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ŽIVILA, HRANILA IN PREHRANA PRIHODNOSTI

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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM MEDICAL PLANTS GROWN IN LIGHT MODIFIED ENVIRONMENT

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Abstract: Thyme, marjoram, lemon balm, mint, and sweet basil were used to determine whether light modification (plants grown under pearl nets with 50% shaded index or un-shaded - open field conditions) could improve the antimicrobial activity of essential oils (EOs). Obtained results were discussed concerning previously determined yield and composition of EOs from five medicinal plants. Seven microorganisms were selected to determine the antimicrobial activity of medicinal plants essential oils. The inhibition zone is dependent primarily on the plant species and the influence of shading is much less expressed. The results revealed that EOs from *Thymus vulgaris* L., proved most active against all isolates with inhibitory zone range from 24 mm (*B. subtilis*) to 56 mm (*C. albicans*). From all species of the plants, only marjoram exhibits inhibition (18-20 mm) in the case of *P. aeruginosa.* EOs from shaded thyme and marjoram expressed higher inhibition effects in comparison to other shaded or unshaded plants against all tested microorganisms. EOs from all shaded plants, except basil, showed higher anti-candida activity than EOs from unshaded plants. The results of this study suggest that the natural products derived from Lamiaceae plantsmay have potential use in the food and/or pharmaceutical industries as antimicrobial agents.

Keywords*:* mint plants; shading; essential oils; composition: antimicrobial activity

PROTIMIKROBNA AKTIVNOST ETERIČNIH OLJ IZ ZDRAVILNIH ZELIŠČ GOJENIH V OKOLJU S SPREMENJENO SVETLOBO

Povzetek: Preučevali smo gojenje timijana, majarona, melise, mete in bazilikev razmerah spremenjene svetlobe (rastline, gojene pod bisernimi mrežami s 50-odstotnim indeksom senčenja ali nezasenčene – dnevna svetloba) z namenom ugotoviti ali senčenje izboljša protimikrobno delovanje eteričnih olj (EO). Dobljene rezultate smo obravnavali glede predhodno določenega donosa in sestave EO iz vseh petih zdravilnih zelišč. Za ugotavljanje protimikrobnega delovanja eteričnih olj zdravilnih zelišč je bilo izbranih sedem mikroorganizmov. Območje inhibicije je odvisno predvsem od rastlinske vrste, vpliv senčenja pa je veliko manj izražen. Rezultati so pokazali, da so se EO iz timijanaizkazale za najbolj aktivne proti vsem izolatom z razponom zaviralnih con od 24 mm (*B. subtilis*) do 56 mm (*C. albicans*). Od vseh izbranih zeliščkaže samo majaron inhibicijo (18-20 mm) v primeru *P. aeruginosa*. EO iz zasenčenega timijana in majarona sta v primerjavi z drugimi zasenčenimi ali nezasenčenimi rastlinami izrazili višje inhibicije proti vsem testiranim mikroorganizmom. EO iz vseh osenčenih rastlin, razen bazilike, so pokazali večjo aktivnost proti kandidi v primerjavi z EO iz nezasenčenih rastlin. Rezultati te študije

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1 INTRODUCTION

Essential oils (EOs) are volatile secondary metabolites extracted from medicinal plants with positive effects on human health, accepted as natural antimicrobials instead of chemical preservatives in food products (Pandeyet al., 2017). The chemical composition of the EOs contributes to their medicinal value which is responsible for the antibacterial properties highly depends on different geographical origin, chemotypes, environment, method of production, maturation stage, plant parts from which EO was derived, as well as harvesting time, isolation techniques, duration, and storage conditions (Helalet al., 2019; Tmušić et al., 2021). EOs are natural origin, with a wide spectrum of antimicrobial activity (Soković et al., 2010) use of small concentrations, do not leave any residual effect on fresh produce (Vermani and Garg, 2002), inexpensive and renewable (Kalemba and Kunicka, 2003). They could be used as alternatives for chemical fungicides because they are known as "reduced-risk" pesticides and they are safe for consumption and environmental (Tzortzakis, 2007a, 2007b).

Climate conditions play very important role in the accumulation and composition of essential oils in the plants (Murillo-Amador et al., 2013). For the medical plant production and synthesis of secondary metabolites, light intensity and quality have great affect. Light modification could improve the quantity and quality of essential oils in medicinal plants (Ilić et al.,2022; Milenković et al., 2021). Shading plants by photo-selective shade nets synthesized more EOs than plants exposed to full sun light (Ilić et al., 2021).

Since the main components of EOs are very complex and variable, it is very difficult to link them to a antimicrobial activity (Milenković et al., 2021). An association between the chemical composition of the most dominant components in the EO and the antimicrobial activity was observed (Carrasco et al., 2012). EOs from *Lamiaceae* species as an oral liquid in dental hygiene procedures demonstrated high antimicrobial activity against pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes) and opportunistic yeast Candida albicans* (Shanaida et al., 2021). EO isolated from Lamiaceae species in the appropriate amount and proportion are increasingly used in food preservation, but also in pharmaceutical and cosmetic products. Special attention should be paid to the protection of biological activities of EO during food processing because they are volatile and can easily oxidize (Beykiet al., 2014). Although the antibacterial and antioxidant abilities of EOs are well documented, studies on environmental impact, especially light intensity, on the antibacterial activity of EOs are still limited. From the economic aspect, it is necessary to create adequate microclimatic conditions in the field for the production of medicinal plants with a high potential of their EOs against bacteria and fungi.

The present paper aimed to investigate the effect of light modification under shading conditions on the EOs composition of thyme, marjoram, lemon balm, mint, and basil and their antimicrobial activity against pathogenic microorganisms.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND GROWING CONDITIONS

The experiment was conducted during 2019-2020 in an experimental garden at the village Moravac in South Serbia (21°42′ E, 43°30′ N, altitude 159 m). *Thymus vulgaris* L. (thyme), *Origanum majorana* L. (marjoram), *Melissa officinalis* L. (lemon balm), *Mentha piperita* L. (mint), and *Ocimum basilicum* L. (sweet basil), were used to determine whether shading conditions (plants cover by color nets) could improve essential oils and antioxidant activity in plants.

The seeds were sown in the field with the task to achieve an optimal plant density of 50 plants/m^2. Treatment combinations were replicated three times with one shading treatment (pearl netswith a shade index of 50%) and non-shaded control treatment in a split-plot design. In the second year, after establishing the plant's production, medicinal plants were harvested for essential oils extraction (main harvest in the middle of August).

2.2 CLEVENGER-HYDRODISTILLATION

Growing of medical plants, under colored shade nets and non-shaded as well as the process of production of EOs by hydrodistillation were performed as described Ilić et al. (2022) and Milenković et al. (2021).The content of essential oil is displayed in % (v/m), which conforms to mL/100 g of air-dried plant material.

2.3 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) AND GAS CHROMATOGRAPHY/FLAME IONIZATION DETECTION (GC/FID) ANALYSIS

The use of gas chromatography-mass spectrometry (GC-MS) and GC-flame ionization detection (GC-FID) for characterization of medical plants essential oils is described in our previous research (Milenković et al., 2021) following methods by Sparkman et al. (2011).

2.4 DPPH ASSAY

The ability of the essential oil to scavenge free DPPH radicals was determined using the DPPH assay. Absorption was measured at 517 nm immediately after adding the DPPH radical and after 20 minutes of incubation with the radical (see Stanojević et al., 2015; 2017).

2.5 ANTIMICROBIAL ACTIVITY

Microorganisms.Seven microorganisms were selected to determine the antimicrobial activity of analyzed EOs: (six bacterial strains) *Escherihia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 8427), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), and *Listeria monocytogenes* (ATCC 15313) and (fungal strain) *Candida albicans* (ATCC 2091). Microorganisms are from the collection of the Laboratory for Microbiology, Faculty of Technology, Leskovac.

2.5.1 Disc-diffusion method

The agar discdiffusion method was used for testing the antimicrobial activity of EOs obtained (Kiehlbauch, 2000). All details about sterilization, prepared initial suspensions, inoculation, and incubation were expressed in our previous explorations (Ilić et al., 2021).

All experiments were carried out in three replications and the results were expressed as the mean value ± standard deviation.

2.6 STATISTICAL METHODS

ANOVA was used to analyze the significance (TIBCO Software Inc. Palo Alto, CA, USA. 2020, version 14.0.015.). Duncan's multiple range test was used for the analysis of significance (with a level of 0.01) of differences between means. For explanatory data analysis, multivariate principal component analysis was used.

3 RESULTS

3.1 GROWING CONDITIONS

In open field conditions during the summer months (July and August) we record values of Photosynthetically active radiation (PAR) over the 2200 µmol $s^{-1}m^{-2}$ and solar radiation around 900 (W m^{-2}). These parameters are quite high due to global warming and plants need some kind of protection against excessive radiation. One of the solutions is the application of shade nets. Net houses have the potential to create an appropriate microclimate that positively affects plants' productivity and quality.Compared to the light parameters, the temperature and relative humidity under the nets are less exposed to changes. Shading significantly affects solar radiation and PAR in relation to the unshading (open field) conditions. More intense reduction was observed in the afternoon, then in the morning, while the decrease in light intensity at noon was about 50% (Table 1).

Table 1. Influence of shading on growing environment (average day in July)

Time (h)	PAR* (µmol m^{-2} s^{-1})		Solar radiation (W m^{-2})		Temperature °C		Relative Humidity %	
	Un shading	Shading Reduction %	Un shading	Shading	Un shading	Shading Reduct.%	Un shading	Shading Reduct.%
6:00	182.5	31.2	162.5	40.5	16.7	0.0	74.7	-4.1
9:00	1325.6	46.0	513.8	281.0	24.7	-0.4	71.8	0.0
12:00	2242.2	49.1	874.5	459.5	31.4	-2.2	47.3	-2.1
15:00	1684.1	51.9	790.5	351.0	31.5	-2.4	48.2	-1.2
18:00	672.0	53.9	375.5	90.9	28.3	-1.0	50.4	-0.2

* PAR.-Photosynthetically active radiation

3.2 ESSENTIAL OILS (EOS) YIELD

The yield of EOs of thyme was 2.57 mL/100g of plant material (p.m) from shaded and 2.32 mL/100g of unshaded plant material. Shaded marjoram (1.68 mL/100g p.m.), lemon balm (0.22 mL /100g p.m.),mint (2.23 mL/100g p.m.) and basil plants (1.32 mL/100g p.m.) showed a higher content of EO than unshaded plants (1.51 mL/100g p.m.: 0.18 mL/100g p. m.; 2.00

mL/100g p.m. and 1.12 mL/100g p.m. respectively), Tab 2. (Ilić et al., 2022; Milenković et al., 2021).

Table 2. Yield of essential oil and EC_{50} values of thyme, marjoram, lemon balm, mint and basil

Plant species	Shading	Essential oil yield, mL/100 g p.m.	EC_{50}, mg mL^{-1}/ 20 min incubation
Thyme	Unshaded	2.32^{b} ±0.03	0.944^{a} ± 0.001
	Shaded	2.57^{a}±0,09	0.852^{a} ± 0.005
Marjoram	Unshaded	1.51^{d}±0.03	54.012^{e} ±1.051
	Shaded	1.68^{c}±0.03	19.972^{d} ± 0.199
Lemon balm	Unshaded	0.18^{f}±0.003	7.957^{c} ± 0.092
	Shaded	0.22^{e}±0.01	7.706^{b} ± 0.091
Mint	Unshaded	2.00^{b}±0.03	13.081^{e} ± 0.103
	Shaded	2.23^{a}±0.01	11.701^{d} ± 0.098
Basil	Unshaded	1.12^{d}±0.01	0.762^{a} ± 0.008
	Shaded	1.32^{c}±0.01	0.682^{a} ± 0.002
ANOVA (p values)			
Plant species		0.00000	0.00000
Shading		0.00000	0.00000
Plan species · shading		0.00518	0.00000

3.3 ESSENTIAL OILS COMPOSITION

The antimicrobial activity of EOs mainly depends on the chemical composition.In this research the main components of the thyme essential oil are thymol (8.05-9.35 mg/mL); γ-terpinene (3.49-4.04%); p-cymene (2.80-3.60%) and caryophyllene oxide (1.54-2.15%). The majority of EO compounds in the marjoram cultivated in unshaded and shaded conditions wereterpinene 4-ol (7.44-7.63%), γ-terpinene (2.82-2.86%), and linalool (2.04-2.65%). The main components of the lemon balm EO are geranial (6.84-7.78%); neral (3.02-3.52%), piperitenone oxide (1.67-5.36%), and caryophyllene oxide (1.54-2.15%). Shaded plants contained a higher content of geranial and neral compared to unshaded plants. The major components identified in mint EO are piperitenone oxide, 1,8-cineole,and myrcene. Accumulation of piperitenone oxide (14.0%), caryophyllene oxide (2.27%) and myrcene (0.91%) was higher in unshaded (control plants) than in shaded plants (12.0%, 1.32% and 0.78%, respectively). BasilEO contained various chemical compounds. The majority of the compounds in the oil were linalool (9.06-10.2%), 1,8-cineole (2.06-1.26%), α-trans-bergamotene (1.21-1.47%), epi-α-cadinol (0.93-1.17%) and eugenol (0.83-1.20%). Shaded plants obtained higher linalool and α-trans-bergamotene yield (Tab. 3) (Ilić et al., 2022; Milenković et al., 2021).

Table 3. Contents of the most common components of medicinal plants depending on the light modification

Method of production	Components %		
Plant species			
Thyme			
	Thymol	γ-Terpinene	p-Cymene
unshade	8.05	3.49	2.80
shade	9.35	4.04	3.60
Marjoram			
	Terpinen-4-ol	Linalool	γ-Terpinene
unshade	7.44	2.04	2.82
shade	7.63	2.65	2.86
Lemon balm			
	Geranial (E-citral)	Piperitenone oxide	Caryophylene oxide
unshade	6.84	5.36	2.15
shade	7.78	1.67	1.54
Mint			
	Piperitenone oxide	1,8-Cineole	Myrcene
unshade	14.0	2.27	0.91
shade	12.0	1.32	0.78
Basil			
	Linalool	1,8-Cineole	α-trans-Bergamotene
unshade	9.06	2.06	1.21
shade	10.20	1.26	1.47

3.4 ANTIOXIDANT ACTIVITY

All medicinal plants covered by shade nets showed higher antioxidant activity than unshaded – control plants. Based on the results given in Table 2. the highest antioxidant activity was observed in basil and thyme EOs from plants covered by nets.The EC_{50} values (efficient concentration of oil)decreased in the following order (the smaller the EC_{50} value, the better the antioxidant): shaded basil (0.682) > shaded thyme (0.852) > shaded lemon balm (7.706) shaded mint (11.701) > shaded marjoram (19.972) (Milenković et al., 2021).

3.5 ANTIMICROBIAL ACTIVITY

The obtained results provide evidence that all EOs from Lamiaceae medicinal plants of Serbia exhibit efficacy against analyzed pathogenic microorganisms. Our results revealed that EOs from *Thymus vulgaris* L., proved most active against all isolates with inhibitory zone range between 22 mm and 56 mm. All five EOs (*Thymus vulgaris* L., *Origanum majorana* L., *Melissa officinalis* L., *Mentha piperita* L., and *Ocimum basilicum* L.) showed significant anti-candida activity (Table 4).

Table 4. Antimicrobial activity (inhibition zone, mm) of essential oils from shading and nonshading medicinal plants

Microorganisms	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 8427	*Staphylococcus aureus* ATCC 25923	*Bacillus subtilis* ATCC 6633	*Listeria monocytogenes* ATCC 15313	*Candida albicans* ATCC 2091
Medical plants	Inhibition zone (mm ± SD)						
A[1] thyme	36.67±1.53	n.z.	24.00±1.73	35.67±2.08	42.33±0.58	54.67±1.15	56.00±0.00
A[2] thyme	37.67±1.15	n.z.	22.00±1.00	36.67±0.58	42.00±1.00	46.00±3.46	54.00±2.00
B[1] marjoram	25.67±0.58	20.00±1.00	23.67±1.53	28.67±0.58	39.33±1.15	24.67±1.15	29.33±1.15
B[2-]marjoram	33.67±1.15	18.67±0.58	30.67±0.58	29.00±1.00	35.33±3.05	30.67±3.05	23.00±1.73
D[2] lemon balm	11.33±0.58	n.z.	11.67±0.58	20.33±0.58	37.33±1.15	/	/
E[1] mint	12.00±1.00	n.z.	14.33±0.58	15.33±0.58	17.00±1.00	12±0.00	45.67±0.58
E[2] mint	n.z.	n.z.	14.33±1.15	16.00±1.00	21.00±1.00	n.z.	38.67±1.15
F[1] basil	n.z.	n.z.	12.33±0.58	16.33±0.58	36.00±1.00	12.00±0.00	26.67±3.05
F[2] basil	18.67±0.58	n.z.	12.33±0.58	14.33±0.58	25.33±0.58	21.33±1.15	34.67±3.05

[1-]Shading plants
[2]-Unshading plants
n.z. –no zone
/ - not treated

The microbial inhibition zone of thyme EO was the largest in the case of *C. albicans*. Marjoram exhibits the most expressed inhibition in the case of *P. aeruginosa*. These two plants exhibit higher inhibition effects in comparison to mint and lemon balm for all other microorganisms included in this investigation (Table4). Inhibition zone was dependent primarily on medicinal plants and the influence of shading was less expressed.

3.6 PCA ANALYSIS

The differentiation based on the first principal component (PC1), explaining 65.14% of total variability, points out that EOs from marjoram and thyme shaded plants exhibit higher inhibition effects in comparison to mint and lemon balm for all other microorganisms included in this investigation. Thyme EO has a higher antimicrobial potential than basil.

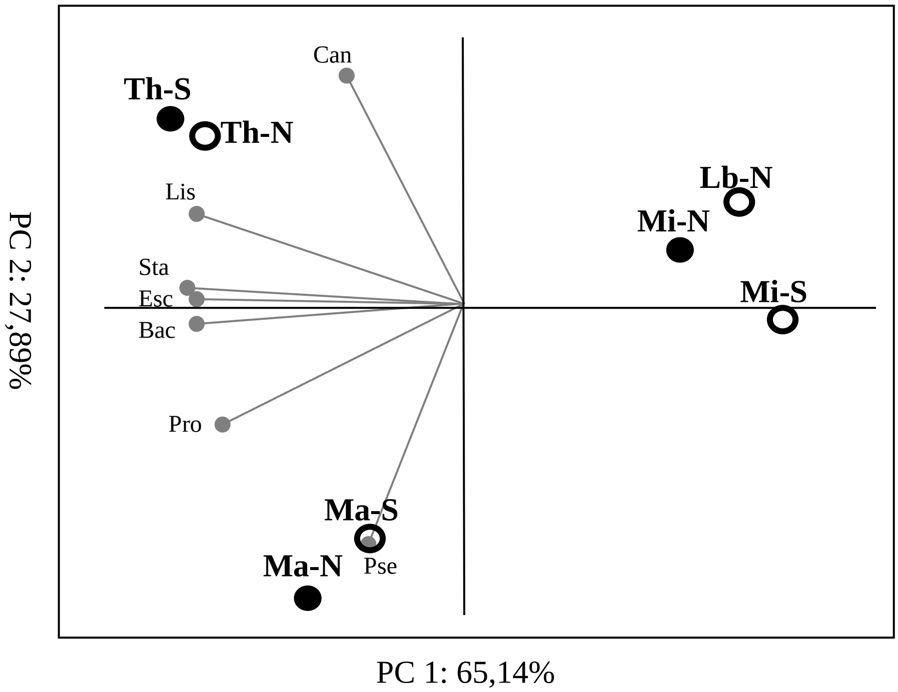

Figure 1: Bi-plot of principal component analysis of antimicrobial potential of essential oils from selected medicinalplants

Esc-*Escherichia coli*; Pse-*Pseudomonas aeruginosa*; Pro-*Proteus vulgaris*; Bac-*Bacillus subtilis;* Sta-*Staphylococcus aureus*; Lis-*Listeria monocytogenes;* Can-*Candida albicans*; **Th-S** (thyme-shaded), **Th-N** (thyme-nonshaded); **Lb-N** (lemon balm-nonshading), **Mi-N** (mint-nonshaded), **Mi-S** (mint-shaded); **Ma-N** (marjoram-nonshaded), **Ma-S** (marjoram-shaded)

4 DISCUSSION

Method of production (open field or protected area) and environmental (light conditions and solar radiation) greatly affect the quality and composition of the medicinal plants. Among all plants, the lowest accumulation of essential oils was observed in the unshaded lemon balm (0.18 mL/100g p.m.) while the highest oil accumulation was achieved in shaded thyme plants (2.57 mL/100g p.m.). In our previous studies, we have observed significantly different yields of the EOs between shaded and unshaded plants. Thus, the lowest accumulation of EOsin sweet basil was observed in the unshaded, control plants (1.02 mL/100g p.m.) while the highest oil yield was achieved in plants from red shade nets (3.23 mL/100g p.m.), Milenković et al., 2019. The spectral changes provided by colored shade nets during medicinal plants production, similar to our research, resulted in a higher yield of EOscontent in sweet basil (Ilić et al., 2021), lemon balm (Oliveira et al., 2016), and sage (Li et al., 1996). Synthesis and accumulation of secondary metabolites from medicinal plants largely depend on the intensity of light as a result of applying shade nets. The chemical components most commonly found as the main ingredients in EOs, among plants presented in Table 3, include piperitenone oxide (thyme), terpinen-4-ol (marjoram), geranial (E-citral) lemon balm, piperitenone oxide (mint), and linalool (basil). Oxygen-containing monoterpenes seem particularly important antifungal component in the plants from Lamiaceae family. Its activity and derivatives, such as caryophyllene oxide are well known. Essential oils containing high concentrations of phenolic monoterpenes (e.g., p-cymene, γ-terpinene,1,8-cineole, myrcene) with great antifungal activities. Important antifungal chemicals often presented in Lamiaceae are also other monoterpenes as alcohol linalool and cyclic 1,8-cineole, limonene, pinenes, and terpinenes. Results from Table 3 show that all of these antifungal substances are common in the presented plants. The oil composition was close to the Mediterranean country such as Tunisia (Sellami et al., 2009), Spain (Ibáñez and Blázquez, 2017), and Greece (Komaitis et al., 1992).

Among five plant species investigated in this work, basil and thyme plants are characterized by the highest level of antioxidant activity and could be used as natural antioxidants for food preservation.These plants species tolerate shading well, and nets provide greater presence and biosynthesis of polyphenolic compounds known to exhibit antioxidant properties (Milenković et al., 2019). The main compounds of essential oil in thymewere thymol, with the content of25%-50% from Serbia or 68.1% from Spain (Gavaric et al., 2015; Rota et al., 2008).

The compositions of the EOs in lemon balm from our research were geranial (E-citral), piperitenone oxide, and caryophylene oxide. EOs of lemon balm from Algeria was dominated by neral, geranial, and citronellal. This composition was qualitatively similar to the oils from Serbia (Mimica-Dukic et al., 2004), Slovakia (Holla et al., 1997), Egypt (Shabby et al., 1995), etc.

Solar irradiance affected the concentration of mint volatile compounds as well as their composition. The main components of the mint oil from our research were piperitenone oxide. The results of EOs from mint plants in the same region (Bosnia and Hercegovina) show the highest content of menthol 43.66%, menthone 20.02% and iso-menthone 7.73% (Marjanović-Balaban et al., 2018) or EOs of mint from Serbia with the content of menthol (37.04%), Soković et al., (2009).Variations in EO content and certain components in medical plants are generally genetic (ecotypes, genotypes, lines, and varieties), but they may be influenced also byenvironmental and cultivation conditions, growing techniques and practices,phenological stages, seasonal variations, etc.(Moghaddam and Mehdizadeh, 2017).

Essential oilsfrom Lamiaceaeplants (*Thymus vulgaris* L., *Origanum majorana* L., *Melissa officinalis* L., *Mentha piperita* L., *Origanum vulgare*,and *Ocimum basilicum* L.) are natural products, against many different types of microbes (Avetisyan et al., 2017), including food-borne pathogens (Kozłowska et al., 2015).

EOs significantly inhibits the growth of microbes by disrupting the cell membranes and their permeability, leads to the leakage of ions, and coagulation of cell contents leading to cell death (Dorman and Deans, 2000).In some of the previous research, species from Lamiaceae family exhibited the strongest antifungal activity with inhibition zones ranging from 39 mm to 45 mm compared with standard antifungal molecules (Ketoconazole, Fluconazole, and Amphotericin 100) which range from 0 mm to 14 mm only (Helal et al., 2019).

Gram-positive bacteria are more susceptible to growth inhibition by plant EOs (because of the single membrane structure) than Gram-negative bacteria due to the great complexity of the double membrane-containing cell envelope (El Abed et al., 2014). In our investigation the most expressed inhibition effect was noticed in case of Gram-positive (*B.subtilis, L.monocytogenes, S. aeraus)*,Gram-negative (*E.coli, P. aeruginosa)* bacterium and one fungus (*C. albicans).*

E. coli is considered to be a dominant bacterium species in the digestive tract. Fecal contamination of water and food is most often caused by the presence of this bacterium. Thyme (36-37 mm) and marjoram (25-33 mm) EOs showed very strong antibacterial activity against *E. coli*. EOs from mint and basil had no antifungal activity against E. coli. Results from our study do not show an increase in the antifungal efficacy against E. coli of EOs obtained from shaded plants. P. aeruginosa an aerobic Gram-negative bacteriumis opportunistic pathogen that rarely causes disease in healthy persons. EOs from all plants has no antibacterial effects against this bacterium, except EO from the marjoram plant. The essential oil of shaded marjoram (39 mm), lemon balm (37 mm), and basil (36 mm) had the strongest inhibition activity against*B. subtilis* than EOs from unshaded plants (35 mm, 17 mm, 25 mm, respectively).

L. monocytogenes is a Gram-positive facultative anaerobic pathogenic bacteria species that leads to listeriosis disease after consumption of contaminated food. EO from shade thyme exhibits the highest antibacterial effects on this bacterium (*L. monocytogenes*) than EOs from other plants. Thyme EOs derived from shaded plants is more effective and has the better antimicrobial ability (54 mm) than EOs from thyme unshaded plants (46 mm).

S. aureus is a gram-positive bacterium that is among many harmful effects to humans. EOs from all plants involved in this research manifests good antimicrobial potential. Method of plant production (shaded or unshaded conditions) had no effects on EOs antimicrobial potential against *S. aureus.*

EOs from all medicinal plants showed strong antifungal activity against *C. albicans*. EOs from plants grown under nets showed the strongest antifungal activity (thyme 56 mm, mint 45 mm; marjoram 29 mm) compared to EOs from plants cultivated in open field (thyme 54 mm; mint 38 mm; and marjoram 23 mm, respectively).

The antibacterial activity of the most widely used EOs from *Thymus* species, which belong to the Lamiaceae family, was also demonstrated against several microorganisms such as *Salmonella typhi, Salmonella typhimurium, E. coli, S. aureus, Streptococcus pneumonia, Bacillus cereus, P. aeruginosa, Proteus mirabilis, K. pneumoniae*, and *L. Monocytogenes* (Golkar et al., 2020; Goudjil et al., 2020). The EOs of *Thymus pulegioides* demonstrated the strongest antimicrobial effects on typical and clinical strains of *Staphylococcus aureus* with the inhibition zones in the range of 24.0-31.0 mm (Shanaida et al., 2021). The antibacterial activities of EOs from *Thymus vulgaris* in a study performed by Pesavento et al. (2015), was found to have the highest antibacterial activity against *L. monocytogenes, S. aureus, S.enteritidis* among EOs from other Lamiaceae species.

Among the all tested natural antimicrobials, the essential oils of *Thymus vulgaris* and *Origanum majorana* as well as their components, thymol, and carvacrol, were the most promising. In combination of various plant-derived antimicrobials probably due to synergistic effects should improve both the spectrum of activity and the level of inhibition.

Similarly, EOs from the Turkish *Thymus vulgaris* with thymol (55.35%) and *p*-cymene (11.2%) as the major components demonstrated high inhibition zones against *Bacillus cereus***,** *Staphylococcus epidermidis*, and *Staphylococcus aureus* **(**Gedikoğlu et al., 2019*). The* EOs of *Thymus vulgaris* from Poland with thymol, *p*-cymene, and **γ**-terpinene as main compounds, show the strong bactericidal activity against *Staphylococcus aureus* (Kot et al., 2019). The EOs of thyme from India are characterized by the great anti-candidal potential (Jafri and Ahmad, 2020)**.**

Essential oil of *Thymus vulgaris* L. as natural preservative with antilisterial activity was shown to be very effective replacementfor nitrates in meat products(Salvaneschi et al., 2021).In the literature, the results of experiments showed that the oil from the *Thymus* exhibited extremely strong activity against all of the clinical strains. Thyme EO has been used to treatwound infectionsas an antibacterial agent in oral hygiene (Nikolic et al., 2014). There is a relationship between the high activity of thymol oil and the presence of phenol components to the bacteria's (Sienkiewicz et al., 2011; Rota et al., 2008).

Peppermint (*Mentha piperita* L.) EO, which was mainly characterized by menthol and menthone, showed significant levels of antibacterial activity against *S. aureus, Micrococcus flavus, B. subtilis, S. enteritidis,Staphylococcus epidermidis* (Kot et al., 2019) and *E. coli* (Gishen et al., 2020). Good inhibition zones against *C. albicans* were also obtained from *Mentha piperita* oils (38.0-45.0 mm). Thyme (16.0-30.0 mm) showed much larger inhibition zones than other oils and streptomycin (0-20.0 mm), Sokovic et al. (2009).

The mode of action of EO goes in the direction of disrupting the permeability of cell membranes caused by changes in its barrier properties that are related to coagulation of the cytoplasm and damage to lipids and proteins and can be letal (Viuda-Martos et al., 2011). *C. albicans* are very often present in hospital-acquired infections (Haque et al., 2018), while *B. cereus* are food born microorganisms (Tewari and Abdullah, 2015).The application of EOs as natural disinfectants in medical facilities or as agents for suppression of microbial activity in food, should be serious considered. EOs from medicinal plants could be used as natural ingredient in pharmaceutical products for the treatment against infection by *C. albicans*(Nobile and Johnson, 2015). Because *C. albicans* very often present microorganism that causes urinary infection, it is recommended that the obtained EO be used not only in prevention but also in therapy (Liu et al., 2017).Natural antifungal agents could be more effective than commercial antifungal preparations which could cause harmful effects (Mazzariol et al., 2017).

Good phytomedical potential with the best antimicrobial activity has show basil essential oil (with the inhibition zone of 40.00 mm) on coagulase-positive *Staphylococcus* (Stanojevic et al., 2017). In research from Ilić et al. (2021) basil essential oils from a plant grown under blue nets are characterized with higher eugenol content and show superior antimicrobial activity against *S. aureus*, *E. coli*, and *P. Vulgaris* and could be a good natural antimicrobial agent. The differences in the antibacterial effects of plant extracts may be due to the differences in their phytochemical compositions. According to literature, the mode of action of EOs against a pathogen or non-pathogen bacteria cannot be attributed to one specific mechanism.

5 CONCLUSIONS

All EOs revealed antibacterial properties, but the degree of bacterial growth inhibition induced by plant materials was shown to be related to bacterial strain and herbal sources. The EOs have antimicrobial activity against a wide range of microorganisms. The EOsof thyme (*Thymus vulgaris* L.) are the most active in terms of antimicrobial and antifungal activity. The EOsof thyme and mint showed significant activity against *C. albicans*. The zone of inhibition of microorganisms in thyme and marjoram is larger concerning other plants for all microorganisms included in this study. Due to the instability of EOs under environmental stresses such as temperature and light, novel technologies (shade nets) might be helpful to protect plants and improve their characteristics and antimicrobial activities. The present study suggested the possible use of EOs as an alternative to synthetic preservatives and chemical additives in food and pharmaceutical products.

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