivation condition in Southern Italy. *Industrial Crops and Products.* 2014, V. 62, P. 242–249. <https://doi.org/10.1016/j.indcrop.2014.08.037>
  19. Mith H., Dure R., Delcenserie V., Zhiri A., Daube G., Clinquart A. Antimicrobial Activities of Essential Oils and Their Components against Food-Borne Pathogens and Food Spoilage Bacteria. *Food Sci. Nutr.* 2014, V. 2, P. 403–416. <https://doi.org/10.1002/fsn3.116>
  20. De Azeredo G. A., Stamford T. L. M., Nunes P. C., Neto N. J. G., De Oliveira, De Souza E. L. Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Res. Inter.* 2011, 44 (5), 1541–1548. <https://doi.org/10.1016/j.foodres.2011.04.012>
  21. Souza E. L., Stamford T. L. M., Lima E. O., Trajano V. N. Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control.* 2007, 18 (5), 409–413. <https://doi.org/10.1016/j.foodcont.2005.11.008>
  22. Salamon I., Poracova J., Hrytsyna M. Oregano Essential Oil (*Origanum vulgare* L.), as a Food-supplement in a Rearing of Piglets. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences.* 2019, 21 (95), 55–61. <https://doi.org/10.32718/nvlvet9510>
  23. Salamon I., Kryvtsova M., Bucko D., Tarawneh Amer H. Chemical characterization and antimicrobial activity of some essential oils after their industrial large-scale distillation. *J. Microbiol. Biotechnol. Food Sci.* 2018, 8 (3), 965–969. <https://doi.org/10.15414/jmbfs.2018.8.3.965-969>
  24. Kryvtsova M. V., Kostenko Ye. Ya., Salamon I. Compositions of essential oils with antimicrobial properties against isolates from oral cavities of parodontium. *Regulatory Mechanisms in Biosystems.* 2018, 9 (4), 491–494. <https://doi.org/10.15421/021873>

### ХІМІЧНИЙ СКЛАД ТА АНТИМІКРОБНІ ВЛАСТИВОСТІ ЕФІРНОЇ ОЛІЇ *Origanum vulgare* L. РІЗНИХ МІСЦЕЗРОСТАНЬ

М. Кривцова<sup>1</sup>, М. Грицина<sup>2</sup>, І. Саламон<sup>3</sup>

<sup>1</sup>Ужгородський національний університет,  
Україна

<sup>2</sup>Львівський національний університет  
ветеринарної медицини та біотехнологій  
імені С. З. Гжицького, Україна

<sup>3</sup>Пряшівський університет, Словачія

E-mail: [maryna.krivcova@gmail.com](mailto:maryna.krivcova@gmail.com)

Ефірні олії широко використовують у різних галузях косметології, медицини, харчової промисловості і вони є високовживаним продуктом. Водночас біохімічний склад, властивості, зокрема антимікробна активність, олій варіюють залежно від місця зростання, кліматичних умов та хемотипу рослин. Метою нашої роботи є дослідження якісного та кількісного складу

### ХИМИЧЕСКИЙ СОСТАВ И АНТИМИКРОБНЫЕ СВОЙСТВА *Origanum vulgare* L. РАЗНЫХ МЕСТООБИТАНИЙ

М. Кривцова<sup>1</sup>, М. Грицина<sup>2</sup>, І. Саламон<sup>3</sup>

<sup>1</sup>Ужгородский национальный университет,  
Украина

<sup>2</sup>Львовский национальный университет  
ветеринарной медицины и биотехнологий им.  
С. З. Гжицкого, Украина

<sup>3</sup>Пряшевский университет, Словачия

E-mail: [maryna.krivcova@gmail.com](mailto:maryna.krivcova@gmail.com)

Эфирные масла широко используются в различных отраслях косметологии, медицины, пищевой промышленности и являются высокопотребляемым продуктом. В то же время биохимический состав, свойства, в том числе антимикробная активность, масел варьируют в зависимости от места произрастания, климатических условий и хемотипа растений.

ефірних олій *O. vulgare* та антимікробних властивостей, рослин, що зібрані у Східній Словаччині та у Львівській області України.

У природних умовах *Origanum vulgare* L. було зібрано в околицях села Тростянець, Львівська область, у 2019 р. У Словаччині рослини були вирощені компанією Агрокарпати, Плавниця. Ефірну олію із сировини відганяли гідродистиляцією (2 години) в апараті Клівенгера згідно з Європейською фармакопеею.

Дослідження біохімічних властивостей ефірних олій популяцій рослин, які ростуть в умовах Львівщини, показали, що вміст ЕО становить  $0,35 \pm 0,05\%$ . Склад ефірних олій вказує на належність рослин природної популяції *Origanum vulgare* L., що зростає у Львівській області, до монотерпенового хемотипу. Монотерпенові вуглеводні  $\alpha$ -терпінен і  $\alpha$ -t-терпинеол становили в сумі 29–33%, ациклічні монотерпени —  $\beta$ -мірцен 7% і 4% — ліналол і лише 15% становила поліфенольна сполука p-цимен.

Рослини *O. vulgare*, вирощені у Словаччині, характеризувались вмістом ефірної олії 0,15–0,50% і компонентним складом, який дав змогу віднести їх до карвакрольного хемотипу, де основним інгредієнтом є феноли — карвакрол і тимол (разом 71%), ізопропілтолуол (4,0%). Монотерпенові вуглеводні терпінен (5,0) і спирт терпинеол (6,0) становили в сумі 11%, ациклічний монотерпен — мірцен (3%) і сесквітерпен  $\beta$ -каріофіллен (4,5%).

Ефірна олія *O. vulgare*, зібрана в Словаччині, виявляла високу антимікробну дію на референтні та клінічні ізоляти умовно патогенних мікроорганізмів. Ефірна олія із зразків, зібраних на Львівщині, виявляла низьку антимікробну активність.

Отже, досліджувані рослини належать до різних хемотипів, що обумовлює перспективу їх використання для різних потреб залежно від біохімічного складу та властивостей.

**Ключові слова:** ефірна олія орігано, антимікробна активність, біохімічні властивості.

Целью работы является исследование качественного и количественного состава эфирных масел *O. vulgare*, а также антимикробных свойств, растений, собранных в Восточной Словакии и во Львовской области Украины.

В естественных условиях *Origanum vulgare* L. были собраны в окрестностях села Тростянец, Львовская область, в 2019 году. В Словакии растения были выращены компанией Агрокарпаты, Плавница. Эфирное масло из сырья отгоняли гидродистилляцией (2 часа) в аппарате Кливенгера согласно Европейской фармакопее.

Исследование биохимических свойств эфирных масел популяций растений, произрастающих в условиях Львовщины, показали, что содержание эфирных масел составляет  $0,35 \pm 0,05\%$ . Состав эфирных масел указывает на принадлежность растений природной популяции *Origanum vulgare* L., которая произрастает во Львовской области к монотерпеновому хемотипу. Монотерпеновые углеводороды  $\alpha$ -терпинен и  $\alpha$ -терпинеол составляли в сумме 29–33%, ациклические монотерпены —  $\beta$ -мирцен (7%) и линалол (4%) и только 15% полифенольное соединение p-цимен.

Растения *O. vulgare*, выращенные в Словакии, характеризовались содержанием эфирного масла 0,15–0,50% и компонентным составом, который позволил отнести их к карвакрольному хемотипу, в котором основным ингредиентом являются фенолы — карвакрол и тимол (в сумме 71%), изопропилтолуол 4,0%. Монотерпеновые углеводороды терпинен (5,0) и спирт терпинеол (6,0) составляли в сумме 11%, ациклический монотерпен — мирцен (3%) и сесквитерпен  $\beta$ -кариофиллен (4,5%).

Эфирное масло *O. vulgare*, собранное в Словакии, проявляло высокое антимикробное действие на референтные и клинические изоляты условно патогенных микроорганизмов. Эфирное масло из образцов, собранных на Львовщине, проявляло низкую антимикробную активность.

Таким образом, исследуемые растения относятся к различным хемотипам, что обуславливает перспективу их использования для различных нужд в зависимости от биохимического состава и свойств.

**Ключевые слова:** эфирное масло орегано, антимикробная активность, биохимические свойства.