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Article

Shading of Medical Plants Affects the Phytochemical Quality of Herbal Extracts

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Abstract: The manipulation of light intensity by shade nets can lead to exchanges in the phytochemical quality and antioxidants ofsome herbs. This study aimed to determine whether shading by pearl nets (50% shade index) could improve the bioactive compounds in several medicinal herbs such as thyme (*Thymus vulgaris L.*), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), lemon balm (*Melissa officinalis* L.), and peppermint (*Mentha piperita* L.), and their correspondingherbal extracts during a four-week maceration process in ethanol. Oregano and thyme provided the highest yield of total extractive substances (TES) from both shaded and non-shaded plants. Among all studied herbs, the highest level of antioxidants, expressed as total phenolic content (TPC) was found in extracts from shaded plants of lemon balm. Herbal extracts produced from non-shaded thyme and marjoram had higher flavonoid contents compared to herbal extracts from shaded plants. Accumulation of the investigated secondary metabolites depends more on the specificity of a plant species rather than light intensity. This study was an initial step in the production of medicinal plants with an increased quantity of antioxidant and other bioactive compounds during the maceration process in extracts, aimed to be used as natural alcoholic productwith added value.

Keywords: shading; mint plants; maceration; herbal extracts; natural antioxidant

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1. Introduction

The mint family (Lamiaceae-Labiatae) includes important plants such as peppermint, thyme, lemon balm, marjoram, and oregano. These plants are used for culinary, medicinal [1], cosmetic, and ornamental purposes in the household or in variousindustries [2]. The medicinal properties of herbs are used in the prevention of various diseases due to the presence of different compounds characterized by high antioxidant capacity [3]. Medicinal plants contain essential oils and phytochemicals as natural remedies that could fulfill people's expectations in helping to cure their diseases [4]. The production of the medicinal plants with the possibility of higher accumulation of bioactive compounds depends on many environmental factors such as light and temperature, the origin of variety, the period of vegetation [5], but also on several additional parameters including harvest period, storage conditions, and extraction technique [6].

Alcoholic beverages comprise a large group of drinks (most often herb liqueurs produced by maceration of various herbs and spices that have a long tradition of use). They are commonly consumed before or after a meal due to their effect to stimulate appetite and improve digestion as a result of their potential functional properties [7]. These drinks are widely used in gastronomy and represent part of the tradition in Serbia too.

Flavonoids are the main phenolic compounds identified in herbal liqueurs consumed in Europe. Among flavonoids, flavanones, flavones, flavonols, and isoflavones are the most abundant subclasses. The identification of most of the phenolic compounds in these beverages support the possibility that the biological effects attributed to the herbal liqueurs are mediated by the antioxidant activity of the phenolic compounds [7].

Alcoholic drinks based on medicinal herbs in small quantities have a positive effect on health [8]. Alcoholic drinks made by maceration or distillation of several aromatic plants are generally a good source of valuable nutrients in herb beverages, primarily due to the presence of polyphenols characterized by antioxidant activity and are still widely used as alcoholic liqueurs [7]. Volatile compounds of medicinal and aromatic plants mainly provide the aroma and taste of strong alcoholic drinks from the corresponding alcoholic macerates. The creation of aromatized wines (vermouth, bermet [9] or some local wine (varieties Prokupac) from medicinal plants [10]) gained additional value with significantly higher photochemical compounds.

The presence of bioactive ingredients from a large number of plant species affects the nutritional composition of alcoholic beverages, and their impact depends on both the solubility and the duration of extraction [11]. For herbal alcoholic beverages, high quality fresh medical plants are required for the maceration process, which will affect the beverage quality with increases of phenolic content and added value for consumers [12,13]. Raw plant material has to be produced in optimal climatic conditions with adequate sunshine. The content of phytochemical compounds in plants depends on many parameters including growing and storage conditions. Light intensity has an important effects on plant growth, development, leaf size, crop yield, and the content of phytochemical compounds [14]. On other side, high light intensity can produce undesirable effects on plants. An economical alternative is the net house or shade net, which protects horticultural plants (leaf and fruit) from strong direct sun radiation, obtaining more vigorous plants, with higher yields and fruits of better quality than in the open field [15]. Light modification can affect a wide range of physiological responses, while the efficiency of light-dependent processes has a large impact on tomato [16], pepper [17–19] lettuce [20], and fresh herbs [21] production. Shade nets are recommended for growing marjoram and oregano sincethey respond well to shading [22]. Shading can also affect the biochemical constituents in plants; thus, the highest content of total phenols, flavonoids, and antioxidant activity was found in basil covered by red net [23]. Based on the obtained results, it could be concluded that basil grown in the shading conditions has an impact on the aroma composition and antioxidant activity of aqueous extracts, regardless of the extraction technique used [24]. Reliable studies about the changes of active ingredients in medicinal plants during light manipulation are still missing.

The present paper aimed to investigate the five most commonly used medicinal plants grown in different light intensities and the impact of the maceration process in production of the alcoholic extract with increased bioactive compounds.

2. Material and Method

2.1. Plant Material and Growing Conditions

The experiment was conducted in 2019 in an experimental garden at the village Moravac in South Serbia (21°42′ E, 43°30′ N, altitude 159 m). The medical plants from the mint family (Lamiaceae—Labiatae) such as thyme (*Thymus vulgaris L.*), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), lemon balm (*Melissa officinalis* L.), and peppermint (*Mentha piperita* L.) wereused to determine whether shading by pearl nets could improve the bioactive compounds in these herbs, which are used for the preparation of liqueurs during the maceration process.

The seeds were sown in the field with the task to achievean optimal plant density of 50 plants/m^2. Treatment combinations were replicated threetimes with one shading treatment (pearl nets) and non-shaded control treatment in a split-plot design. During vegetation, the plants were covered (net house) with nets from an Israeli company (Polysack

Plastics Industries) with a shade index of 50%. In the second year, after establishingthemedicinal plants production, medicinal plants were harvested for essential oils extraction (main harvest at middle of August). Plants from an additional, second harvest (beginning of October) were used in preparing hydroalcoholic extracts of mint plants.

2.2. Light Interception by Nets

To measure the intensity of light (PAR-photosynthetically active radiation -µmol m^{-2} s^{-1}) a probe was used intervals the Sun Scan probe SS1-UM-1.05 (Delta-T Devices Ltd., Cambridge, UK).

The Solarimeter-SL 100 (KIMO, France) was used to measure the insolation of the sun during the day at specified time intervals.

After measuring the light intensity parameters every second day during one month, the average values for the month of July were calculated.

2.3. Reagents and Chemicals

Folin–Ciocalteu's reagent, gallic acid, rutin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, aluminium (III) chloride hexahydrate, potassium acetate (Sigma Chemical Company, St. Louis, MO, USA). All other chemicals were of analytical reagent grade.

2.4. Plant Material and Extraction

Chopped and homogenized plant materialharvested a few days before maceration, 5 g above-ground part of each species was immersed in 50 mL (40% v/v ethanol solution; ration $1/10$ m/v) at room temperature (25 °C). From various literature sources [11], we decided that the ethanol content of the maceration process should be -40% v/v ethyl alcohol for a duration of fourweeks. After extraction, the plant material was separated from the liquid extract by vacuum filtration on a Buchner funnel.The liquid extracts were kept in the refrigerator at +4 °C. The yield of total extractive substances (TES, dry extract) was determined by drying of liquid extract (2 mL) in an oven at 105 °C to constant mass. TES was calculated based on dry residue (TES yield was expressed as g of TES per 100 g of fresh plant material (g TES/100 g f.p.m.).

2.5. Determination of Content of Total Phenols in Extracts

UV-VIS spectrophotometry was used to determine the content of total phenols and flavonoids and the antioxidant activity of ethanol extracts. All measurements were performed on a Cole Parmer Spectrophotometer.

The content of total phenols was determined in herb extracts by the Folin-Ciocalteu procedure [25–29] using gallic acid as a standard (the concentration range 0.00625 to 0.2 mg/mL). The results are expressed as gallic acid equivalents per gram of dry extract (mg GAE g^{-1} d.e.).

2.6. Determination of Content of Total Flavonoids in Extracts

The content of total flavonoids in mint plant extracts was determined by the spectrophotometric method with aluminum chloride [28–30] with certain modifications.

2.7. Antioxidant Activity of Extracts—DPPH Test

The ability of the ethanolic herb extracts to scavenge free DPPH (1.1-diphenyl-2-picrylhydrasil) radical was determined using the DPPH assay. A series of solutions of various concentrations of ethanolic herb extracts were made. An ethanolic solution of DPPH radical (1 mL, 300 µmol solution (3×10^{-4} mol/L)) was added to 2.5 mL of extracts of different concentrations. The reaction mixture (sample) was incubated at room temperature in the dark for 20 min and the absorbance measured at 517 nm.

Free radical scavenging capacity is calculated according to Stanojević et al., [28].

$$\text{DPPH radical scavenging capacity}(\%) = 100 - [(A_S - A_B) \times \frac{100}{A_C}]$$

where A_B is absorbance of non-treated ethanolic solution of extract, without DPPH radical added (2.5 mL of extract diluted with 1 mL of ethanol—"blank").

Ac is ethanolic solution of DPPH radical (2.5 mL of DPPH radical diluted with 1mL of ethanol—"control").

The absorbance was annulated against the ethanol as a blank.

A_S is the absorbance of the sample, A_B is the absorbance of the blank, and A_C is the absorbance of the control at 517 nm.

The DPPH test is based on the reduction reaction of the intensely purple-colored DPPH radical to its corresponding hydrazine, which can be spectrophotometrically followed by the absorbance decreasing at 517 nm.

2.8. Statistical Analysis

ANOVA was used to analyse the significance (TIBCO Software Inc. Palo Alto, CA, USA. 2020, version 14.0.015.). Duncan's multiple range test used for analysis of significance (with level of 0.01) of differences between means.

3. Results

3.1. Growing Conditions

Climatic conditions characterizing the growing season, collected from the closest meteorological data recording unit (Kruševac, 21°20′ E, 43°34′ N), (http://www.hidmet.gov.rs/latin/meteorologija/klimatologija_godisnjaci (accessed on 15 July 2021)) indicate that the growing season in which the experiment was conducted was characterized by close to average or above average values for average daily temperatures and under average total sum of insolation in comparison to multiannual mean values.The climatic conditions of southern Serbia are very favorable for the production of medicinal plants throughout the growing season (Table 1).

Table 1. Climatic conditions characterizing production season in which investigations were conducted.

Month	Number of Summer Days (over 25 °C in June; over 30 °C for July and August)	Average Temperature Difference from Multiannual Average (°C)	Sum of Insolation Difference from Multiannual Average (h)
June	27	0.8	−51.4
July	10	−0.2	−72.5
August	28	2.0	−1.9

Source: Republic Hydrometeorogical Service of Serbia (http://www.hidmet.gov.rs/ (accessed on 15 July 2021)).

The reduction of PAR depends on the period during the day. Thus, a greater reduction in PAR was observed in the late afternoon (53.9%) compared to morning (31.2%), and noon. Results from Table 2 show that from the open field during a average sunny day in July, the solar radiation reached 874 W m^{-2}. The solar radiation inlate afternoon was reduced by more than 50% under pearl nets compared tononshading conditions. Light parameters, temperature and relative humidity were less exposed to changes under the shading nets (Table 2).

Table 2. Influence of shading on the growing environmentparameters (average day in July).

Time (h)	PAR * (μmol m^{-2} s^{-1})		Solar Radiation (W m^{-2})		Temperature °C		Relative Humidity %	
	Unshading	Reduction by Shading %	Unshading	Shading	Unshading	Reduction by Shading %	Unshading	Reduction by Shading %
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	−3.4	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. Content of Total Extactive Substances (TES)

In the present work, the extraction of extractive substances from the aerial parts of mint plants was performed by classical solvent extractions (maceration) using an aqueous ethanol solution (40% vol.) as the extracting solvent was studied. Operating extraction conditions by maceration of total extractive substances (TES) were: 40% v/v ethanol and solvomodule 1/10 m/v. Classical maceration is a mechanism via normal diffusion through cell walls. This process requires a significantly longer extraction time. It can be used for the extraction of thermolabile components.

In this exploration, the highest yield of TES was observed from non-shaded oregano (12.72 g/100 g f.m.) and thyme (10.26 g/100 g f.m.) plants. The light intensity also positively influenced the TES yield of marjoram and peppermintbut had no influence on the TES yield of lemon balm (Table 3).

Table 3. The yield of total extractive substances(TES) in alcoholic extract from medical plants.

Plant Species	TES (g/100g f.p.m. *) Non Shading	Shading Pearl Nets (50%)
A—Thyme (*Thymus vulgaris* L.)	10.26 ^{b} ± 0.09	6.30 ^{g} ± 0.09
B—Marjoram (*Origanum majorana* L.)	7.06 ^{e} ± 0.08	5.33 ^{h} ± 0.07
C—Oregano (*Origanum vulgare* L.)	12.72 ^{a} ± 0.07	9.02 ^{c} ± 0.08
D—Lemon balm (*Melissa officinalis* L.)	6.48 ^{f} ± 0.12	6.48 ^{f} ± 0.05
E—Peppermint (*Mentha piperita* L.)	8.38 ^{d} ± 0.08	6.47 ^{f} ± 0.14
ANOVA		
Plant		$p < 0.01$
Shading		$p < 0.01$
Plantx shading		$p < 0.01$

Values followed by the same letter do not significantly differ between the treatments, at $p = 0.01$ according to Duncan's multiple range test. * TES (total extractive substances) g/100 f.p.m.—gram per 100 g of fresh plant material.

3.3. Total Phenolic Content

The total phenolic (TP) contents in the different plant extracts are presented in Table 4. The liqueur richest in phenolics after fourweeks of maceration process was the lemon balm extract from shading plants (170.58 mgGAE/g d.e). Peppermint, the herbal plant with the lowest total phenolic content, contained a slightly lower sum of phenols in ethanolic extracts from non-shaded (57.31 mg GAE/g d.e) and shaded (34.08 mg GAE/g d.e) plants (Table 4).

Table 4. Total phenols (TP) content of alcoholic extract from medical plants.

Plant Species	TP(mg GAE/g.d.e. *) Non Shading	Shading Pearl Nets (50%)
A—Thyme (*Thymus vulgaris* L.)	128.64 ^{b} ± 0.50	127.42 ^{b} ± 0.82
B—Marjoram (*Origanum majorana* L.)	124.27 ^{c} ± 0.68	96.55 ^{ef} ± 0.89
C—Oregano (*Origanum vulgare* L.)	95.81 ^{f} ± 0.41	121.22 ^{d} ± 0.57
D—Lemon balm (*Melissa officinalis* L.)	98.22 ^{e} ± 0.78	170.58 ^{a} ± 1.36
E—Peppermint (*Mentha piperita* L.)	57.31 ^{g} ± 1.31	34.08 ^{h} ± 1.59
ANOVA		
Plant		$p < 0.01$
Shading		$p < 0.01$
Plant × shading		$p < 0.01$

Values followed by the same letter do not significantly differ between the treatments, at $p = 0.01$ according to Duncan's multiple range test.* mg GAE/g d.e.: milligram of gallic acid equivalents/g dry extract.

3.4. Total Flavonoid Content

The results of total flavonoids (TF)content analysis indicated that net shading could alter these compositions in a species (genotype) dependent manner. Herbal alcoholic extract from non-shaded thyme, marjoram, and oreganoplants resulted in significantly higher flavonoid contents 27.51 mg/g, 15.92 mg/g and 12.15 mg/g, respectively, compared to shaded plants (Table 5).

Table 5. Total flavonoids (TF) content of alcoholic extract from medical plants.

Plant Species	**TF (mg RE/g.d.e. *)** **Non Shading**	 **Shading Pearl Nets (50%)**
A—Thyme (*Thymus vulgaris L.*)	$27.51^{a} \pm 0.31$	$6.67^{e} \pm 0.77$
B—Marjoram (*Origanum majorana* L.)	$15.92^{b} \pm 0.72$	$1.60^{g} \pm 0.55$
C—Oregano (*Origanum vulgare* L.)	$12.15^{c} \pm 0.12$	$10.43^{d} \pm 0.20$
D—Lemon balm (*Melissa officinalis* L.)	$3.17^{f} \pm 0.28$	$6.88^{e} \pm 0.28$
E—Peppermint (*Mentha piperita* L.)	$3.92^{f} \pm 0.22$	$3.87^{f} \pm 0.49$
ANOVA		
Plant		$p < 0.01$
Shading		$p < 0.01$
Plant × shading		$p < 0.01$

Values followed by the same letter do not significantly differ between the treatments, at $p = 0.01$ according to Duncan's multiple range test. * mg RE/g d.e.: milligram of routine equivalents/g dry extract.

Lemon balm alcoholic extract from shading plants contains twice the amount of flavonoids than unshaded plants.

3.5. Total Antioxidant Capacity (TAC)

Phenolics and flavonoids are the main part of total antioxidant capacyty (TAC) in herbs.Herbal extracts of lemon balm and oregano plants shaded by pearl nets have better antioxidant properties (lower EC_{50} DPPH values) compared to unshaded herbs from open fields.

Herbal ectract from lemon balm plants covered by pearl nets had the highest antioxidant capacity ($EC_{50} = 0.024$ mg·mL^{-1}). The lowest antioxidant capacity was measured in extract from shaded peppermint plants ($EC_{50} = 0.303$ mg·mL^{-1}), Table 6.

Table 6. Total antioxidant capacity(TAC) of alcoholic extract from medical plants.

Plant Species	**EC_{50} Values (mg·mL^{-1} *)** **Non Shading**	 **Shading Pearl Nets (50%)**
A—Thyme (*Thymus vulgaris L.*)	$0.027^{a} \pm 0.0001$	$0.055^{d} \pm 0.0006$
B—Marjoram (*Origanum majorana* L.)	$0.061^{e} \pm 0.0003$	$0.089^{f} \pm 0.0005$
C—Oregano (*Origanum vulgare* L.)	$0.186^{g} \pm 0.0021$	$0.041^{c} \pm 0.0002$
D—Lemon balm (*Melissa officinalis* L.)	$0.033^{b} \pm 0.0003$	$0.024^{a} \pm 0.0002$
E—Peppermint (*Mentha piperita* L.)	$0.243^{h} \pm 0.0040$	$0.303^{i} \pm 0.0040$
ANOVA		
Plant		$p < 0.01$
Shading		$p < 0.01$
Plant × shading		$p < 0.01$

Values followed by the same letter do not significantly differ between the treatments, at $p = 0.01$ according to Duncan's multiple range test. ^{c} EC_{50}, mg·mL^{-1}. * = concentration of extract necessary to neutralize 50% of initial concentration of DPPH radicals.

Figure 1 shows the percentage of DPPH radical neutralization with increasing extract concentration without and with incubation (20 min), while Table 6 presents the EC_{50} values for all extracts.

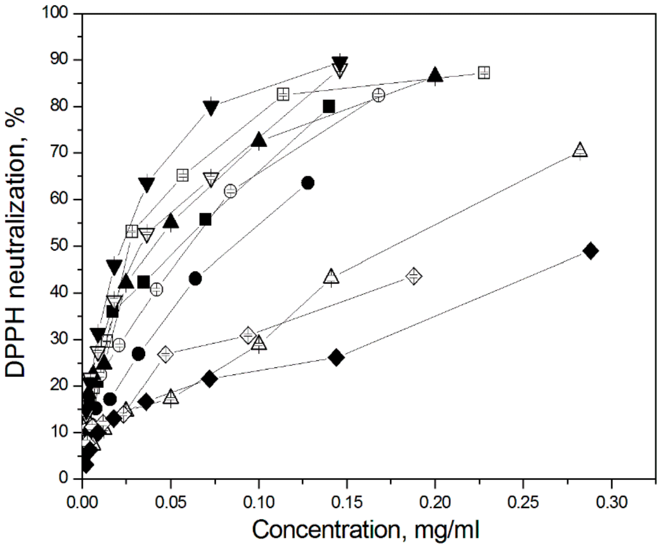

Figure 1. Antioxidative activity of thyme (*Thymus vulgaries*) alcohol extract (A1-□,) unshaded plants,(A2-■) shaded plants; marjoram (*Origanum majorana* L.) alcohol extract (B1-○) unshaded plants, (B2-•) shaded plants; origanum (*Origanum vulgare* L.) alcohol extract (C1-Δ) nonshaded plants(C2-▲), shaded plants; lemon balm (*Melissa officinalis* L.) alcohol extract (D1-∇) unshaded plants, (D2-▼) shaded plants; peppermint (*Mentha piperita*) alcohol extract (E1-◊) unshaded plants, (E2-♦) shaded plants.

Correlations were not observed between total flavonoids (TF) and EC_{50} value, as demonstrated by the low values of determination coefficients (R^2 = 0.121). One of the reasons for the lack of correlation between flavonoids and EC_{50} value can be attributed to secondary metabolites that could not be detected in this study, such as antioxidant vitamins (vit C and E), carotenoids, quercetin, tannins, etc. [29]. Higher content of total phenol compounds resulted in higher antioxidant activity indicating a higher correlation for TP content (R^2 = 0.774) (Figure 2).

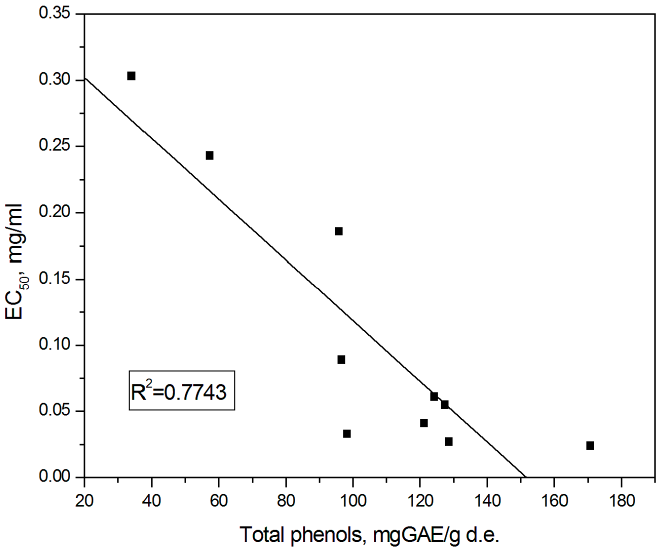

Figure 2. Correlation between content of total phenols and EC_{50} value in alcoholic extract.

4. Discussion

An adequate amount of plant material, appropriate alcohol concentration, and sufficient maceration time are the most important parameters for successful extraction into a liqueur [13].Compared with the large number of published references about the analytical and sensory characteristics of young distillates, herbal liqueurs have had few studies reported [12,13]. References reporting on the technology of production of fresh material (medicinal herbs) for the maceration process are especially deficient. Unlike the research of Rodriguez et al., [13] which emphasizes volatile components and parameters of taste and smell in local Orujo herbal drinks, we have in our research emphasized the raw material from which natural alcoholic beverages are made, as well as their health and nutritional value through the presence of bioactive compounds with antioxidant capacity.

Our results indicate that extracts obtained at room temperature (25 °C) by maceration for fourweeks exhibitgood medicinal properties. The growth, development, and biologically active content of medicinal plants may be significantly influenced by genetic (internal) and environmental (external) factors. Optimal environmental conditions are needed to enhance the intensity of growth and achieving higher biomass, but also to enhance the levels of biogenic and medicinal components in less stressful conditions under shading nets than in the open field [31].

A low-cost protected agricultural environment such asthe nethouse is an alternative and a supplement to field production [31]. Studies on the bioactive compounds under restriction of light intensity have shown that each species responds to shade nets differently [32]. Due to environmental influences (primarily light) on the synthesis of secondary metabolites, detailed investigations are necessary to understand how the quality of medical plants can be improved by cultivation methods [32].

Shade conditions can induce stress in the plants. As a result, an increased flavonoid concentration is observed, which explains the increase in flavonoids in oregano, marjoram, and coriander under pearl nets [22]. During the summer season, the black nets have been shown to improve the antioxidant activity in coriander, oregano, and marjoram [22].

The increase of phytonutrients compounds, in medical plants may be caused by multiple responses to light quantity and quality. Light modification under the photoselective nets retaining or increasing antioxidant activity after storage [19,20]. Antioxidant activity was higher in fresh herbs grown under the yellow and red nets after storage. Also, no significant differences were noted in leaves from the plants grown under the different nets with respect to antioxidant content during postharvest process [22].

Duletić-Laušević et al. [33] have shown that the highest content of phenolics is found in aqueous extracts of the Serbian *O. majorana* (122.71 mg GAE/g dry extract), and in ethanolic extracts of the Egyptian plant (139.98 mg GAE/g dry extract). Similarly, Benchikha et al. [34] found high phenolic content in the ethanolic extract of *O. majorana* from Algeria (266.86 mg GAE/g), while the extract was poor in flavonoids.

Our study found the shaded plants (lemon balm and oregano) to be rich in phenolic compounds, which could be useful for the herb alcoholic extract production technique. Other plants (thyme, marjoram and peppermint) were found to produce higher levels of phytochemicals in non-shaded conditions.

Environmental conditions, production methods, time, and method of harvesting affect the level of antioxidants found in medicinal plants.Total phenol content was significantly higher in shaded lemon balm and oregano plants covered by pearl nets compared to unshaded plants from the open field. Flavonoids in natural herbal extracts from unshaded plants (thyme, marjoram, and peppermint) have more expressed antioxidant properties compared to the flavonoids found in alcoholic extract from herbs covered by pearl shade nets. Furthermore, this investigation suggests that the use of shade nets during production of these plants improves the phytochemical quality.

Changes in light intensity through the utilization of shade nets can change the synthesis of phenolic compounds in plants. However, different plants respond differently to shade levels, which alter the production of TP and TF. Previous studies have shown that change in light intensity can modify the production and accumulation of TF and TP in herbs [23].

Light intensity manipulation by shade nets has proven to be avery successful management method for the quality maintenance of medical plants. However, what may constitute a successful management strategy in one species and/or in a particular country may have a severely limited commercial potential for quality management in a different country and/or ina different plant species. The complexity and variability of natural radiation on the one hand and the multiple reactions of plant response on the other hand make it difficult to predict how a given manipulation of natural solar radiation will affect the production of bioactive compounds.

According to Bergquist et al. [35] shade conditions promote or induce some stress and, as a result, an increased flavonoid concentration is observed (which explains the increase in flavonoids (quercetin) in oregano, marjoram, and coriander under pearl nets after harvest) [22]. Although there is a slight decline in phytochemical content during postharvest, a higher level of accumulation of phytochemicals at harvest enables the herbs to retain the phytochemical quality during postharvest [22].

Our previous study confirms that increased levels of β-carotene provide photoprotection by dissipation of the excess energy of the excited chlorophyll, thereby removing the reactive oxygen species [18]. Furthermore, this investigation suggests that the use of shade nets during production of these plants improves phytochemical quality.The observed lower flavonoid concentrations in all herbs under shaded conditions could be associated with the lower antioxidant scavenging activity. Generally, the phenol content has a stronger effect on antioxidant activity than the flavonoid content [33]. In recent years, there has been a growing interest in the extraction of phytonutrients from these plants since they are not expensive, are of natural origin, and represent an adequate replacement for synthetic antioxidants [36].

The Maceration and Soxhlet extraction are commonly used methods for small manufacturing enterprise. The aforementioned species are among the most frequently investigated species of the family *Lamiaceae* besides thyme, peppermint, lemon balm, and oregano. Moreover, their antioxidant activity has been demonstrated in numerous studies [21,32,37–39].

In this study we demonstrated an increase of antioxidant scavenging activity in oregano and lemon balm plants grown under pearl nets. The antioxidant activity decreased in the following order: shading lemon balm (0.024) > nonshading thyme (0.027) > nonshading lemon balm (0.033) > shading oregano (0.041) > shading thyme (0.055) > nonshading marjoram (0.061) > shading marjoram (0.089) > nonshading oregano (0.186) > nonshading peppermint (0.243) > shading peppemint (0.303). Liqueurs produced at home by maceration in small quantities are rich sources of antioxidants with high biological effects (rich α-glucosidase, low/moderate AChE, and low tyrosinase inhibition) [40]. More comprehensive studies are needed to determine adequate amounts of herbs that would be sufficient in the production of homemade liqueurs with concentrations of compounds without adverse effects on human health [40].

It can be intuited that due to the analysis in essential oils of the mentioned plants in previous works (such as *terpinen-4-ol* in marjoram; *thymol* in thyme; *geranial (E-Citral)* in lemon balm; *caryophyllene oxide* in oregano; and *piperitenone oxide* in mint) the results obtained in the current macerations or plant extracts may suggest that certain terpenes present in these plants may be those, among others, that provide bioactive properties [21,32]. To reduce the sweet note in herbal liqueurs based on mint plants, some herbal species such as hops (*Humulus lupulus* L.) can be added to improve specificity and achieve a slightly bitter taste [41].

Natural antioxidants and medicinal plants are increasingly used in the food and pharmaceutical industries [21,32,42]. Since oxidative stress has been involved in the development of different human medical problems and the occurrence of various side effects of synthetic antioxidants, researchers have focused on the beneficial health effects of bioactive compounds from herbal drinks [43], including those isolated from mint family plants.

In strengthening the immune system in protection against the current COVID-19 pandemic, it is also possible to strengthen the immune system through the use of herbal extracts as a source of additional natural plant secondary metabolites that are bioactive [44].

5. Conclusions

Different plant species respond differently to shading in terms of TES (total extractive substances). All studied plants reacted positively to TES content by shading except for lemon balm. The shading of oregano and lemon balm increased the phenol content. Thyme is a light-neutral plant, so shading does not affect the phenol content of the extracts. Similarly, shading only in lemon balm increased flavonoids content but did not affect the flavonoids content of the all others plants extracts. Among the five plant species investigated in this work, thyme and lemon balm extracts were characterized by the highest level of antioxidant activity. Lemon balm tolerates shading well, so it is recommended to grow under shaded nets. In addition to containing significant amounts of phytonutrients, all mint species also have a strong smell and taste that add aroma value to the final herbal extracts.

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