Anatolian Journal of Botany
6(2): 115-121 (2022)
Research article *doi: 10.30616/ajb.1181724*

Phenolic and flavonoid amounts and antioxidant capacity of *Lavandula officinalis* (lavender) callus grown in different growth regulator combinations

Abayhan BURAN*, Aykut TOPDEMİR[2]
[1,2]*Fırat University, Engineering Faculty, Department of Bioengineering, Elazığ, Türkiye*
**a.buran@firat.edu.tr*, [2]*atopdemir@firat.edu.tr*

Received : 30.09.2022
Accepted : 12.11.2022
Online : 13.11.2022

Farklı büyüme düzenleyici kombinasyonlarda yetiştirilen *Lavandula officinalis* (lavanta) kalluslarının fenolik ve flavonoid miktarları ve antioksidan kapasitesi

Abstract: In this study, it was aimed to determine the total antioxidant capacity and phenolic and flavonoid amounts of *Lavandula officinalis* (lavender), which is an aromatic plant, callus extracts. Combinations of Naphthalene Acetic Acid (NAA), Benzylaminopurine (BAP), Kinetin (KIN) and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) plant growth regulators at different concentrations were used in the growth medium. The specified analyzes were carried out for the extracts obtained from the callus of plants grown in different media. The callus were extracted with water and ethanol solvents. Total phenolic amount analyzes were accomplished using the Folin-Ciocalteu method. The spectrophotometric $AlCl_3$ method was used for the total flavonoid amount analysis and the antioxidant capacity of lavender callus extracts was measured considering the radical cation capture ability of 2,2′-azino-bis -3- ethylbenzthiazoline -6- sulphonicacid (ABTS). The highest antioxidant capacity (9.24 ± 0.14 mmol TEAC/g dry callus weight) was obtained from the callus of plants grown in medium containing 0.5 mg/L BAP + 0.5 mg/L 2,4-D combination; the highest amount of phenolic substance (35.74± 0.48 mg GAE/g dry callus weight) was obtained from the callus of plants grown in medium containing the combination of 0.5 mg/L BAP + 0.5 mg/L NAA; the highest amount of flavonoid substance (32.42 ± 0.46 mg QE/g dry callus weight) was obtained from the callus of plants grown in medium containing the combination of 0.5 mg/L BAP + 1 mg/L 2,4-D. The results are compared for the combination of plant growth regulators and the effects of the different growth medium ingredients were specified.

Key words: Antioxidant, callus, flavonoid, plant growth regulator, *Lavandula officinalis*, phenolic

Özet: Bu çalışmada, tıbbi ve aromatik bir bitki olan *Lavandula officinalis* (lavanta) kallus ekstraktlarının toplam antioksidan kapasitesinin ve fenolik ve flavonoid madde miktarlarının belirlenmesi amaçlanmıştır. Büyüme ortamında Naftalin Asetik Asit (NAA), Benzilaminopurin (BAP), Kinetin (KIN) ve 2,4-Diklorofenoksi Asetik Asit (2,4-D) bitki büyüme düzenleyicilerinin farklı konsantrasyonlardaki kombinasyonları kullanılmıştır. Farklı ortamlarda yetiştirilen bitkilerin kalluslarından elde edilen ekstraktlar için belirtilen analizler yapılmıştır. Kalluslar su ve etanol solventleri ile ekstrakte edilmiştir. Toplam fenolik miktarı analizleri Folin-Ciocalteu yöntemi kullanılarak yapılmıştır. Toplam flavonoid miktarı analizi için spektrofotometrik $AlCl_3$ yöntemi kullanılmış ve lavanta kallus ekstraktlarının antioksidan kapasitesi 2,2′-azino-bis -3-etilbenztiazolin -6- sülfonik asidin (ABTS) radikal katyon yakalama kabiliyeti dikkate alınarak ölçülmüştür. En yüksek antioksidan kapasite (9,24 ± 0,14 mmol TEAC/g kuru kallus ağırlığı) 0,5 mg/L BAP + 0,5 mg/L 2,4-D kombinasyonunu içeren ortamda yetiştirilen bitkilerin kalluslarından elde edilmiştir; en yüksek fenolik madde miktarı (35,74± 0,48 mg GAE/g kuru kallus ağırlığı) 0,5 mg/L BAP + 0,5 mg/L NAA kombinasyonunu içeren ortamda yetiştirilen bitkilerin kalluslarından; en yüksek flavonoid madde miktarı (32,42 ± 0,46 mg QE/g kuru kallus ağırlığı) 0,5 mg/L BAP + 1 mg/L 2,4-D kombinasyonunu içeren ortamda yetiştirilen bitkilerin kalluslarından elde edilmiştir. Sonuçlar, bitki büyüme düzenleyicilerinin kombinasyonu için karşılaştırılmış ve farklı yetiştirme ortamı bileşenlerinin etkileri belirlenmiştir.

Anahtar Kelimeler: Antioksidan, kallus, flavonoid, bitki büyüme düzenleyici, *Lavandula officinalis*, fenolik

Citation: Buran A, Topdemir A (2022). Phenolic and flavonoid amounts and antioxidant capacity of *Lavandula officinalis* (lavender) callus grown in different growth regulator combinations. Anatolian Journal of Botany 6(2): 115-121.

1. Introduction

It is known that medicinal and aromatic plants have been used to protect or improve human health. Throughout history, mankind has discovered the therapeutic properties of parts of plants such as roots, stems, leaves, flowers and fruits by chance. It is stated in Ninova (3000 BC) tablets that Mesopotamian civilizations such as Assyrians, Akats and Sumerians benefited from medicinal aromatic plants and animal therapeutic products for medicinal purposes (Genç & Kaçar, 2012). Medicinal aromatic plants and therapeutic drugs of animal origin have formed the basis of drugs used in modern pharmacy and medicine today. It is known that most of these drugs are of plant origin. With the developing technology, the chemical components of the plants and the effective substances they contain have been clarified and isolated purely. In this way, the foundations of the concept of "pure and standard medicine" were laid (Tanrıseven, 2013).

Lavender, which is among the medicinal and aromatic plants, is rich in essential oils. It is also commercially cultivated for the extraction of spices and essential oils. Lavender essential oils, with their antiseptic and anti-inflammatory properties, are used primarily in soaps, bubble baths, shampoos, etc. It is also used in perfumes, massage oils, and creams, including bath products (Moon et al., 2006). The lavender plant is a perennial and semi-

bush plant of the Mediterranean geography. It is a dicot plant whose roots can reach up to 1 m depending on the environmental conditions. The stem is hairy or bare. The lavender body has a gray-green color and gives many side branches (Koç, 2002).

Lavandula officinalis, which is considered as an important medicinal and aromatic plant, is included in the Lamiaceae family. It contains 150 bioactive components (El Sherif at al., 2020). The essential oils of the lavender plant, which is one of the most produced essential oils in the world, are used extensively in the pharmaceutical industry, perfumery and cosmetics industry. The dried flowers and leaves of the lavender plant are generally used in the pharmaceutical industry. The Lamiaceae family includes approximately 224 genera and 5600 species worldwide (Hickey and King, 1997), and Turkey is an important geography for the Lamiaceae family. Turkey has an important place in the world in terms of geography and species diversity. It is a great opportunity for the country to benefit from this richness where medicinal and aromatic plants (lavender, rosemary, basil etc.) are widely spread.

From a biotechnological point of view, the basic system that is considered in plant tissue processes and genetic improvements is the regeneration of the plant. In the plant regeneration process, tissue culture and callus formation are observed. Callus formation and further biotechnological developments depend on the plant resources and growth medium content in the culture. Hormones, generally called plant growth regulators, are added to the growth medium. Different results can be obtained with callus obtained with plant growth regulators added in different concentrations and combinations in callus formation or in later stages.

In this study, it was aimed to determine the total amount of antioxidant capacity and phenolic and flavonoid amounts in lavender, which is a medicinal and aromatic plant, by extracting the calluses obtained from media containing various plant growth regulator combinations with water and ethanol solvents.

2. Materials and method

In this study, *L. officinalis* (lavender) plant, which is a member of the Lamiaceae family, was used. Lavender seeds were obtained and identified at Firat University Plant Tissue Culture Laboratory and Greenhouse, Elazığ, Türkiye. Plants were grown from seed to grown-up plants under stable conditions at 22±2 ℃, 16-h d^{-1} photoperiod, 3000 lux light intensity. Node parts of the plant were used to obtain callus. Node explants were obtained on the day they were taken into the medium.

2.1. Method

Lavender explants were collected with the help of a scalpel. The explants were washed with tap water. They were kept in 70% ethanol for 30 seconds and kept in 50% sodium hypochlorite solution for 5 minutes. 5% commercial bleach was used as sodium hypochlorite. Then, the bleach was removed by washing 3 times with sterile distilled water.

Murashige and Skoog (MS) medium was used as the growth medium. MS medium meets almost all the basic nutritional needs for plant tissue culture. It also contains vitamins and minerals, macro and micro elements, which are the basic needs of plants.

In this study, combinations of Naphthalene Acetic Acid (NAA), Benzylaminopurine (BAP), Kinetin (KIN) and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) plant growth regulators at different concentrations were used in addition to the MS medium. These combinations are shown in Table 1 and Table 2.

Table 1. Plant growth regulators in combination with BAP

Medium Code	2,4-D(mg/L)	BAP(mg/L)	NAA(mg/L)	KIN(mg/L)
0,5B-0,5N	-	0.5	0.5	-
0,5B-1N	-	0.5	1	-
0,5B-2N	-	0.5	2	-
0,5B-0,5D	0.5	0.5	-	-
0,5B-1D	1	0.5	-	-
0,5B-2D	2	0.5	-	-

Table 2. Plant growth regulators in combination with KIN

Medium Code	2,4-D(mg/L)	BAP(mg/L)	NAA(mg/L)	KIN(mg/L)
0,5K-0,5N	-	-	0.5	0.5
0,5K-1N	-	-	1	0.5
0,5K-2N	-	-	2	0.5
0,5K-0,5D	0.5	-	-	0.5
0,5K-1D	1	-	-	0.5
0,5K-2D	2	-	-	0.5

Growth medium was prepared with 4.4 g/L MS and 30 g/L sucrose. 7.8 g/L agar was added after the pH was stabilized in the range of 5.6-5.8. In case of any confusion, the coding process was applied to the prepared media as in Table 1 and Table 2. Explants were planted in the growth medium that was sterilized. The cultivated explants were kept at +24 ± 2 ℃ for 16 hours of light and 8 hours of dark.

The callus obtained were left to dry for 48 hours at 55 °C. Dry callus were crushed into small pieces with the help of a pestle. From the obtained dry callus, 0.1 g was weighed for each sample. For each 0.1 g mass of dry callus, 4 mL of water and ethanol solvents were added separately. Callus with added solvents were kept at +4 ℃ for 72 hours and were used for analysis after filtering by filter paper (Whatman No:1).

All experiments were done in 3 repetitions. The obtained results were tabulated and their standard deviations were calculated. The results are given with their standard deviations.

2.1.1. Analysis of total phenolic amount

In this study, total phenolic content analyzes were accomplished using the Folin-Ciocalteu method (Singleton & Rossi, 1965). For the analyses, 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent was mixed with 300 µL of lavender callus extract. After a waiting period of about 2 minutes, 1.2 mL of 7.5% Na_2CO_3 solution was added and mixed thoroughly. Then, the absorbance values of the mixtures, which were kept in the dark for 90 minutes, were measured against pure water at a wavelength of 765 nm. The total amount of phenolics was calculated with the equation obtained from the gallic acid calibration curve and given as gallic acid equivalent (GAE) per g of dry callus weight

(d.c.w.). The absorbance values of gallic acid measured at different concentrations (0.025-0.05-0.1-0.2-0.4 mg/mL) were used for the calibration chart.

2.1.2. Analysis of total flavonoid amount

The spectrophotometric $AlCl_3$ method was used in the analysis of the total flavonoid content of lavender callus extracts (Lamaison et al., 1990). While preparing 2% $AlCl_3$ solution glacial acetic acid/methanol (50%, v/v) was used as solvent. In this analysis, 1 mL of lavender callus extracts and 1 mL of 2% $AlCl_3$ solution were mixed and kept at room conditions for 10 minutes. The absorbance values of the samples were then measured against the blank (2% $AlCl_3$) at a wavelength of 394 nm. The equation obtained from the quercetin calibration curve for the total amount of flavonoid substance was used and the results were given as quercetin equivalent (QE) per g of d.c.w. The absorbances of quercetin at different concentrations (0.025-0.05-0.1-0.2-0.4 mg/mL) were used for the calibration plot.

2.1.3. Determination of total antioxidant capacity

The antioxidant capacity of lavender callus extracts was measured considering the radical cation capture ability of 2,2′-azino-bis -3- ethylbenzthiazoline -6- sulphonicacid (ABTS). ABTS•+ radical is formed as a result of the oxidation of ABTS with persulfate.

The discoloration of the ABTS solution mixed with the plant extract indicates the presence of antioxidant activity. With the effect of this antioxidant presence, the ABTS•+ cation was fragmented and the dark blue color became lighter (Miller et al., 1995).

In this method, ABTS stock solution (7 mM) was diluted with phosphate buffer until its absorbance was approximately 0.7 and kept away from light as much as possible. Before the absorbance readings, 100 µL of lavender callus extract and 1900 µL of ABTS solution were mixed and kept at room conditions. At the end of the 6th minute, absorbance values were read against phosphate buffer at 734 nm wavelength. The results were given as trolox equivalent antioxidant capacity (TEAC) per g of d.c.w. using the line equation in the trolox calibration curve. The absorbances of trolox at different concentrations (0.05-0.1-0.2-0.3-0.4 mmol/L) were used for the calibration curve.

2.2. Statistical analysis

Statistical analyses of the data were performed by one way ANOVA and post hoc multiple comparisons (SPSS 21, Statistical Software, IBM). A probability value of $p<0.05$ was considered to denote a statistically significant difference. All determinations were performed in three replicates. Data were presented as mean values ±Standard Deviations (SD).

3. Results

The total antioxidant capacity, total phenolic and flavonoid substance amounts of the samples obtained by extracting the callus of the lavender plant with water and ethanol were obtained as a result of the analysis. Obtained results are shown on graphs and the differences between solvents and plant growth regulators are compared. In callus cultures of the study, the best callus formation was observed in growth medium containing 0.5 BAP + 1 mg/L 2,4-D.

3.1. Total phenolic content

While calculating the total content of phenolics, the line equation obtained from the gallic acid calibration graph (Fig. 1) was used. The absorbances measured during the analysis were substituted in the equation and the results were obtained as mg GAE/g.

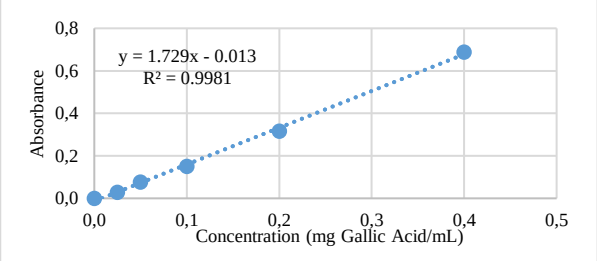

Figure 1. Gallic acid calibration curve

The results obtained from the samples using water and ethanol as solvents are given in graphs (Fig. 2 and Fig. 3). According to the results, the highest phenolic substance (35.74± 0.48 mg GAE/g) was obtained from 0.5 mg/L BAP + 0.5 mg/L NAA water extracts. The lowest amount of phenolic substance (24.22 ± 1.03 mg GAE/g) was obtained from the ethanol extract of the sample taken from the growth medium containing the combination of 0.5 mg/L KIN + 2 mg/L 2,4-D.

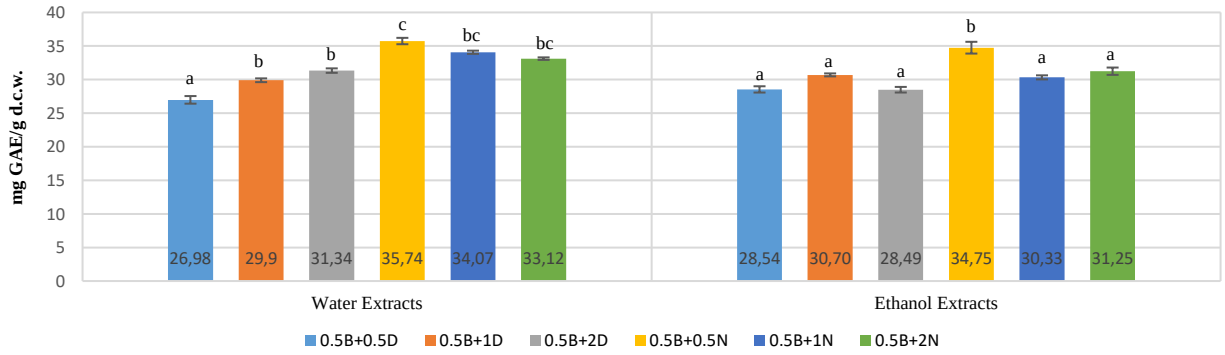

Figure 2. Total Phenolic Contents (mg GAE/g d.c.w.) of *L. officinalis* callus extracts according to grown different plant growth regulators (B: BAP, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

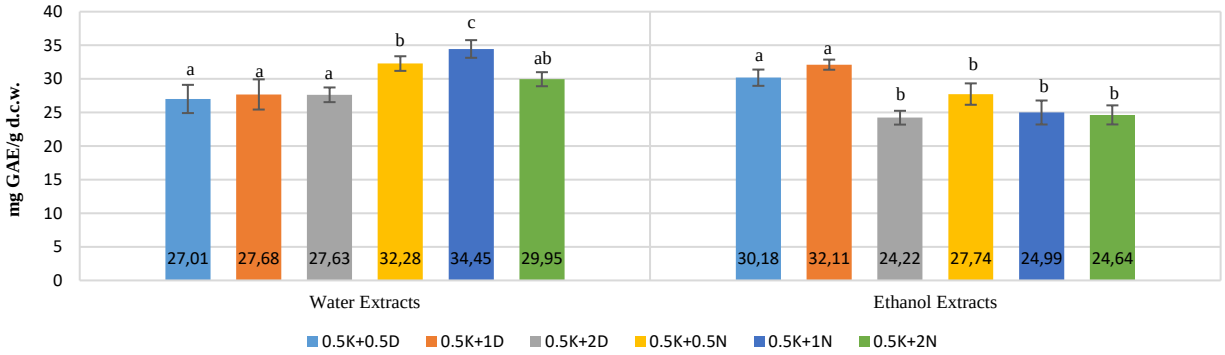

Figure 3. Total Phenolic Contents (mg GAE/g d.c.w.) of *L. officinalis* callus extracts according to grown different plant growth regulators (K: KIN, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

3.2. Total flavonoid substance content

The line equation obtained in the quercetin calibration graph (Fig. 4) was used to calculate the total content of flavonoid substance.

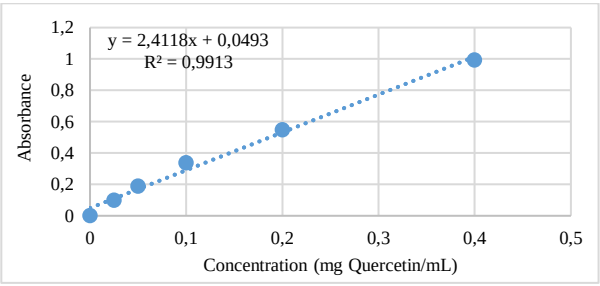

Figure 4. Quercetin calibration curve

The absorbance values measured during the analysis were substituted in the equation obtained from the graph, and the results were obtained as mg QE/g.

The results obtained from the samples using water and ethanol solvents are given in graphs (Fig. 5 and Fig. 6). According to the results, the highest amount of flavonoid content (32.42 ± 0.46 mg QE/g) was obtained from the water extract of the callus sample taken from the growth medium containing 0.5 mg/L BAP + 1 mg/L 2,4-D combination. The lowest amount of flavonoid content (11.64 ± 0.80 mg QE/g) was obtained from the ethanol extract of the callus sample taken from the growth medium containing the combination of 0.5 mg/L BAP + 2 mg/L NAA.

3.3. Total antioxidant capacity

The equation obtained in the trolox calibration graph (Fig. 7) was used to calculate the total antioxidant capacity. The absorbances measured during the analysis were substituted in the equation obtained from the graph, and the results were obtained as mmol/g TEAC.

The results obtained from the samples using water and ethanol solvents are given in a single graph (Fig. 8 and Fig. 9). According to the results, the highest antioxidant capacity (9.24 ± 0.14 mmol/g TEAC) was obtained from the water extract of the callus sample taken from the growth medium containing the combination of 0.5 mg/L BAP + 0.5 mg/L 2,4-D.

The lowest amount of antioxidant capacity (5.17 ± 0.41 mmol/g TEAC) was obtained from the ethanol extract of the callus sample taken from the growth medium with a combination of 0.5 mg/L KIN + 1 mg/L NAA.

4. Discussions

Growth media containing plant growth regulators at different concentrations affect callus development. The combination of different concentrations of these regulators also changes the development of the callus and the amount of total antioxidant, phenolic and flavonoid substances that the callus has.

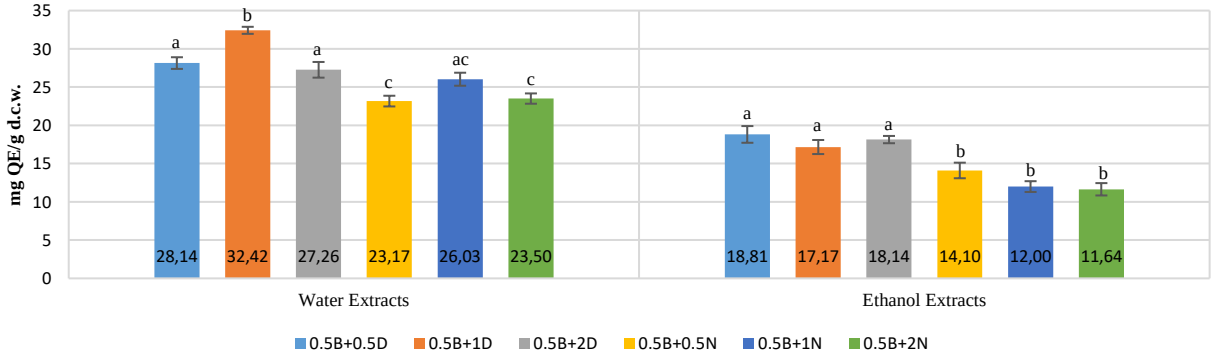

Figure 5. Total Flavonoid Contents (mg QE/g d.c.w.) of *L. officinalis* callus extracts according to grown different plant growth regulators (B: BAP, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

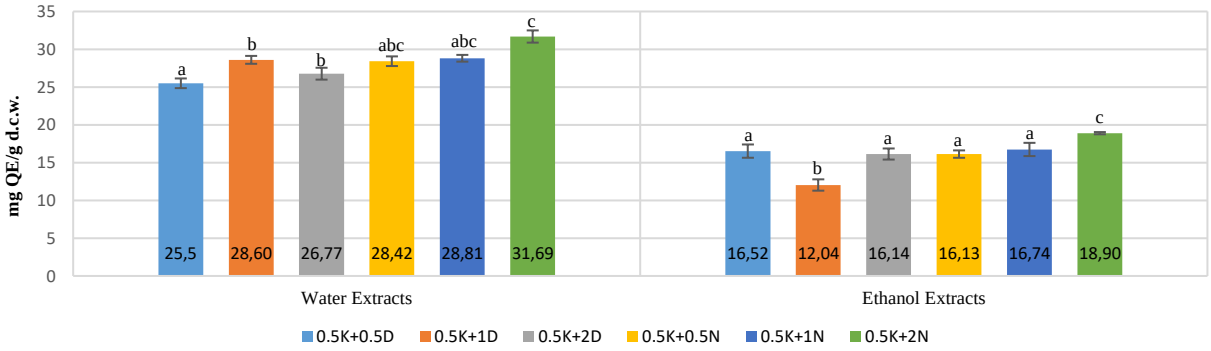

Figure 6. Total Flavonoid Contents (mg QE/g d.c.w.) of *L. officinalis* callus extracts according to grown different plant growth regulators (K. KIN, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

In callus cultures of this study, the best callus formation was observed in growth medium containing 0.5 BAP + 1 mg/L 2,4-D. On the other hand, Meric et al. (2019) observed callus formation from shoot explants of *Lavandula angustifolia* and used different growth regulators. They reported that the best callus development was obtained in MS medium containing 2 mg/L 2,4-D + 2 mg/L BAP.

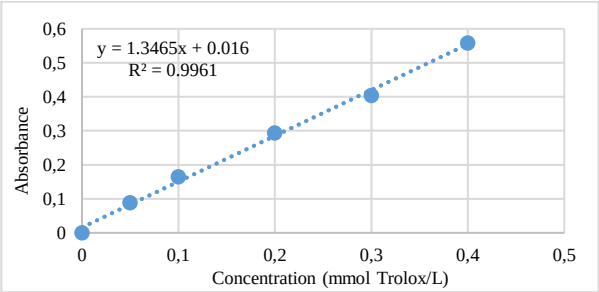

Figure 7. Trolox calibration curve

When the antioxidant activity studies of *L. officinalis* callus culture were examined, almost no study was found comparing growth medium combinations with antioxidant activity, phenolic and flavonoid content above the standards, as in this study. However, in studies with similar plants, studies comparing the relationships between plant growth regulator combinations and antioxidant activity were found. When the antioxidant activity studies with different types of callus are examined, it is seen that different results are generally obtained from the callus samples taken from different environments. The results obtained from callus extracts found in medicinal and aromatic plants generally prove that callus also shows antioxidant activity as in the total plant. It has also been reported in some studies that the presence of antioxidant activity in callus extracts was determined according to the materials used in natural antioxidant source research (Grzegorczyk et al., 2007; Yesil-Çeliktaş et al., 2007).

Kim et al., (2003) obtained 1.57 mg QE/g flavonoid substance in the water extract of the callus of *Stevia rebaudiana* plant. They found the amount of phenolic substance as 43.99 µg catechin equivalents/mg.

Kovacheva et al., (2006) in a study they conducted, found the amount of phenolics in ethanol extracts of *Lavandula vera* callus to be 42.3±4.2 mg GAE/g. They found the antioxidant activity to be 5.76 µm Trolox/g based on the trolox standard. In this study, 32.42 ± 0.46 mg QE/g was obtained in the water extract of *L. officinalis* callus obtained from the combination of 0.5 mg/L BAP + 1 mg/L

2,4-D. The amount of phenolic substance was found to be 35.74 ± 0.48 mg GAE/g based on the gallic acid standard. In a study by Kousalya and Bai (2016), they obtained callus from the explants of *Canscora decussata* plant with different growth regulators in the growth medium. As a result, the highest antioxidant capacity was obtained as 12.23 mmol/g TEAC in the callus obtained from the growth medium containing 1/2 MS + 0.5 mg/L NAA combination. In this study, the highest antioxidant capacity was obtained as 9.08 ± 0.13 mmol/g TEAC from the combination of 0.5 mg/L BAP + 0.5 mg/L 2,4-D.

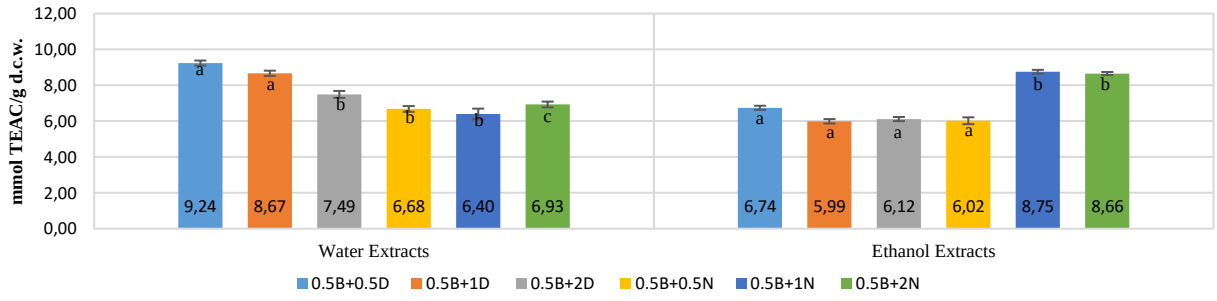

Figure 8. Total Antioxidant Capacity (mmol TEAC/g d.c.w) of *L. officinalis* callus extracts according to grown different plant growth regulators (B: BAP, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

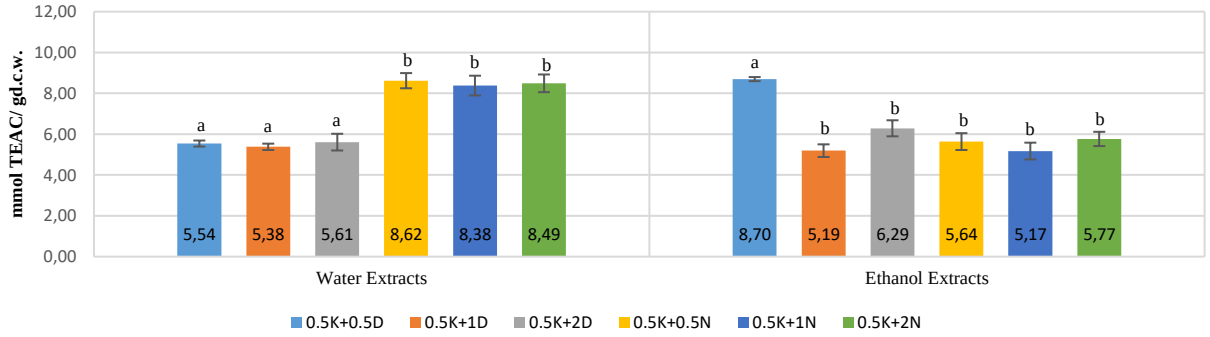

Figure 9. Total Antioxidant Capacity (mmol TEAC/g d.c.w) of *L. officinalis* callus extracts according to grown different plant growth regulators (K: KIN, N:NAA, D: 2,4-D.) combinations (X mg/L+X mg/L). The values with different letters within column designate statistically significant differences, $p < 0.05$ by ANOVA. Error bars represent ± Standard Deviations (SD).

Yoon et al. (2006) conducted a study investigating the antioxidant activities of *Rosmarinus officinalis* and *Lavandula spica* L. callus. They observed that the antioxidant activity of the plants changed according to the growth medium. They reported the highest effect in the lavender plant as 0.1 mg/L 2,4-D extract (37.6±0.9 µg/mL RC50).

Topdemir (2018), in her thesis study with *Melissa officinalis* L., examined the amount of highest antioxidant capacity of *M. officinalis* callus. Accordingly, it was reported that the combination of 1 mg/L 2,4-D + 1 mg/L PIC (Picloram) + 0.5 mg/L KIN plant growth regulator was obtained as 9.15±0.74 mmol/g TEAC from the water extract. When the ethanol extracts of callus were examined, it was reported that the highest antioxidant capacity was obtained as 14.61± 1.14 mmol/g TEAC from the plant growth regulator combination of 1.5 mg/L 2,4-D + 1 mg/L PIC + 0.5 mg/L KIN.

In a different thesis study with the callus of the basil (*Ocimum basilicum* L.) plant, the highest antioxidant content was 0.5 mg/L KIN – 0.5 mg/L 2,4-D plant growth regulator combination was 5.74±0.20 mmol/g TEAC from the ethanol extract of callus. was obtained. The amount of flavonoid substance was obtained as 3.01±0.34 mg quercetin/g from the ethanol extract of callus obtained from 0.5 mg/L BAP – 0.25 mg/L 2,4-D plant growth regulator combination. The highest total phenolic content was obtained from the callus ethanol extract obtained from the combination of 0.5 mg/L KIN – 0.5 mg/L NAA plant growth regulator as 2.55±0.11 mg GAE/g (Topdemir et al., 2019a,b).

5. Conclusion

When the results of the study are considered, it is seen that the extracts obtained with both solvents, water and ethanol,

have antioxidant activity. There are different uses of plant growth regulator in plant tissue culture. As a result, antioxidant activity and phenolic and flavonoid content of *L. officinalis* callus extracts obtained from growth media containing different plant growth regulator combinations were revealed.

Considering the results, it is seen that the callus show antioxidant capacity and contain phenolic and flavonoid substances. According to the general results of lavender calluses, it is seen that higher yields are obtained from the extracts using water as a solvent, especially considering the total antioxidant capacity and total flavonoid content. Growth medium and conditions should be considered in plant tissue engineering studies and in plants to be grown for commercial purposes. Plant cell suspensions can be used commercially for the production of secondary metabolites. For this purpose, more efficient productions can be obtained by transferring from flasks or petri dishes to bioreactors. It is possible to obtain higher results by optimizing the growth conditions of these callus and by more efficient extraction methods.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Acknowledgements

We would like to thank Dr. İlay KAVURAN (Ph.D) for the excellent technical support.

References

El Sherif F, Albotnoor N, Yap YK, Meligy A, Khattab S (2020). Enhanced bioactive compounds composition in *Lavandula officinalis* in-vitro plantlets using NaCl and Moringa oleifera, *Aloe vera* and *Spirulina platensis* extracts. Industrial Crops and Products 157: 112890.

Genç L, Kaçar O (2012). Tıbbi ve aromatik bitkilerin tarım ve hayvancılık alanlarında kullanımı. In: Kınay A, Özyılmaz B (eds). Birinci Tıbbi Aromatik Bitkiler Sempozyumu Bildirileri Kitabı Tokat, Türkiye. Pp. 7-28.

Grzegorczyk I, Matkowski A, Wysokinska H (2007). Antioxidant activity of extracts from in vitro cultures of *Salvia officinalis* L. Food Chemistry 104(2): 536-541.

Hickey M, King C (1997). Common Families of Flowering Plants. Cambridge: Cambridge Univ. Press.

Kim HY, Kim OH, Sung MK (2003). Effects of phenol-depleted and phenol-rich diets on blood markers of oxidative stress, and urinary excretion of quercetin and kaempferol in healthy volunteers. Journal of the American College of Nutrition 22(3): 217-223.

Koç H (2002). Bitkilerle Sağlıklı Yaşama. Ankara: T.C. Kültür Bakanlığı Kültür Eserleri Dizisi, No: 373.

Kousalya L, Bai VN (2016). Effect of growth regulators on rapid micropropagation and antioxidant activity of Canscora decussata (Roxb.) Roem. & Schult.–A threatened medicinal plant. Asian Pacific Journal of Reproduction 5:(2), 161-170.

Kovacheva E, Georgiev M, Pashova S, Angelova M, Ilieva M (2006). Radical quenching by rosmarinic acid from *Lavandula vera* MM cell culture. Zeitschrift fur Naturforschung C - Journal of Biosciences 61(7-8): 517-520.

Lamaison JLC, Carnet A (1990). Teneurs en principaux flavonoides des fleurs de *Crataegus monogyna* Jacq et de *Crataegus laevigata* (Poiret D. C) en fonction de la vegetation. Pharmaceutica Acta Helvetia, 65: 315-320.

Meriç S, Tuman CB, Ayan A, Atak Ç (2019). Optimization of tissue culture media-inducing essential oil production of levander (*Lavandula angustifolia* Mill.). Physiology and Molecular Biology of Plants 25(1): 85-99.

Miller NJ, Diplock AT, Rice-Evans CA (1995). Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. Journal of Agricultural and Food Chemistry 43(7): 1794-1801.

Moon T, Wilkinson JM, Cavanagh HM (2006). Antiparasitic activity of two *Lavandula* essential oils against *Giardia duodenalis*, *Trichomonas vaginalis* and *Hexamita inflata*. Parasitology Research 99(6): 722-728.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144-158.

Tanrıseven A (2013). Tıbbi aromatik bitkiler biyo sağlık ve ekonomi. Batı Akdeniz Tarımsal Araştırma Enstitüsü Dergisi 3: 44-58.

Topdemir A, Gür N, Demir Z (2018). *Melissa officinalis* L. kallus kültürlerinin antoksidan özellikleri. Eurasian Journal of Biological and Chemical Sciences 1(1): 12-15.

Topdemir A, Buran A, Gür N (2019a). *Ocimum basilicum* L. (Fesleğen) kallus kültürlerinin antioksidan kapasitesinin belirlenmesi. In: Doğan M & Atay E (eds.). 2nd International Eurasian Conference on Biological and Chemical Sciences, Ankara, Türkiye, Proceeding Book Pp. 920-928.

Topdemir A, Buran A, Gür N (2019b). Fesleğen (*Ocimum basilicum* L.) bitkisi kallus kültürlerinin toplam fenolik ve flavonoid miktarının tespiti. In: Doğan M, Atay E (eds.). 2nd International Eurasian Conference on Biological and Chemical Sciences, Ankara, Türkiye, Proceeding Book. Pp. 929-936.

Yeşil-Çeliktaş O, Nartop P, Gürel A, Bedir E, Vardar-Sukan F (2007). Determination of phenolic content and antioxidant activity of extracts obtained from *Rosmarinus officinalis* L. Journal of Plant Physiology 164(11): 1536-1542.

Yoon JH, Jong-II L, Chi-Kun O, Zhi C, Seung-Yul Y, Won-Seob S (2006). Effect of Plant Growth Regulator on Antioxidant Activity of *Rosmarinus officinalis* L. and *Lavandula spica* L. Korean Journal of Plant Resources 19(2): 315-322.