Chromatographic profiles and antimicrobial activity of the essential oils obtained from some species and cultivars of the Mentheae tribe (Lamiaceae)

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Abstract

The present study was focused on the chemical composition and antimicrobial activity of the essential oils (EsO) obtained from five Lamiaceae representatives grown in the south of Ukraine. Among them are Salvia sclarea L., Monarda didyma (cultivar 'Cambridge Scarlet'), Thymus pulegioides (cultivar '2/6-07'), Thymus vulgaris (cultivar ‘Jalos’), and Thymus serpyllum L. The component analysis of the EsO was carried out by gas chromatography method coupled with mass spectrometry (GC–MS). The antimicrobial properties of the EsO were determined using the agar diffusion test against widespread pathogenic bacterial strains (Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes) and opportunistic yeast Candida albicans. The EsO of Thymus serpyllum and Thymus vulgaris (cultivar ‘Jalos’) displayed noteworthy antibacterial properties against a wide spectrum of the microorganisms. These antimicrobial properties could be attributed to the high content of aromatic monoterpene thymol (52.56% and 47.33%, respectively). The EsO of Salvia sclarea with the dominance of linalyl acetate (45.51%) and linalool (38.98%) as well as Thymus pulegioides (cultivar ‘2/6-07’) containing α-citral (27.10%) and β-citral (17.11%) demonstrated the strongest antimicrobial effects on clinical strains of Candida albicans. In conclusion, the present study revealed the chemical composition of five Lamiaceae species and cultivars grown in the south of Ukraine and considerable antimicrobial activity of the tested EsO, especially against the typical and clinical strains of Staphylococcus aureus and Candida albicans. The obtained results could be perspective for applying in the pharmaceutical industry and for the conservation of food and cosmetic products.

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1. Introduction

Natural substances derived from medicinal plants represent a rich source for the development of new herbal medicinal products. Plant-derived compounds could be appropriate alternatives to synthetic active substances for the treatment of many diseases (Moghtader, 2012). EsO are considered as an alternative to synthetic antimicrobial medicines, especially antibiotics (Sharifi-Rad et al., 2017; Tariq et al., 2019). The ability of EsO to inhibit the growth of undesirable microorganisms in cosmetic and food products could solve the problem of the substitution for synthetic...
preservatives which can cause serious problems for human health (Liu et al., 2017; Long et al., 2019; Park et al., 2012).

Among the different groups of Angiosperms, Lamiaceae Marti
nov family is a large taxonomical unit of plants. This family is unoff
icially divided into two groups according to the phytochemical peculiarities: the first group mainly produces EsO, while the sec
ond one biosynthesize components of the polar fraction (di-
and triterpenoids, iroidoids and polyphenols) (Marchioni, 2020). Such
genera as Thymus L., Salvia L., and Monarda L. belong to the Mentheae Dumort. tribe of the Nepetoideae Burnett subfamily of the Lamiaceae family. They are characterized by the distinctive aro
matic smell due to the accumulation of EsO in epidermal glandular trichoms (Lange and Turner, 2013). The representatives of these
genera are important medicinal plants widely used in traditional and complementar medicine. The Thymus species are native to temperate regions in Europe, North Africa and Asia where they are used mainly to cure bronchitis and oral cavity infections (Jaric´ et al., 2015; Mohammadi et al., 2020; Mostafa et al., 2018). Representatives of the Salvia genus are widely spread in temperate and subtropical regions on the different continents and are often used for treating the illnesses related to the gastrointestinal and respiratory systems (Cui et al., 2015; Hudz et al., 2019; Koshyoyi et al., 2020). The Monarda species are native to North America where they are widely used in folk medicine for the treatment of flu, cold, skin wounds, etc. (Fraternale, et al., 2006; Mattarelli et al., 2017). Many species from the abovementioned genera are widely cultivated throughout the world. The plant raw materials of such species as Thymus serpyllum (Serpill herba) and Thymus vulgaris (Thymi herba) as well as Salviae sclareae aetheroleum are included in the 10th Edition of the European Pharmacopoeia (2020). The appearance of the new cultivars and chemotypes encourage the studies of their chemical composition and biological activity (Myadelets et al., 2014, Hudz et al., 2019; Hudz et al., 2020).

The comparative analysis of EsO and antimicrobial activity of promising species from the above-mentioned genera is of consid
erable scientific interest to researchers in the field of botany, plant physiology, microbiology, pharmacognosy and pharmaceutical technology. A different number of original papers and reviews about the EsO composition and activities of the tested species were published up to 15 December 2020 (data retrieved from PubMed via searching the words ‘latin name of plant’ and ‘essential oil’). ‘Monarda didyma’ (cultivar ‘Cambridge Scarlet’) (C2), Thymus vulgaris (cultivar ‘Jalos’) (CJ), and Thymus serpyllum were collected in the flowering stage in Kherson region (Ukraine) in 2016. The voucher specimens were deposited at the Herbarium of the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute of the NAAS (Plodove, Kherson region, Ukraine) and the Department of Analytical Chemistry of Opole University (Poland).

2. Materials and methods

2.1. Plant material

The aerial parts of Monarda didyma (cultivar ‘Cambridge Scarlet’) (CCS), Salvia sclarea, Thymus pulegioides (cultivar ‘2/6–07′) (C2), Thymus vulgaris (cultivar ‘Jalos’) (CJ), and Thymus serpyllum were collected in the flowering stage in Kherson region (Ukraine) in 2016. The voucher specimens were deposited at the Herbarium of the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute of the NAAS (Plodove, Kherson region, Ukraine) and the Department of Analytical Chemistry of Opole University (Poland).

2.2. Essential oil isolation procedure

The EsO were obtained via hydrodistillation for 4 h with a Cle
venger-type apparatus according to European Pharmacopoeia (2020) using fresh raw materials. The oils were kept at 4 °C in seated amber bottles before analysis by GC–MS and antimicrobial studies.

2.3. General experimental procedures of GC–MS

The Hewlett Packard HP 6890 series GC system chromatograph (Hewlett Packard, Waldbronn, Germany) was employed for this study. It was coupled with the Hewlett Packard 5973 mass selective detector (Hewlett Packard, Waldbronn, Germany). The chromatograph was equipped with the non-polar, high-temperature ZB-5HT (5% diphenyl- and 95% dimethylpolysiloxane) capillary column Phenomenex (Torrance, CA, USA) of length of 30 m with inner diameter of 0.32 nm and film thickness of 0.25 μm. The split ratio was 20:1 and 1 μL of a sample was introduced. Helium was a carrier gas, its flow rate was 2 ml/min. The analyses were performed at the temperature range of 40–280 °C and the heating rate was 10 °C/min; injector temperature was 250 °C (Hudz et al., 2020).

The volatile compounds of the tested EsO were identified by comparing the mass spectra data with spectrometer database of the NIST 11 Library and their retention index calculated against n-alkanes (C₉–C₂₀). Each chromatographic analysis was repeated three times. The average value of the relative composition of the EsO percentage was calculated from the peak areas.

2.4. Antimicrobial assay

Antimicrobial activity was determined using the agar diffusion test (Kryvtsova et al., 2019). The bacterium inocula in the physi
ological solution were adjusted to the equivalent of 0.5 McFarland standard. Then 100 μL of bacteria inoculates were evenly spread on the surface of Muller-Hinton agar (incubated at a temperature
of 37 ± 2 °C for 24 h). 100 µL of the yeasts inoculates were spread on SDA agar (incubated at 35 ± 2 °C for 48 h). 10 µL of the EsO were introduced into wells (6 mm in diameter). The diameters of the inhibition zones were measured in millimeters including the well diameter.

The following bacteria and yeasts from the American Type Culture Collection (ATCC) were used: Candida albicans ATCC 885–653; Staphylococcus aureus ATCC 25923; Escherichia coli ATCC 25922; Enterococcus faecalis ATCC 29212; Streptococcus pyogenes ATCC 19615; reference Staphylococcus aureus CCM 3953 biofilm-forming strain. The clinical strains of bacteria and yeasts (Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Enterococcus faecalis, Candida albicans) isolated from the oral cavities of patients suffering from inflammatory periodontium and pharynx. We identified the bacteria and fungi based on macromorphology, micro-morphology, physiological and biochemical tests using ENTEROtest, STREPTO-test, STAPHYLO-test produced by Erba Lachema. The clinical strains with multiple resistances at least to two classes of antibiotics were chosen. The following antibiotics were used as positive controls: gentamicin (10 mcg/disk); ampicillin (10 mcg/disk); amoxicillin/clavulanic acid (20/10 mcg/disk); nystatin (100 UI) and itraconazol (30 mcg/disk) for Candida strains. Dimethyl sulfoxide (DMSO) was used as a negative control.

Each antimicrobial assay was performed at least three times. The obtained data were expressed as mean ± standard deviation (x ± SD) of three measurements. The Tukey's test was applied for comparisons of means; differences were considered significant at p < 0.05.

### 3. Results

#### 3.1. GC–MS analysis of essential oil

GC–MS analysis of the tested EsO revealed their component compositions (Table 1).

As can be seen from the obtained results, thymol was the predominant component of the EsO isolated from the Thymus vulgaris CJ (47.33%), Thymus serpyllum (52.56%) and Monarda didyma CCS (47.54%) herbs. α-Cymene (14.34%) and γ-terpinene (11.57%) were the major components of Thymus vulgaris CJ besides thymol. Isothymol methylether (11.28%) was the important component of Thymus serpyllum in addition to thymol. α-Citral (27.10%) and β-citral (17.11%) were the major components of Thymus pulegioides C2 EsO.

Eucalyptol (17.82%) was the second predominant component of the EsO of Monarda didyma CCS besides thymol. The tested Salvia scarea EsO was characterized by the dominance of linalyl acetate (45.51%) and linalool (38.98%).

It should be noted that thymol, α-cymene, linalool, caryophyllene, terpinen-4-ol, α-terpinene, and 1-octen-3-ol are the common compounds of the EsO of all the investigated species. Some of minor compounds can be regarded as the specific markers of the investigated species, for instance, aromadendrene (0.12%) was found only in Thymus serpyllum EsO, pinocarveol (0.11%) in Thymus pulegioides C2, whilst β-thujene (0.60%), 3-carene (0.10%) and many others were found only in the EsO of Thymus vulgaris CJ.

### Table 1

| Component | IR | Area [%] ± SD |
|-----------|----|--------------|
| β-thuene  | 929| 0.60 ± 0.02 |
| α-pinene  | 935| 0.32 ± 0.03 |
| camphene  | 950| 0.39 ± 0.01 |
| β-phellandrene | 975| 0.09 ± 0.01 |
| β-pinene  | 978| 0.20 ± 0.01 |
| 1-octen-3-ol | 981–982| 0.68 ± 0.02 |
| 3-octane  | 987–988| 0.03 ± 0.00 |
| β-myrcene | 991–992| 1.37 ± 0.04 |
| 3-octanol | 995| 0.11 ± 0.00 |
| α-pinene  | 1000| 0.16 ± 0.01 |
| 3-carene  | 1010| 0.10 ± 0.00 |
| terpinolene | 1017| 1.02 ± 0.02 |
| o-cymene  | 1025–1029| 14.34 ± 0.21 |
| β-pinene  | 1030| 0.40 ± 0.02 |
| limonene  | 1030| 0.22 ± 0.08 |
| eucalyptol| 1032–1033| 2.65 ± 0.03 |
| trans-β-ocimene | 1040–1041| 0.31 ± 0.02 |
| β-ocimene | 1050–1051| 0.06 ± 0.01 |
| γ-terpinene | 1060–1063| 11.57 ± 0.09 |
| cis-β-terpinol | 1070| 0.20 ± 0.00 |
| cis-linalol oxide | 1074| 0.07 ± 0.00 |
| 1-nonen-3-ol | 1082| 0.52 ± 0.04 |
| 1,8-cineole | 1089| 0.12 ± 0.00 |
| trans-linalol oxide | 1090| 0.14 ± 0.02 |
| trans-β-terpinol | 1094–1097| 0.51 ± 0.03 |
| linalool | 1100| 0.20 ± 0.01 |
| thujone | 1114| 0.12 ± 0.00 |
| trans-p-menth-2-en-1-ol | 1124| 0.06 ± 0.02 |
| pinocarveol | 1141| 0.11 ± 0.01 |
| 2-bornanone | 1152| 0.44 ± 0.03 |
| camphor | 1153| 1.19 ± 0.06 |
| verbolen | 1154| 0.11 ± 0.01 |
| p-menthone | 1156| 0.09 ± 0.01 |
| cis-verbolen | 1157| 0.16 ± 0.02 |
| endo-borneol | 1169| 2.06 ± 0.70 |

(continued on next page)
The results of the antimicrobial activities study of the EsO of five Lamiaceae species against typical and clinical strains of pathogenic microorganisms are presented in Table 2.

As can be seen from the results of antimicrobial activity studies, the EsO of all the analyzed species possess the marked antimicrobial properties against the typical and clinical strains of pathogenic bacteria as well as yeast Candida albicans. It should be noted that the clinical isolates of microorganisms used in this study were insensitive to such antibiotics as ampicillin and gentamicin, and that the clinical isolates of microorganisms used in this study were insensitive to such antibiotics as ampicillin and gentamicin.

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The highest antimicrobial activity against both the typical and clinical strains of S. aureus was detected for the EsO of Thymus vulgaris CJ and Thymus serpyllum Salvia sclarea. The other EsO showed antimicrobial activity against the EsO of Thymus vulgaris CJ and Thymus serpyllum Salvia sclarea. Generally, all the tested EsO are characterized by the presence of antifungal activity against Candida albicans as the growth inhibition zones were greater than 20.0 mm for most of them. Thus, Thymus serpyllum and Thymus vulgaris CJ EsO were found to possess the antimicrobial activity.

4. Discussion

It was found that thymol and its isomers (p-thymol, carvacrol) were the predominant compound of three tested EsO (Thymus vulgaris CJ, Thymus serpyllum and Monarda didyma CCS) as well as many other Nepetoideae species of the Lamiaceae family (Hudz et al., 2020; Moghtader, 2012; Nutrizio et al., 2020; Vaičiulytė et al., 2016). Predominant compounds can be considered as biologically active components of EsO while the minor ones can discriminate species, subspecies, cultivars or chemotypes.

There is no doubt that it is very important to know the limit content of some bioactive compounds in EsO with undesired properties. The main components of the tested EsO do not possess any toxic properties like psychoactive neoclerodane diterpene salvini- norin A of Salvia divinorum (Siebert, 2004). The content of the toxic component thujone in the Salvia sclarea EsO oil is regulated by the EU (maximum 0.2% in the EsO) (European Pharmacopoeia, 2020). As can be seen from the Table 1, thujone was presented in the tested EsO in the very small quantities: 0.08% in Thymus vulgaris CJ and 0.07% in Thymus pulegioides C2.
Such ecological factors as climate, altitude, pathogen attack to plants, cultivation systems, in combination with the genetic background can considerably modify EsO composition through interfering with a plant’s secondary metabolism (Marchioni et al., 2020; Mohammadi et al., 2020). The season of harvesting had also the significant effect on the chemical composition of EsO (Verma et al., 2011).

Nutrizio et al. (2020) revealed that the EsO of Croatian *Thymus serpyllum* accumulated linalool (0.30–15.03%), thymol (0.48–9.43%) and carvacrol (0.47–15.20%). The major components of the *Thymus serpyllum* EsO grown in Western Himalaya were thymol (19.4–60.1%), γ-terpinene (0.3–13.8%) and p-cymene (3.5–10.4%). (E)-nerolidol, Caryophyllene oxide, myrcene and borneol chemotypes of wild *Thymus serpyllum* were found in Estonia (Paaver et al., 2008). The contents of (E)-nerolidol, Caryophyllene oxide, myrcene and borneol were in the range of 1.7–70.1%, 1.4–45.0%, 0–20.2% and 0–19%, respectively, while the contents of thymol and carvacrol were very low (0–4.0%, and mean 0.96 and 0.62%, respectively) (Paaver et al., 2008). Paaver et al. (2008) concluded that thymol and carvacrol are not the main components of the Estonian wild thyme EsO. Loziene et al. (1998) revealed five chemotypes of *Thymus serpyllum* on the base of hierarchical cluster analysis: linalool chemotype, geranial/geraniol/neral one, thymol one, carvacrol/terpinene/p-cymene one and thymol/carvacrol/p-cymene/γ-terpinene. The geranial/geraniol/neral chemotype is the most frequently found, followed by the phenolic types. The linalool chemotype was recorded only one time. To whatever degree, we can state that the tested EsO of *Thymus serpyllum* cultivated in Ukraine is the thymol/isothymol methyl ether chemotype.

The predominant compound of *Thymus pulegioides* EsO from Serbia was linalyl acetate (40.0%) (Stojanović et al., 2014). The percentage of carvacrol and its precursors γ-terpinene and p-cymene in the EsO of Lithuanian *Thymus pulegioides* ranged between 16.88 and 29.29%, 20.60–24.43%, and 5.54–11.33% respectively, depending on the influence of meteorological conditions and growth stage (Vaičiūlytė et al., 2016). Mockute and Bertoniene (1999) revealed that the main components of the citral-geraniol chemotype of *Thymus pulegioides* EsO collected in wild habitats of Lithuania were geraniol (14.9–30.8%), geranial (x-citral) (9.7–19.7%), β-caryophyllene (6.0–11.4%) and nerol (4.1–11.8%). The EsO of carvacrol chemotype of *Thymus pulegioides* EsO contained carvacrol (16.0–22.2%), β-bisabolene (11.1–20.2%), β-caryophyllene (11.1–19.1%), γ-terpinene (5.8–16.2%), p-cymene (5.5–10.4%), and thymol (3.3–9.8%) (Mockute and Bertoniene, 1999). The revealed components of this carvacrol chemotype of *Thymus pulegioides* are characteristic for the *Thymus genus* (Mockute and Bertoniene, 1999). Five chemotypes of *Thymus pulegioides* ssp pulegioides collected in Vilnius city according to their odour and chemical composition were revealed by Mockute and Bertoniene (2003). The first one was the citral/geraniol chemotype (lemon odour) contained 18.4–30.2% of cis- and trans-citral (neral and geranial), 14.4–23.2% of geraniol, 9.4–13.1% of β-caryophyllene and 5.5–12.1% of nerol. The second chemotype was geranial (“sweet” odour) which contained 23.1–25.5% of geraniol, 10.8–13.5% of β-caryophyllene, and 9.5–10.2% of β-bisabolene. The third chemotype was caryophyllene one with “spicy” odour and contained 18.0–19.7% of β-caryophyllene, 14.5–15.9% of β-bisabolene, 12.5–14.6% of germacrene D and 8.2–13.8% of (E)-nerolidol. The fourth γ-terpinene/p-cymene chemotype had “hydrocarbon” odour and composed of 15.5–17.2% γ-terpinene, 7.7–17.0% p-cymene, 4.6–13.5% carvacrol and 9.6–10.5% β-caryophyllene. The fifth chemotype was carvacrol one which possess the “phenolic” odour and contained carvacrol (24.4–33.3%), β-caryophyllene (9.5–14.9%) and β-bisabolene (10.3–12.9%) (Mockute and Bertoniene, 1999).

| Test culture | Thymus vulgaris | Thymus serpyllum | Salvia sclarea | Monarda didyma |
|--------------|----------------|-----------------|---------------|---------------|
| ATCC 25923   | 30.0 ± 0.25a   | 30.0 ± 0.25a    | 31.0 ± 1.00a  | 30.0 ± 0.75a  |
| S. aureus     | 31.0 ± 0.20a   | 24.0 ± 0.20a    | 28.0 ± 0.50b  | 30.0 ± 1.00a  |
| ATCC 29212   | 25.0 ± 0.50b   | 11.0 ± 0.25g    | 30.0 ± 1.25a  | 20.0 ± 1.00d  |
| E. faecalis   | 19.0 ± 1.00d   | 10.0 ± 0.25g    | 17.0 ± 0.30g  | 17.0 ± 0.75e  |
| E. faecalis   | 12.0 ± 0.25e   | 17.0 ± 0.50d    | 21.0 ± 0.80e  | 20.0 ± 0.75d  |
| S. pyogenes   | 12.0 ± 0.35e   | 17.0 ± 0.75d    | 20.0 ± 0.50f  | 18.0 ± 0.25e  |
| E. coli       | 30.0 ± 0.75a   | 15.0 ± 0.25e    | 25.0 ± 0.20c  | 19.0 ± 0.55f  |
| C. albicans   | 24.0 ± 0.20b   | 19.5 ± 0.30c    | 23.0 ± 1.20d  | 23.0 ± 0.15c  |
| ATCC 885–653 | 23.0 ± 0.35c   | 21.0 ± 1.00b    | 24.5 ± 0.50c  | 25.0 ± 0.45b  |
| S. pyogenes   | 12.0 ± 0.35e   | 17.0 ± 0.75d    | 20.0 ± 0.50f  | 18.0 ± 0.25e  |
| E. coli       | 30.0 ± 0.75a   | 15.0 ± 0.25e    | 25.0 ± 0.20c  | 19.0 ± 0.55f  |
| C. albicans   | 24.0 ± 0.20b   | 19.5 ± 0.30c    | 23.0 ± 1.20d  | 23.0 ± 0.15c  |

Notes: the data were statistically significant as compared with the control (P < 0.05); letters indicate the inhibition zones significantly different one from other according to Tukey’s test. AM – ampicillin, AMO – amoxicillin/clavulanic acid, GEN – gentamicin, FCS – formic acid for C. albicans; “0” – no inhibition.
EsO of *Monarda* species can accumulate a high content of aromatic compounds (up to 90%) (Myadelets et al., 2014). The composition of EsO from *Monarda didyma* and *Monarda fistulosa* aerial parts grown in two Italian sites differed significantly, especially in thymol content (62% vs. 31%, respectively) (Mattarelli et al., 2017). Thymol (64.9%), methyl ether thymol (7.6%), and p-cymene (9.5%) were revealed among 37 identified compounds in the EsO of the herb of *Monarda didyma* cultivated in the Omsk Region (Russia) (Myadelets et al., 2014). It seems that Myadelets et al. (2014) were dealing with the thymol chemotype of *Monarda didyma* as well. The most abundant compounds of *Monarda didyma* oil grown in Italy were oxygenated monoterpenes, and thymol (19.4%), its methyl ether (19.9%) and linalool (17.1%) prevailed among them (Marchioni et al., 2020). Linalool was the main component of the specific chemotype of *Monarda didyma* grown in France (Carnat et al., 1991). It should be speculated that the tested EsO isolated from the Ukrainian *Monarda didyma* CCS belongs to the thymol-eucalyptol chemotype due to prevailing these two compounds (47.54% and 17.82%, respectively).

GC–MS analysis of EsO isolated from the Indian *Salvia sclarea* herb showed that monoterpenoids linalyl acetate (61.33%) and linalool (17.59%) were its major components (Kumar Singh et al., 2019). Rafat and Habib (2018) revealed the availability of five chemotypes of Libyan *Salvia sclarea* due to their EsO composition which differed significantly in the contents of linalool (10.75–38.07%), α-terpineol (6.45–13.40%) and linalyl acetate (1.0–35.28%) as the most abundant compounds. Cai et al. (2006) determined the chemical composition of the EsO of four *Salvia sclarea* samples obtained from Huabao Fragrance & Flavor Co. Ltd (Shanghai, China). The growing places of them are therefore not known. These samples of EsO mainly contained: linalyl acetate (29.50–49.83%) and linalool (17.03–28.76%) (Cai et al., 2006). Hudail et al. (2001) stated about the following composition of the tested samples of the EsO of *Salvia sclarea* growing in Italy and Russia: linalyl acetate (42.75–55.72%), linalool (9.01–11.97%), germacrene D (4.35–7.58%), and β-caryophyllene (3.00–4.24%). These authors conclude that EsO from infected materials of *Salvia sclarea* of Italian origin contained higher percentages of sesquiterpene hydrocarbons (e.g. germacrene D and β-caryophyllene), monoterpenes alcohols (e.g. α-terpineol) and diterpenoids (mainly sclareol) than non-infected ones. The most abundant components of the Turkish *Salvia sclarea* EsO were caryophyllene oxide (24.1%), sclareol (11.5%), and α-pinene (11.2%) (Yuce et al., 2014). The main components of four EsO of the aerial parts of *Salvia sclarea* collected from eleven provinces in Iran were linalool (12.17–21.41%), linalool acetate (13.06–52.61%) and D-germacrene (0–17.68%) (Rajabi et al., 2014).

It could be speculated that the major compounds of the investigated EsO impart their specific odour and biological properties, while minor components define the chemosystematic significance. Thymol (5-methyl-2-(propan-2-yl)phenol) is a monoterpenic phenol, which is used in traditional medicine due to various pharmacological properties, including antibacterial, antifungal, antioxidant, and antiinflammatory (Nagoor Meeran et al., 2017). Thymol is as a component of herbal medicinal preparations, cosmetic products, including mouthwashes, and as a food flavoring (Memar et al., 2017).

A lot of studies have revealed the antibacterial and antifungal properties of thymol and its isomer carvacrol (2-methyl-5-(propan-2-yl)phenol), especially against antibiotic-resistant pathogenic bacteria and *Candida albicans* (Memar et al., 2017). Thymol and carvacrol are active ingredients of the EsO of many Lamiaceae species with promising antimicrobial as well as antioxidant effects (Memar et al., 2017; Shanaida et al., 2018). They can inhibit the growth of both gram-positive and gram-negative microorganisms and have the antibiofilm effects (Sharif-Rad et al., 2017). Thymol and carvacrol, the major compounds of *Thymus vulgaris* and *Oregano vulgare* EsO collected in Serbia, showed the highest antimicrobial activity against food-borne pathogens such as *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Staphylococcus aureus* (Soković et al., 2010). Rattanachaikunsopon and Phumkhachorn (2010) revealed that aromatic monoterpenephlorop-cymene enhanced the inhibitory effects of carvacrol in the studies against *Vibrio cholerae* and other foodborne pathogens.

Linalool (3,7-Dimethyl-1,6-octadien-3-ol) is a monoterpenic alcohol presented in many Lamiaceae species (Herman et al., 2016; Hudz et al., 2020). The addition of linalool increased the efficac-ity of *Thymus vulgaris* EsO against *Pseudomonas aeruginosa* (Herman et al., 2016). Linalool and α-terpineol demonstrated strong antimicrobial effect against the cariogenic and periodontopathic bacteria in low concentrations (below 0.4 mg/mL) (Park et al., 2012).

Citril (3,7-dimethyl-octa-2,6-dienal) has a specific lemon odour and antimicrobial activity. It significantly reduced the levels of such cytokines as IL-1β, IL-6, TNF-α and such oxidative factors as malondialdehyde and hydroxyl radicals in methicillin-resistant *Staphylococcus aureus*-infected mice and diminish their lung inflammatory infiltrates that indicate antioxidant, antimicrobial and anti-inflammation activities of citral (Long et al., 2019). The combinations of citral and linalool in nanoemulsion with sesame oil possessed the antibacterial effects against *Listeria monocytogenes* as a known food-borne pathogen (Prakash and Vadivel, 2020). Mulyaningsih et al. (2011) found that eucalyptol, or 1,8-cineole (1,3,3-Trimethyl-2-oxahydrinocyclo[2.2.2]octane), which was the second predominant component of the tested *Monarda didyma* CCS EsO, was very active against multidrug-resistant gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* (Mulyaningsih et al., 2011). Sharifi-Rad et al. (2017) concluded that antimicrobial activity of EsO correlated mostly to the presence of molecules with aromatic structure or aldehydes, alcohols, ethers such as thymol, carvacrol, linalool, 1,8-cineole, etc.

Among investigated 72 Lamiaceae species, the EsO of *Thymus* genera were the most active against pathogenic microorganisms due to prevailing aromatic compounds (Karpinski, 2020). Among 79 EsO screened for antibiotic activity and reducing virulence of the uropathogenic strains of *Escherichia coli*, thymol-rich *Thymus vulgaris* oil and carvacrol-rich *Origanum vulgare* oil as well as their major constituents, carvacrol and thymol, inhibited this pathogenic microorganism significantly (Lee et al., 2017). EsO of the Ethiopian *Thymus serrulatus* and *Thymus schimperi* containing thymol and carvacrol as the major components demonstrated the significantly high inhibition zones against tooth decay bacteria *Streptococcus mutans* and *Lactobacillus* (Damtie et al., 2020). Thymol (55.35%) and p-cymene (11.2%) were the major components of the EsO hydrosolventilatt from the Turkish *Thymus vulgaris*. This EsO demonstrated high antimicrobial activity. The highest inhibition zones were against *Bacillus cereus* (NRRL B3711), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 9144) (Gedikoglu et al., 2019). The EsO of the Polish *Thymus vulgaris* which contained thymol (61.9%), p-cymene (10.0%), and γ-terpinene (10.0%) as predominant compounds demonstrated the strong bactericidal activity against multidrug-resistant strain of *Staphylococcus aureus* (Kot et al., 2019). The EsO of the Indian *Thymus vulgaris* and its major active compound thymol are charac-terized by the great anti-candidal potential alone and in combination with antifungal medicines (Jafri et al., 2020). Antimicrobial properties of *Thymus vulgaris* EsO were found against several clinical isolates of opportunistic infections (Krytsova et al., 2019).

The EsO of Monarda didyma (cultivar ‘Scorpio’) grown in Poland was rich in linalool, p-cymene, thymol and thymol methyl ether, and showed the prominent antimicrobial effect against *Candida albicans*, *Bacillus cereus* and *Pseudomonas fluorescens* (Wróblewska et al., 2020).
should be mentioned that the main components of the Monarda species (M. fistulosa, M. didyma, and M. didyma var. 80-1A) exhibited the inhibitory effects on mold Botrytis cinerea. It should be mentioned that the main components of the M. didyma EsO were thymol (41.17%), γ-terpinene (15.88%), and carvacrol (15.20%). Var. 80-1A of M. didyma was characterized by the domination of linalool (55.38%) and geraniol (20.71%), and M. fistulosa contained mainly geraniol (61.83%) and geranyl formate (16.61%). (Adelayo et al., 2013). Gram-positive cocci Staphylococcus aureus and yeast Candida albicans were very sensitive to the EsO obtained from the Monarda fistulosa cultivated in Ukraine due to the prevailing aromatic compounds such as thymol (42.01%) and p-cymene (15.45%). (Shanyaida and Pokryshko, 2015).

It was revealed that the Salvia sclarea EsO and antibiotic oxacillin have the synergistic effect against methicillin-resistant Staphylococcus epidermidis. This EsO contained such main components as linalyl acetate (38.67%), linalool (20.42%) and germacrene (5.31%) (Chovanová et al., 2016). The antimicrobial activity of the Salvia sclarea EsO was related to damaging the bacterial cell membrane, leading to the release of ATP or even DNA (Cui et al., 2015). The inhibitory effect of the EsO against Candida albicans, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli. The inhibition effect increased progressively with increasing of contact time (Peana et al., 1999).

5. Conclusion
The analyzed EsO of five Ukrainian Lamiaceae representatives could be characterized as such chemotypes: ‘thymol-α-cymene, γ-terpinene’ of Thymus vulgaris C., ‘thymol-isothymol methyl ether of Thymus serpyllum, ‘x-citral–β-citral’ of Thymus pulegioides C2, ‘linalyl acetate-linalool’ of Salvia sclarea, and ‘thymol-eucalyptol’ of Monarda didyma C.CS. Among the tested EsO, Thymus serpyllum and Thymus vulgaris C followed by Salvia sclarea demonstrated the widest spectrum of action and highest antimicrobial activity against the most of tested typical and clinical strains of widespread pathogenic microorganisms. The found antimicrobial effects could be assigned to the chemical composition of the tested EsO. The sensitivity of the tested microorganisms is related to specific compounds of the EsO or synergistic effects of different compounds. Overall, all the chemotypes of the tested Lamiaceae species are highly promising herbal substances for the development of safe and effective herbal products in the form of oral liquid or sprays for the complementary treatment of the diseases of the oral cavity caused by drug-resistant pathogenic bacteria (Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes) and opportunistic yeast Candida albicans. These EsO can be also natural preservatives for cosmetic and food products.

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Declaration of Competing Interest
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