Aroma Profile and Antioxidant Activity of Sweet Basil Aqueous Extracts Affect by Light Modification

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Received 21 January 2022; Received in revised form 11 September 2022; Accepted 12 September 2022

Abstract: This study examines the action of light modifications by different shade nets (pearl, red and blue color nets with a shade index of 50%) in relation to unshaded plants from the open field on the relative effects on changing basil aroma profile and antioxidant activity. The most abundant components were linalool, 1,8-cineole and myrcene. Aqueous extracts were isolated from basil leaves using maceration and ultrasound-assisted extraction. Yield of total extractive matters depends on both the extraction technique applied and the growing conditions used. The highest yield of total extractive matter (30.50 g/100g) was obtained by maceration from open field-grown basil. The highest content of total phenols (106.52 mg GAE/g), flavonoids (19.74 mg RE/g) and antioxidant activity (EC50 - 7 µg/mL) was found under a red net. The isolated water extracts are a source of natural antioxidants with potential application in the food and pharmaceutical industries.

Keywords: Ocimum basilicum L., extraction methods, light intensity, volatile, quality components.

Introduction
Sweet basil (Ocimum basilicum L.) is an important culinary and medicinal plant, but at the same time, important essential oil crop in the world. Serbian sweet basil production have been introduced with the new culinary uses as an addition to food preparation and may be potential sources of new aroma compounds 1,2 to add a distinctive flavor to foods such as tomato pastes, ketchup, sauce, and soups 3. The perfume, pharmacy, and food industries use aromatic essential oils, extracted from the basil.

Cultivar, but also the agronomical practices 4 and environmental conditions 1 as well as harvesting time, isolation techniques, duration, and storage conditions 5,6 affect the basil composition of sensory characteristics. Shading plants by photo-selective shade nets synthesized more EOs than plants exposed to full sun light 2. Light play an important role in the synthesis of essential oils and have been shown to affect volatile compounds in basil 7. Basil grown under plastic tunnels obtained highest value of antioxidant activities than plants from the open field 8. Essential oil from sweet basil (cv. ‘Genovese’) grown under blue shade nets are characterized by the highest eugenol content and highest antioxidant activity 1.

Essential oils composition determines the specific aroma and the flavor of basil. The
European type of sweet basil, in relation to others, considered to have the highest quality aroma, containing linalool and methyl chavicol as the major constituents. After harvest, during the drying process, through oxidation, evaporation, and various physicochemical changes of aromatic volatiles, aroma intensity and quality are lost. Research from Klimankova et al. confirms that fresh basil leaves are characterized by a higher amount of extracted volatile compounds than dried or frozen leaves. The basil extracts contained appreciable levels of total phenolic contents and antioxidants, with high correlation between these parameters. Being rich in compounds with high antioxidant activity, basil extract can be used to replace synthetic antioxidants used in vegetable oils.

The present paper aimed to investigate the effect of light modification by color shade nets on the aroma profile (volatile compounds) in sweet basil leaves and antioxidant activity of the aqueous extracts obtained by different extraction methods.

**Material and methods**

**Plant material and growing conditions**
The experiment was conducted in 2018-2019 in an experimental garden at the village Moravac in South Serbia (21°42′ E, 43°30′ N, altitude 159 m). Sweet basil (*Ocimum basilicum* cv. ‘Genovese’) seed is distributed by Suba Seeds Company SPA Srl. Longiano, Italia. Sowing of basil was done on May 20th and after germination the crop was thinned so that a plant density of the 50 plants/m² was achieved. Basil was used to determine whether shading conditions (plants cover by pearl, red and blue colour nets with a shade index of 50%, – (Polysack, Israel), could improve aroma profile and antioxidant activity in plants. Combinations of plant shading treatments and un-netted control treatment were replicated 3 times in a split-plot design. The shade nets were mounted on a structure placed about 2.0 m above the plants (net house) at middle of June until end of August. The basil plants were harvested at the beginning of August, at the commercial maturity stage (eight to ten weeks after germinating). After harvest leaves were selected and dried without the presence of light and ventilation at room temperature (about 25-30°C) as air-dry herbage for analysis.

**Light interception by nets**
For PAR-photosynthetically active radiation (μmol m⁻² s⁻¹) under different nets and open field condition (control) was used the Sun Scan probe SS1-UM-1.05 (Delta-T Devices Ltd., UK). Solar radiation was measured at different intervals during the days with Solarimeter-SL 100 (KIMO, France).

**Extraction of volatile compounds from basil leaves**
SPME manual holder and fused silica fiber coated with Carboxen®/ Polydimethylsiloxane. (the length of CAR/PDMS, fiber used was 1 cm, 75 μm thickness; Supelco, Bellefonte, PA, USA) were used for the volatile compounds’ extraction from chopped dried basil leaves by HS-SPME. The fiber was preconditioned according to the manufacture’s instruction (1 h at 300°C). One gram of grounded dry plant material was placed in a glass bottle (10 mL), closed with a rubber septum, and sealed with parafilm. The fiber was inserted into the headspace of the sample and the volatiles were extracted for 20 minutes at 23°C. The desorption of the fiber was performed in split/splitless inlet set at 250°C with 20:1 split mode for 10 minutes and further analyzed by GC/MS and GC/FID.

**Gas chromatography/mass spectrometry (GC/MS) analysis and gas chromatography-flame ionization detection (GC/FID) of volatile compounds in dried basil leaves**
The Agilent Technologies 7890B gas chromatograph, coupled with a 5977A mass detector was used for GC/MS analysis. Separation of the components was achieved on HP-5MS (5% diphenyl- and 95% dimethylpolysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Santa Clara, USA) silica capillary column, using helium as the carrier gas at a constant flow rate of 1 mL min⁻¹. The oven program was as follows: held isothermally at 40°C for 2 min, increased to 250°C at the rate of 7°C min⁻¹, and finally held at 250°C for 2 min. The analysis lasted 34 min. The
ion source, quadruple mass analyzer, and MSD transfer line temperatures were set as described by Vladimirov et al.\textsuperscript{13}. The ionization voltage was 70 eV and mass detection was done in the Scan mode, in \( m/z \) range from 25 to 550.

Semi-quantitative results were obtained on the gas chromatograph used for the GC/MS analysis and equipped with the flame-ionization detector (FID). The GC/FID conditions and data processing were the same as given by Vladimirov et al.\textsuperscript{13}. The volatile compounds were identified by comparing their retention index and their mass spectra on the HP-5MS column with those of commercial spectra database.\textsuperscript{25}

### Maceration process

The homogenized plant material (3 g of ground basil leaves) was extracted by maceration with water (60 mL). Maceration process was performed with slight modifications by a previously described procedure.\textsuperscript{15} Maceration was performed for 120 minutes with hydromodule (plant material/water ratio) 1/20 m/v at 25°C. The resulting liquid extract was separated from the plant material by vacuum filtration on a Büchner funnel and stored in the refrigerator at +4°C until analysis. The yield of total extractive matter (TEM, dry extract) was determined by drying of liquid extract (2 mL) in an oven at 105°C to constant mass. TEM was calculated based on dry residue (TEM yield was expressed as g of TEM per 100 g of plant material, gTEM/100 g p.m.).

### Ultrasound-assisted aqueous extraction (USAE)

The homogenized plant material (3 g of ground basil leaves) was extracted with water (hydromodule, 1/20 m/v) in the presence of low-frequency ultrasound. Ultrasound-assisted extraction was performed with slight modifications by a previously described procedure.\textsuperscript{15} Extraction was performed for 30 minutes using an ultrasonic bath (Sonic, Niš, Serbia; internal dimensions: 30 \( \times \) 15 \( \times \) 20 cm; total nominal power: 3 \( \times \) 50 W; and frequency: 40 kHz) at 25°C. The resulting extract was treated by the same procedure as described in section Maceration.

### Determination of total flavonoids content in aqueous basil leaves extracts

The total flavonoids content, in the aqueous extracts of basil leaves, was determined according to the aluminum chloride colorimetric method with slight modifications by a previously described procedure.\textsuperscript{17}

### Antioxidant activity (DPPH assay) of aqueous basil leaves extracts

The antioxidant ability of the essential oils has also been evaluated using DPPH assay. The DPPH assay was carried out according to Stanojević et al.\textsuperscript{18}

### 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.

### Results and discussion

#### Chemical composition of volatile compounds in basil leaves

Environmental conditions such as temperature, relative humidity or cultivation method may reflect changes in physiological and morphological parameters and production of secondary metabolites. Genetics and breeding are the main efforts by scientist in creating perfect aroma and flavor. Also, the influence of environment conditions (light intensity, temperature, and photoperiod) is no less importance in modulation of basil aromatic compounds. Net houses have the potential to reduce the risks in basil production from the open field (high temperatures, hail, wind, pathogens, pests, birds) and create a microclimate which positively affects productivity and quality. Photosynthetically active radiation (PAR) was significantly lower (50%) under pearl nets compared to the control (open field condition).

Results from Table 1 show that the maximum solar radiation in the open field, during a average sunny day in July reached 874 Wm\textsuperscript{2}. The solar radiation at noon was significantly reduced (47.5%) under pearl nets, compared to control.

Light manipulation is commonly applied using...
coloured shade nets or different photoselective plastic films. The use of photoselective cover materials (i.e. nets or films) could be a suitable agro-technological alternative and promising tool for improvement of basil secondary metabolites and their activities.

Different colors of shade nets reflect or absorb different wavelengths of light. Blue shade nets absorb ultraviolet, red, and far-red light spectral bands, enriching the blue light spectra, while red shade nets absorb ultraviolet, blue, and green light spectral bands, enriching red and far-red light spectra. Black shade nets do not modify the spectral composition of light received by plants.

Spectral bands received by plants can affect crop quality, yield, and biosynthesis of metabolites. Light quality also affects complicated responses in plant physiology, morphology, and gene expression by initiating the signaling of phytochromes, phototropins, and cryptochromes in photoreceptors to regulate plant growth, morphological processes, chloroplast accumulation, and secondary metabolite biosynthesis such as phenolic acids, carotenoids, flavonoids, anthocyanins, and α-tocopherol.

Aroma profile highly affected by seasonal effects and depends on environmental conditions during the growing period. Basil demonstrated significant differences in volatile accumulation based on the light intensity. Many studies have examined blue combined with red as light source for growth of crops in controlled conditions but there is a lack of research on the effect of light modification by color shade nets on the aroma profile of basil when grown in an open field.

Micro-extraction from the gaseous phase was used to extract the volatile components from the chopped dried basil leaves. The chemical composition of the volatile components is shown in Tables S1-S4.

An extremely large number of components (49 to 71) have been identified in basil leaves samples, which is a major advantage of the HS-SPME/GC-MS method. The obtained results on the composition of volatile components of dry sweet basil leaves can be veryimportant when later choosing the method of distillation of essential oil from basil leaves. Based on previous research and unpublished results, the chemical composition of basil essential oil from the same samples differs from the composition of volatile components identified by the HS-SPME/GC-MS technique. Hydro-distillation, during heating, most likely causes the loss of individual components, so that the content of these components is lower in the oil itself concerning their content in the plant material.

In these study, the linalool, 1,8-cineole, myrcene, and α-trans-bergamotene are constituents responsible for the distinct aroma of basil plants. Based on the results from Tables 2-5, it can be concluded that the light modification of basil affect the chemical composition of the volatile components of basil leaves. The different quantitative and qualitative composition of volatile components was determined in the

| Time (h) | PAR* (μmol m⁻² s⁻¹) | Solar radiation (W m⁻²) | Temperature °C | Relative Humidity % |
|----------|---------------------|-------------------------|----------------|---------------------|
|          | Non-Shading Shading Reduction % | Non-Shading Shading Reduction % | Non-Shading Shading Reduction % | Non-Shading Shading Reduction % |
| 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
basil leaves. Thus, 99.9% of the total number of components in basil leaves from the open field was identified. At the same time in basil leaves under the pearl, red and blue net 95.2; 98.5; and 99.9% of the total composition was identified, respectively. The most common components were oxygenated monoterpenes (67.5-72.3%), followed by monoterpene hydrocarbons (15.8-26.5%), sesquiterpene hydrocarbons (3.4-6.3%), and aromatic compounds (0.7-1.0%).

The highest content of oxygenated monoterpenes was found in all samples. Similar findings were found for the Iranian and Turkish basil samples by Sonmezdag et al.25 Higher content of these components was found in non-shading basil plants and under a pearl net (72.7 and 72.3%, respectively) in comparison to basil plants grown under blue and red nets (68.2 and 67.5%, respectively). The highest content of monoterpene hydrocarbons was determined in the sample grown under the blue nets, while the highest content of sesquiterpene hydrocarbons was determined in the plants from the open field (Tables S1-S4).

Based on the obtained results, it could be seen that the most represented components in all samples are: linalool, 1,8-cineole, and myrcene. Linalool is the dominant component in all samples, being most abundant in the basil leaves grown under pearl nets. The second most common component was 1,8 cineole. The highest content of this component was found in basil leaves grown under blue nets. The content of myrcene was approximately the same in the basil leaves grown under the red and blue nets (15.7 and 15.3%, respectively), while it was the lowest in the basil sample grown under the pearl nets (Table 2).

The content of the main components is higher in basil grown under shading than in non-shading (open field) conditions. Light modification under photo-selected nets contributes to the enhanced synthesis of the considered groups of compounds. At the same time, the shadow effect and slightly lower temperature below the nets may also be responsible for the more efficient synthesis of individual groups of molecules. Depending on the purpose of the basil leaves, it is possible to determine the light modification under which this plant should be grown, that is, to choose the type of nets under which the target component is most synthesized. Thus, if it is necessary to isolate essential oil from basil leaves with better antioxidant activity, basil should be grown under red nets. Under the given conditions, linalool is synthesized the most, which is largely responsible for the antioxidant activity of the oil 26,27.

### The yield of total extractive matter isolated from basil leaves
The yield of total extractive matter (TEM) expressed in grams of dry extract per 100 g of plant material (g/100g) from basil leaves grown under shading by nets (A-pearl, B-red, and C-blue) and non-shading - open field (D) during the process of maceration and ultrasound-assisted extraction was presented in Table 3. Maceration and ultrasound-assisted extraction (hydromodule 1/20 m/v, extraction time 120 min for maceration and 30 min for ultrasound-assisted extraction) were performed at room temperature (25°C).

The applied extraction technique has an impact

### Table 2. Contents of the most common volatile components of basil leaves depending on the light modification

| Method of basil production | Myrcene | 1,8-Cineol | Linalool |
|---------------------------|---------|------------|---------|
| A (pearl nets)            | 9.0b    | 19.6b      | 46.1a   |
| B (red nets)              | 15.7a   | 22.0b      | 41.6a   |
| C (blue nets)             | 15.3a   | 29.2a      | 34.8b   |
| D (Control: non-shading – open field) | 11.0b | 21.9b | 44.2a |

Values followed by the same letter do not significantly differ between the treatments, at P = 0.05 according to Duncan’s multiple range test
on the yield of TEM from basil leaves, as well as the shading conditions of basil (Table 3). Ultrasound-assisted extraction achieves slightly lower values of TEM yield in comparison to maceration, but 4 times shorter extraction time under the same other operating conditions (hydromodule 1/20 m/v and temperature 25°C). The obtained results show that the effect of ultrasound has a positive effect on the rate of extraction of total extractives from basil leaves, which is in accordance with previous research 28,29,15.

The highest yield of TEM was achieved by maceration from basil leaves grown in an open field (30.5 g/100 g pm), while the lowest yield was achieved by ultrasound-assisted extraction from basil leaves grown under pearl photo-selective nets (27.87 g/100 g p.m.). TEM yield from basil leaves increases in the following order: basil grown in the open field (D, control) > basil grown under red nets (B) > under blue nets (C) > under pearl nets (A), regardless the extraction technique applied. The influence of basil growing conditions is higher on the TEM yield (for the same extraction technique) than the influence of the applied extraction technique (only 0.1-1% higher yields obtained by maceration than the yields obtained by ultrasound-assisted extraction for the same basil sample). The results of research with basil and the influence of shading on the extraction yield is very rare in the available literature 30 - which gives importance to the results obtained in this study.

### Table 3. Yield of total extractive matter (TEM), total phenols (TP), and total flavonoids (TF) obtained by maceration and ultrasonic extraction with water from basil leaves (hydromodule 1:20 m/v)

| Technique of extraction | A | B | C | D |
|-------------------------|---|---|---|---|
| **Yield of TEM, g/100 g plant material** | | | | |
| Maceration (120 min, 25°C) | 28.01±0.16a | 29.20±0.20a | 28.73±0.06a | 30.50±0.20a |
| Ultrasound assisted extraction (30 min, 25°C) | 27.87±0.15a | 28.90±0.22a | 28.70±0.10a | 30.33±0.15a |
| **Total phenols (TP), mg GAE/g d.e** | | | | |
| Maceration (120 min, 25°C) | 70.47±0.66a | 106.57±0.38a | 87.20±0.38b | 80.15±0.38b |
| Ultrasound extraction (30 min, 25°C) | 66.73±0.38b | 87.20±0.38b | 76.19±0.038b | 76.63±0.38b |
| **Total flavonoids (TF), mgRE/g d.e** | | | | |
| Maceration (120 min, 25°C) | 13.93±0.14b | 19.74±0.24b | 14.25±0.14b | 15.11±0.14b |
| Ultrasound extraction (30 min, 25°C) | 14.33±0.24b | 16.68±0.24ba | 14.80±0.24b | 16.36±0.14ba |

ANOVA
- Extraction technique p < 0.01
- Color nets p < 0.01
- Extraction technique · Color nets p < 0.01

A: pearl nets, B: red nets, C: blue nets, D: Control (non-shading-open field)

mg GAE/g d.e.: milligram of gallic acid equivalents/g dry extract
mgRE/g d.e. : milligram of routine equivalents /g dry extract

Values followed by the same letter do not significantly differ between the treatments, at P = 0.05 according to Duncan’s multiple range test.

Total phenols and total flavonoids content in the aqueous basil leaves extracts

The content of total phenols (TP) in the basil leaves extracts depends on both the extraction technique used and the growing conditions...
Regardless of the growing conditions, a higher content of these components (in all samples) was determined in the extracts obtained by maceration in comparison to the ultrasound-assisted extracts. Previous research of the influence of extraction technique on the content of TP from different plant materials also showed that the TP content was lower in the extracts obtained by ultrasound-assisted extraction than by maceration method. In water-ethanol extracts of nettle leaves, the same dependence of TP content on the applied extraction technique was determined as in this study.

In both extraction techniques, a higher content of TP (106.57 mg GAE/g dry extract obtained by maceration and 87.20 mg GAE/g dry extract obtained by ultrasound-assisted extraction) was observed in the extract from shaded basil plants by red nets, than in extracts from shaded plants by blue and pearl nets and basil from the open field.

Gavarić et al. in their research determined the phenol content in the aqueous-ethanolic extract of basil obtained by maceration (extraction time 5 days) in the range of 116.63 to 148.55 mg GAE/g dry extract, which is slightly higher than the content in the extract obtained by maceration for 2 hours, from plant material shaded by a red net. The results presented here demonstrate that TP content is significantly higher in shaded basil plants by red nets than in non-shaded (control plants) from the open field in both extraction technique used.

Maceration is the most common extraction method for a low-capacity. More recently though, new extraction techniques, such as ultrasound-assisted extraction are being increasingly used.

The content of total flavonoids (TF) in aqueous extracts of basil leaves depends on the extraction technique but also on the growing conditions of basil (plant material used to isolate the extracts). The highest TF content was found in the extracts isolated from basil leaves grown under red nets, regardless of the extraction technique used (19.74 and 16.68 mgRE/g of the dry extract obtained by maceration and ultrasound-assisted extraction, respectively), Table 3.

In these investigations, the extraction technique did not exhibit significant dependence on the TF content as is the case with TP content. The TF content is higher in the extracts obtained by ultrasound-assisted extraction, in all cases except for basil grown under the red net (Table 3), while the TP content in all four samples is higher in the extracts obtained by maceration (Table 3). Earlier research Dimitrijević found that the content of flavonoids is lower in extracts of sour cherry stems obtained by ultrasound-assisted extraction than in extracts obtained by maceration, which is in accordance with the results in this paper. The deviation in the case of the sample of basil grown under the red net is probably a consequence of the effect of ultrasound, but also of a different light composition under photoselective red color nets.

The changes in light intensity through the utilization of shade nets were able to change the synthesis of phenolic compounds in plants. However, different plants had diverse reactions to shade levels, which alter the production of TP and TF. Previous studies showed that change in light intensity was able to modify the production and accumulation of TF and TP in herbs. The content of flavonoids and phenolics was higher in basil collected from plants grown under foil tunnels (0.36, and 0.78 g·100g−1 DW, respectively) than from the open field (0.29, and 0.59g·100g−1 DW), respectively. Similarly, TP content is significantly higher in lettuce cultivars from net-house comparing to open field production. Thus, the highest content of TP is in the lettuce cv. Discoa cultivated in the red net-house (76.70±1.9 mg∙g-1 GAE D.M dry extract). The use of shade netting is also acceptable for the production of baby spinach in relation to improving flavonoid concentration and composition.

Based on the obtained results, it could be concluded that maceration is a better technique for the isolation of extracts with a high TF content while growing conditions under shading by red nets is the best solution. Data on the content of phenols and flavonoids in aqueous extracts obtained from basil grown under photoselective nets is very limited in the available literature. It is known that biosynthesis of flavonoids takes
place in plants, where they participate in the light phase of photosynthesis, during which they catalyze electron transfer. The formation of flavonoids, as well as other phenolic compounds, depends on the light, temperature, soil, climatic conditions, and other factors. The same plant material was used for extraction in this research, but the basil was grown under different nets, the consequence of different content of both, phenols and flavonoids, is not only the application of different extraction techniques, but also, different growing conditions. Basil grown under red nets has the highest content of phenols and flavonoids and having in mind that the red region is photosynthetically extremely active, it leads to the conclusion that the efficiency of photosynthesis has a decisive influence on the content of the considered compounds.

**Antioxidant activity of aqueous basil extracts**

Aqueous basil leaves extracts showed good, concentration-dependent, antioxidant activity. All extracts showed better activity after incubation with DPPH radical (Figure 1 and 2). If we compare the EC$_{50}$ values of the incubated extracts, it could be seen that the antioxidant activity of the extracts depends on both the extraction technique used and the growing conditions of basil (Table 4). The influence of these factors is very complex and could be an interesting topic for future research.

The best antioxidant activity was shown by

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**Figure 1.** Degree of neutralization of DPPH radicals with aqueous extracts of basil leaves obtained by maceration (hydromodule 1:20 m/v, extraction time 120 minutes, 25°C)

a: pearl nets, b: red nets, c: blue nets, d: Control (non-shading – open field)
Figure 2. Degree of neutralization of DPPH radicals with aqueous extracts of basil leaves obtained by ultrasonic extraction (hydromodule 1:20 m/v, extraction time 30 minutes, 25°C) a: pearl nets, b: red nets, c: blue nets, d: Control (non-shading – open field)

The aqueous extract of basil plants grown under shading by red nets, obtained by maceration. The extracts obtained by maceration from basil grown in open field and under a blue net show similar antioxidant activity (EC_{50} values: 0.034 and 0.035 mg/ml, respectively) while the extract from basil grown under a pearl net shows less activity (0.044 mg/ml).

Ultrasound extracts show similar (0.030-0.031 mg/ml) antioxidant activity, except for the extract isolated from a control non-shading basil (from open field) which shows less activity (EC_{50}=0.041 mg/ml). The results show that the cultivation of basil under shading by red nets can be used for the production of plant material with a high content of phenolic compounds. Under these conditions, a more intensive synthesis of phenolic components, compounds from which the antioxidant activity of the extracts largely originates, probably takes place. The highest antioxidant activity was recorded after 20 min of incubation (EC_{50} is 7 µg/ml) by the extract obtained from leaves of basil cover by red nets (Table 4). In previous research, we found that the highest antioxidant activity was recorded by essential oil isolated from basil plants cover by blue nets after 30 min of incubation.

The aqueous extract of basil grown under red nets obtained by maceration, shows better antioxidant activity than the synthetic antioxidant
BHT, while the other extracts have slightly lower antioxidative activity than BHT (Table 8). Since BHT is one of the most commonly used antioxidants, but with harmful effects to human health\(^\text{39}\), the tested extracts can be an alternative to synthetic antioxidants with potential use in the pharmaceutical and food industry.

This study found the interaction between \(EC_{50}\) value and content of total phenols and total flavonoids in aqueous extracts of basil obtained by different extraction methods. A stronger correlation between phenol (\(R^2=0.797\)) and flavonoids (\(R^2=0.917\)) and \(EC_{50}\) values, as measures of antioxidant activity of extracts, in aqueous extracts is achieved by maceration (Figures 3 and 4). Higher content of total flavonoid compounds results in higher antioxidant activity indicating a higher correlation for TF content. The correlation was significantly lower in aqueous extracts obtained by ultrasound-assisted extraction (for phenols: \(R^2=0.230\) and flavonoids: \(R^2=0.166\)). These results are a consequence of the effects of ultrasound during the extraction process (Figures 3 and 4). The antioxidant effect of aqueous basil extracts does not originate only from phenolic compounds but is probably a consequence of the synergistic effect of these compounds with other biomolecules isolated from plant material. Changes in light intensity through the utilization of shade nets were able to change the synthesis of phenolic compounds in plants. Different shade levels, with the resultant changes in plant morphology and physiological characteristics, affected the secondary metabolites such as phenolic compounds in plants. These results confirm that shading by red nets significantly increases the total phenolics and flavonoids content and antioxidant activities of basil than other nets and plants from open field (non-shading plants).

Light quantity and quality can strongly affect the concentration of volatile compounds as well as their composition. In our previous explorations, under 40% shading, the majority of compounds in sweet basil oil from unshaded and shaded plants were oxygen-containing monoterpenes (63.4-63.7%); sesquiterpene hydrocarbons (19.4-18.5%); oxygen-containing sesquiterpenes (7.1-7.9%); and aromatic compounds (4.9-6.6%). The majority of the compounds in the oil were linalool (9.06-10.2%), 1,8-cineole (2.06-1.26%), \(\alpha\)-trans-bergamotene (1.21-1.47%), epi-\(\alpha\)-cadinol (0.93-1.17%), and eugenol (0.83-1.20%). Shade-grown plants had a high relative content of eugenol (the highest under blue nets – 20.9%) and highest antioxidant activity, whereas

| Technique of extraction                  | Without incubation | 20 min incubation |
|-----------------------------------------|--------------------|-------------------|
|                                         | A      | B      | C      | D      | A      | B      | C      | D      |
| Maceration (120 min, 25°C)              | 0.126b | 0.123b | 0.116b | 0.119b | 0.044b | 0.007a | 0.035b | 0.034b |
| Ultrasonic extraction (30 min, 25°C)    | 0.058a | 0.129b | 0.121b | 0.160b | 0.030b | 0.031b | 0.031b | 0.041b |
| BHT                                     |        |        |        |        | 0.021  |        |        |        |

**Table 4. \(EC_{50}\) values of aqueous extracts of basil leaves and synthetic antioxidant BHT**

**ANOVA**

| Extrac | \(EC_{50}\) mg/mL |
|--------|-------------------|
| A      | 0.058a            |
| B      | 0.129b            |
| C      | 0.121b            |
| D      | 0.160b            |

**Extraction technique**

- A: pearl nets,  B: red nets,  C: blue nets,  D: Control (non-shading – open field)

**\(EC_{50}\):** concentration of extract in the mixture required to neutralize 50% of the initial concentration of DPPH radicals, BHT - The synthetic antioxidant was included in experiments as a positive control.
the relative contents of linalool were high in plants without shading (control plants – 53.9%). Some components were present only in unshaded plants, like caryophyllene oxide (0.01-0.03%), (E)-iso-γ-bisabolene (0.05%); β-bisabolene (0.05%); α-terpinene (0.01%); p-cymene (0.01%). On the contrary, in shaded plants we registered the presence of δ-3-carene (0.01%) 1.

Linalool is a common acyclic monoterpenoid compound that can be synthesized from α-pinene or β-pinene. In plants, the enzyme linalool synthase catalyzes geranyl pyrophosphate (GPP) to linalool. In addition, GPP can be catalysed by 1,8-cineole synthase to 1,8-cineole 40,41 and α-terpinol 40 catalysed by monoterpene cyclase to pinenes, 3-carene and limonene 41. In the present study, there were differences in the relative contents of main constituents under different shading treatments. Basil plants cover by blue shade nets obtained the lowest content of linalool (34.8%) than plants from other color nets and unshaded-control plants. On other hand, blue nets obtained the highest content of 1,8-cineol (29.2%) in comparison with another nets. Significant differences were found for linalool and 1,8-cineol, suggesting that further research should be carried out to determine how the light quality affects the activity of linalool synthase and 1,8-cineole synthase and thereby regulating the relative content of linalool and 1,8-cineol.

Some other chemicals maintained a relatively stable level under different conditions,
Conclusion
The linalool, 1,8-cineole, myrcene, and α-trans-bergamotene are constituents responsible for the distinct aroma of basil plants. Monoterpene hydrocarbons were predominant in the sample grown under the blue nets, while the highest content of sesquiterpene hydrocarbons was obtained in the plants from the open field. The highest content of TP and TF was found in the extract obtained by maceration of basil grown under a red net. 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. The isolated extracts are a source of natural antioxidants with potential application in the food and pharmaceutical industries. Thus, the consideration for the commercialization of these products is crucial.

Acknowledgements
This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the Program of financing scientific research work, number 451-03-9/2021-14/20013.

Supplementary data
Table S1-S4 are provided as supplementary information.

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