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ORIGINAL PAPER

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DETERMINATION OF ANTIOXIDANT ACTIVITY AND PHENOLIC AND FLAVONOID CONTENT OF *Ocimum basilicum* L. CALLUS CULTURES OBTAINED BY DIFFERENT PLANT GROWTH REGULATORS

Aykut Topdemir ✉, Abayhan Buran

Fırat University, Faculty of Engineering, Department of Bioengineering, Elazığ, Turkey

ABSTRACT

In this study, binary combinations of five plant growth regulators (PGR) were used for the cultivation of *Ocimum basilicum* L. calluses. Antioxidant properties, phenolic and flavonoid contents of *O. basilicum* L. calluses were determined by keeping the concentrations and solvents of PGR constant.The total phenolic content of ethanol extracts of *O. basilicum* L. calluses was between 1.044 ±0.188 and 0.417 ±0.049 mg of GAE/g; total phenolic content of methanol extracts was between 2.547 ±0.110 and 0.701 ±0.095 mg of GAE/g. The total flavonoid content of ethanol extracts was between 2.058 ±0.122 and 0.446 ±0.063 mg of quercetin/g, whereas the total flavonoid content of methanol extracts was between 3.010 ±0.336 and 0.341 ±0.041 mg of quercetin/g. The antioxidant content of ethanol extracts was between 2.826 ±0.141 and 5.736 ±0.201 mmol/g of Trolox equivalent antioxidant capacity (TEAC) and the antioxidant content of methanol extracts was between 4.186 ±0.394 and 1.050 ±0.211 mmol/g of TEAC.

Key words: *Ocimum basilicum* L., callus, phenolic substance, flavonoid, antioxidanta

INTRODUCTION

The commercial development of plants as sources of antioxidants for enhancing health and food preservation is of global interest [Rice-Evans et al. 1997]. Epidemiological studies have shown a positive relationship between the consumption of phenolic-rich foods and beverages and disease prevention [Scalbert and Williamson 2000].

Antioxidants, when present in very low concentrations in the food or body, are substances that delay, control or prevent oxidative processes that cause the initiation and reproduction of degenerative diseases in the body or the deterioration of food quality. Antioxidants are used to reduce the risk of illness and support health by protecting the body against oxidative damage in healthy parts of the body. These antioxidants are free radical scavengers, singlet oxygen absorbers, peroxide inactivators, metal ion chelating agents, dampers of secondary oxidation products and inhibitors of pro-oxidative enzymes [Shahidi and Zhong 2007].

Phenolic compounds or polyphenols are a group of compounds that contain a benzene ring with one or more attached hydroxyl groups. All compounds with at least one aromatic ring and a large number of hydroxyl substituents in this ring are termed phenolic compounds. Polyphenolic compounds contain mainly phenol and are organic compounds formed in the leaves, branches, fruits and flowers of plants with

✉ atopdemir@firat.edu.tr

the help of sunlight. They are more soluble in organic solvents than in water. The antioxidant properties of polyphenols are associated with their reducibility and hydrogen donor characteristics [Bursal 2009]. Phenolic compounds are found in several plant organs, thus constituting an integral part of human nutrition. Various studies have shown that phenolics exhibit antioxidative effects and are responsible for the inhibition of DNA oxidation and low-density lipoprotein cholesterol in vitro systems [Adom and Liu 2002, Chandrasekara and Shahidi 2010, Liyana-Pathirana et al. 2006, Madhujith and Shahidi 2007].

Flavonoids have a 2-phenyl benzopyran (diphenylpropane) structure (C6-C3-C6) with 15 carbon atoms and form a ring with oxygen by a triple carbon bridge between the phenyl groups (flavan ring). Differences between various flavonoids are due to the number of hydroxyl groups bound to them, the degree of unsaturation and the oxidation level of the triple carbon segment. Although the skeletal structure of flavonoids is different, there are different subclasses termed flavones, flavonols, flavanones, flavanols, isoflavones, anthocyanidins and chalcones [Bors et al. 1990, Formica and Regelson 1995, Ptittin 1987].

These effects are attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids, among others [Rice-Evans et al. 1996].

Basils (*Ocimum* spp., *Lamiaceae*) contain a wide range of essential oils consisting of a variety of natural products including polyphenols such as phenolic compounds, flavonoids and anthocyanins [Phippen and Simon 2000, Simon et al. 1990]. Several studies have evaluated the antioxidant activity of basil extracts and essential oils [Juliani and Simon 2002].

MATERIAL AND METHODS

In this study, the body and leaf explants obtained from *O. basilicum* L. plant were transferred to cultures containing Murashige–Skoog (MS) medium using sterile materials. All the calluses obtained from these cultures were analysed. The cultured *O. basilicum* L. explants were incubated in special climate rooms at +22°C under 2500 lux fluorescent light for 16 h in light and 8 h in dark and callus development was observed. The contaminated culture vessels were removed from the environment immediately and the culture vessels without callus development were kept in the climate room for a prolonged duration.

Solvents. A 10-fold diluted version of the 2 N Folin–Ciocâlteu reagent stock solution was used; 7.5 g of solid Na_2CO_3 was weighed and dissolved in 100 mL of purified water. Using a protective mask, 2 g of solid $AlCI_3$ was weighed and dissolved in 100 mL of an equally mixed methanol/glacial acetic acid (50/50 mL) mixture.

A total of 50 mg solid gallic acid was weighed and placed in a beaker, and a few drops of ethyl alcohol and distilled water were added to dissolve. To adjust the concentration of the solution, it was transferred to a 100-mL volumetric flask and diluted to the mark of 100 mL with distilled water. The solution was then used as the stock solution for the calibration curve.

A total of 50 mg solid quercetin chemical was weighed and transferred to a 100-mL flask and diluted to the mark with distilled water. This solution was then used for the calibration curve.

A total of 3.57 g dipotassium hydrogen phosphate (K_2HPO_4) and 0.615 g potassium dihydrogen phosphate (KH_2PO_4) were weighed in 500-mL volumetric flasks and diluted with distilled water. The prepared buffer solution was stirred until the pH was fixed at 7.2–7.4.

A total of 20-mL phosphate buffer solution was added to 77 mg 2,2 az-azonobis (3-ethylbenzothiazoline-6-sulfonate, ABTS) and stirred until it dissolved. In a different beaker, 13 mg of potassium persulfate and 20 mL of phosphate buffer solution were mixed and dissolved with a stirrer. The two solutions were mixed and transferred to a foil-coated flask. The mixture was then allowed to stand at room temperature for at least 16 h until a dark blue colour appeared.

A total of 32-mg solid Trolox was weighed and transferred to a 50-mL volumetric flask and diluted with phosphate buffer. This was then used for the calibration curve.

In each callus extract used in the analyses, the concentration values were calculated by taking triplicate readings for each measurement in separate analysis conducted for ethanol–methanol solvents, and the explants separated as stem–leaf and the standard deviations of these concentration values were calculated. All the calculated results were noted in tables. Graphs were plotted and comparisons were made with these tables.

Table 1. Combinations of plant growth regulators used-1 (BAP)

Growth culture medium code	2,4-D (mg/L)	IBA (mg/L)	BAP (mg/L)	NAA (mg/L)	KIN (mg/L)
0,5B-0,25N	–	–	0.5	0.25	–
0,5B-0,5N	–	–	0.5	0.5	–
0,5B-1N	–	–	0.5	1	–
0,5B-2N	–	–	0.5	2	–
0,5B-0,25D	0.25	–	0.5	–	–
0,5B-0,5D	0.5	–	0.5	–	–
0,5B-1D	1	–	0.5	–	–
0,5B-2D	2	–	0.5	–	–
0,5B-0,25 I	–	0.25	0.5	–	–
0,5B-0,5 I	–	0.5	0.5	–	–
0,5B-1 I	–	1	0.5	–	–
0,5B-2 I	–	2	0.5	–	–

Table 2. Combinations of plant growth regulators used-2 (KIN)

Growth culture medium code	2,4-D (mg/L)	IBA (mg/L)	BAP (mg/L)	NAA (mg/L)	KIN (mg/L)
0,5K-0,25N	–	–	–	0.25	0.5
0,5K-0,5N	–	–	–	0.5	0.5
0,5K-1N	–	–	–	1	0.5
0,5K-2N	–	–	–	2	0.5
0,5K-0,25D	0.25	–	–	–	0.5
0,5K-0,5D	0.5	–	–	–	0.5
0,5K-1D	1	–	–	–	0.5
0,5K-2D	2	–	–	–	0.5
0,5K-0,25 I	–	0.25	–	–	0.5
0,5K-0,5 I	–	0.5	–	–	0.5
0,5K-1 I	–	1	–	–	0.5
0,5K-2 I	–	2	–	–	0.5

Table 3. Analysis results of ethanol extracts of the main plant

Plant part	Total phenolic (mg GAE/g)	Total flavonoid (mg quercetin/g)	Antioxidant capacity (mmol/g TEAC)
Leaf	9.72 ±1.21	10.10 ±1.16	12.30 ±1.09
Body	7.05 ±1.12	9.39 ±1.10	10.66 ±0.88

Table 4. Analysis results of methanol extracts of main plant

Plant part	Total phenolic (mg GAE/g)	Total flavonoid (mg quercetin/g)	Antioxidant capacity (mmol/g TEAC)
Leaf	10.35 ±1.20	9.77 ±0.89	13.51 ±1.20
Body	9.61 ±1.14	7.69 ±0.71	11.13 ±0.98

For the plant tissue culture, the MS medium that is often used in all studies and meets the basic nutritional requirements was used. The MS growth medium contains water and vitamins as well as macro–micro nutrients that are required by plants.

In this study, the combinations of naphthalene acetic acid (NAA), 2,4 dichlorophenoxyacetic acid (2,4-D), benzyl amino purine (BAP), indole butyric acid (IBA) and kinetin (KIN) plant growth regulators (PGR) were used in the MS growth medium (Tabs 1 and 2).

While preparing the medium, 4.4-g/L MS and 30-g/L sucrose was weighed for medium content and then dissolved with shaker after mixing [Murashige and Skoog 1962].

Total phenolic content analysis. The total phenolic analysis was performed as per the Folin–Ciocâlteu method applied by Singleton and Rossi [1965]. The total phenolic content of callus extracts obtained from *O. basilicum* L. plant species was analysed. The absorbance values were expressed as milligrams gallic acid equivalent per gram (mg GAE/g) as per the gallic acid calibration curve. In the equation $y = 2.092x + 0.080$ obtained from the gallic acid calibration curve, the concentration results were calculated by substituting 'x' with the absorbance values.

Total flavonoid content analysis. The methanolic form was used for analysing the total flavonoid content [Lamaison et al. 1990]. The total flavonoid content of callus extracts obtained from *O. basilicum* L. plant was analysed. The absorbance values were expressed in milligrams quercetin per gram (mg quercetin/g) as per the quercetin calibration curve. The concentration results were calculated by substituting 'x' with the absorbance values in the equation $y = 0.340x - 0.037$ obtained from the quercetin calibration curve.

Antioxidant capacity analysis. The antioxidant capacity of *O. basilicum* L. extracts was measured based on the ability of ABTS to capture radical cations.

To determine the antioxidant capacity of the callus extracts obtained from *O. basilicum* L. plant, the Trolox equivalent antioxidant capacity (TEAC) assay was performed. The absorbance values were expressed as millimole per gram (mmol/g) TEAC as per the Trolox calibration curve. The concentration values were calculated by substituting 'x' with the absorbance values obtained from the ethanol and methanol callus extracts in the equation $y = 0.680x - 0.003$ obtained from the calibration curve.

Total phenolic and total flavonoid content and antioxidant capacity analyses on *O. basilicum* L. calluses were also performed on the main plant parts (Tabs 3 and 4). Consequently, the results obtained from the calluses were compared with those from the main plant. For this purpose, the leaf and body parts of *O. basilicum* L. plant were included in the analysis.

RESULTS AND DISCUSSION

The antioxidant properties of *O. basilicum* L. plant were determined and the total phenolic substance, the total flavonoid and antioxidant capacity data of callus extracts with the same extractions, concentrations and

analyses methods were calculated. The results obtained are shown in Tables 5–10.

Generally, when comparing PGR combinations for ethanol extracts, more phenolic substances were observed to be detected in KIN combinations. The maximum amount of phenolic substance was reached in KIN – 2,4-D combinations. As per the results, the amount of phenolic substance was found to be the lowest at low concentrations of KIN – NAA PGR combination, BAP – NAA and BAP – 2,4-D PGR combinations (Tab. 5).

In BAP – NAA combinations, as the NAA concentration increased, an unstable result was obtained, and when other PGR concentrations increased, the total amount of phenolic substances increased proportionally. Similarly, it was observed that unbalanced results were obtained in KIN – 2,4-D combinations as the 2,4-D concentration increased. In the other combinations of PGR at different concentrations, as the concentration increased, the results of the amount of phenolic substance increasing in proportion to the concentration were obtained (Tab. 5).

For the analysis of the total phenolic substance in methanol extracts, in general, it was observed that more phenolic amount was determined on average in KIN combinations. The amount of phenolic substance reached was the highest in the combinations of KIN–NAA and BAP – 2,4-D PGR. As per the results, the amount of phenolic substance was determined to be the lowest in the concentrations of BAP – NAA PGR combination and BAP – IBA PGR combinations (Tab. 6).

In ethanol extracts, on average, more flavonoid substances were found in calluses grown in KIN-containing medium. The maximum amount of flavonoids was reached in KIN – 2,4-D combinations. As per the results, the flavonoid amount was determined to be the lowest in the low concentrations of KIN – NAA and BAP – NAA and BAP – 2,4-D PGR combinations (Tab. 7).

In BAP – NAA combinations, the optimum value of NAA concentration was 1 mg/L NAA for both leaf and body explants, whereas the amount of flavonoid substance decreased as the concentration increased or decreased. Similar values were obtained for different concentration values of KIN – IBA PGR combination (Tab. 7).

As per the results of the analysis, generally close results were obtained in total flavonoid substance analysis for methanol extracts. However, optimum values were seen in the combination of BAP – 2,4-D PGR for both leaf and body explants (Tab. 8). Considering the total antioxidant activity results (Tabs 9 and 10), the results in ethanol extracts were higher than the methanol extracts compared to the general average.

The concentration values were calculated by making three replicate readings for each measurement in each callus extract used in the analysis for total phenolic substance, total flavonoid substance and antioxidant capacity determination, and standard deviations of the values are given by calculation.

As per the results obtained, the highest antioxidant capacity was 4.186 ±0.394 mmol/g of TEAC for the methanol extracts and 5.736 ±0.201 mmol/g of TEAC (Fig. 1) for the ethanol extracts. As per the gallic acid calibration curve, the total phenolic content of ethanol extracts of *O. basilicum* L. calluses was between 1.044 ±0.188 and 0.417 ±0.049 mg of GAE/g. The total phenolic content of the methanol extracts was between 2.547 ±0.110 and 0.701 ±0.095 mg of GAE/g. As per the quercetin calibration curve, the total flavonoid content of the ethanol extracts of *O. basilicum* L. calluses was between 2.058 ±0.122 and 0.446 ±0.063 mg of quercetin/g. The total flavonoid content of the methanol extracts was between 3.010 ±0.336 and 0.341 ±0.041 mg of quercetin/g. Also, when the body–leaf explants were compared, the maximum antioxidant capacity was found to have been reached in the methanol solutions of the leaf explants (Figs 2 and 3). The amount of phenolic content was detected to be the lowest in the BAP – 2,4D combination, and the antioxidant capacity and flavonoid content were found to be the lowest in the BAP – IBA combination (Fig. 4).

However, as per the values obtained from the graphs, the optimum amount of matter for antioxidant capacity was detected in the BAP – NAA combination (Fig. 5). More so, when the body–leaf explants were compared, it was observed that the maximum amount of the total phenolic and flavonoid content values were reached in the methanol solutions of the body explants (Figs 6 and 7).

As per the values obtained from the graphs, the optimum amount of the total phenolic content was obtained from the KIN – NAA PGR combination (Fig. 8).

The antioxidant capacity was found to be the highest in the KIN – 2,4D combination. The phenolic

Table 5. Total phenolic matter values of ethanol callus extracts (mg GAE/g)

PGR combinations	Total phenolic matter (mg GAE/g)/ethanol
0,5B-0,25N (L)	0.490 ±0.123
0,5B-1N (B)	0.519 ±0.060
0,5B-1N (L)	0.569 ±0.153
0,5B-2N (B)	0.460 ±0.044
0,5B-2N (L)	0.477 ±0.144
0,5B-0,5D (B)	0.482 ±0.033
0,5B-0,5D (L)	0.417 ±0.049
0,5B-1D (B)	0.477 ±0.101
0,5B-2D (B)	0.632 ±0.095
0,5B-2D (L)	0.766 ±0.091
0,5K-0,25N (L)	0.419 ±0.063
0,5K-0,5N (L)	0.446 ±0.047
0,5K-1N (B)	0.455 ±0.051
0,5K-1N (L)	0.472 ±0.060
0,5K-2N (B)	0.720 ±0.077
0,5K-0,25D (L)	0.799 ±0.082
0,5K-0,5D (L)	0.701 ±0.094
0,5K-1D (B)	0.773 ±0.077
0,5K-1D (L)	0.624 ±0.069
0,5K-2D (L)	1.044 ±0.188
0,5K-0,25 I (B)	0.605 ±0.087
0,5K-1 I (B)	0.434 ±0.044
0,5K-1 I (L)	0.517 ±0.063
0,5K-2 I (B)	0.545 ±0.160
0,5K-2 I (L)	0.561 ±0.026

L – leaf part callus, N – NAA, B – BAP, D – 2,4-D, B – body part callus, I – IBA, K – KIN

Table 6. Total phenolic matter values of methanol callus extracts (mg GAE/g)

PGR combinations	Total phenolic matter (mg GAE/g)/metanol
0,5B-0,25N (B)	0.854 ±0.182
0,5B-0,5N (L)	0.812 ±0.093
0,5B-0,5N (L)	0.650 ±0.078
0,5B-1N (B)	1.060 ±0.034
0,5B-1N (L)	0.950 ±0.145
0,5B-2N (B)	0.752 ±0.088
0,5B-0,25D (B)	0.894 ±0.097
0,5B-0,25D (L)	1.201 ±0.208
0,5B-0,5D (B)	2.036 ±0.184
0,5B-0,5D (L)	1.235 ±0.155
0,5B-1D (L)	1.168 ±0.121
0,5B-2D (B)	1.653 ±0.097
0,5B-2D (L)	1.487 ±0.064
0,5B-0,5 I (B)	0.900 ±0.149
0,5B-0,5 I (L)	0.806 ±0.175
0,5B-1 I (L)	0.890 ±0.088
0,5B-2 I (B)	0.739 ±0.177
0,5B-2 I (L)	0.701 ±0.095
0,5K-0,25N (B)	1.258 ±0.069
0,5K-0,25N (L)	1.143 ±0.100
0,5K-0,5N (B)	2.547 ±0.110
0,5K-1N (B)	1.348 ±0.071
0,5K-2N (B)	0.911 ±0.091
0,5K-2N (L)	0.940 ±0.133
0,5K-0,25D (L)	1.100 ±0.159
0,5K-0,25D (B)	0.875 ±0.040
0,5K-0,5D (L)	1.441 ±0.089
0,5K-0,5D (B)	1.214 ±0.199
0,5K-1D (B)	1.670 ±0.053
0,5K-2D (B)	1.160 ±0.062
0,5K-2D (L)	0.927 ±0.157
0,5K-1 I (B)	1.406 ±0.111
0,5K-2 I (B)	0.877 ±0.086

Table 7. Total flavonoid matter values of ethanol callus extracts (mg quercetin/g)

PGR combinations	Total flavonoid matter (mg quercetin/g)/ethanol
0,5B-0,25N (L)	0.446 ±0.063
0,5B-1N (B)	1.106 ±0.132
0,5B-1N (L)	1.653 ±0.096
0,5B-2N (B)	0.806 ±0.044
0,5B-2N (L)	0.786 ±0.031
0,5B-0,5D (B)	2.004 ±0.148
0,5B-0,5D (L)	0.517 ±0.121
0,5B-1D (B)	1.184 ±0.066
0,5B-2D (B)	0.950 ±0.047
0,5B-2D (L)	1.466 ±0.142
0,5K-0,25N (L)	0.950 ±0.059
0,5K-0,5N (L)	2.058 ±0.122
0,5K-1N (B)	0.606 ±0.070
0,5K-1N (L)	0.704 ±0.061
0,5K-2N (B)	0.912 ±0.084
0,5K-0,25D (L)	0.514 ±0.028
0,5K-0,5D (L)	0.657 ±0.039
0,5K-1D (B)	1.656 ±0.079
0,5K-1D (L)	0.912 ±0.103
0,5K-2D (L)	0.970 ±0.076
0,5K-0,25 I (B)	1.490 ±0.140
0,5K-1 I (B)	1.320 ±0.074
0,5K-1 I (L)	1.503 ±0.033
0,5K-2 I (B)	1.245 ±0.080
0,5K-2 I (L)	1.644 ±0.103

Table 8. Total flavonoid matter values of methanol callus extracts (mg quercetin/g)

PGR combinations	Total flavonoid matter (mg quercetin/g)/metanol
0,5B-0,25N (B)	1.939 ±0.230
0,5B-0,5N (B)	1.874 ±0.156
0,5B-0,5N (L)	2.010 ±0.244
0,5B-1N (B)	1.286 ±0.163
0,5B-1N (L)	1.126 ±0.100
0,5B-2N (B)	1.378 ±0.128
0,5B-0,25D (B)	3.010 ±0.336
0,5B-0,25D (L)	1.673 ±0.184
0,5B-0,5D (B)	1.755 ±0.200
0,5B-0,5D (L)	2.234 ±0.193
0,5B-1D (L)	1.630 ±0.096
0,5B-2D (B)	1.585 ±0.176
0,5B-2D (L)	1.354 ±0.139
0,5B-0,5 I (B)	1.187 ±0.186
0,5B-0,5 I (L)	0.409 ±0.076
0,5B-1 I (L)	1.435 ±0.089
0,5B-2 I (B)	0.341 ±0.041
0,5B-2 I (L)	1.122 ±0.098
0,5K-0,25N (B)	1.551 ±0.134
0,5K-0,25N (L)	0.701 ±0.061
0,5K-0,5N (B)	1.150 ±0.103
0,5K-1N (B)	0.426 ±0.041
0,5K-2N (B)	1.148 ±0.133
0,5K-2N (L)	1.129 ±0.207
0,5K-0,25D (L)	1.133 ±0.164
0,5K-0,25D (B)	0.524 ±0.060
0,5K-0,5D (L)	1.072 ±0.144
0,5K-0,5D (B)	0.776 ±0.063
0,5K-1D (B)	1.439 ±0.179
0,5K-2D (B)	0.568 ±0.033
0,5K-2D (L)	1.194 ±0.099
0,5K-1 I (B)	1.854 ±0.145
0,5K-2 I (B)	1.333 ±0.212

Table 9. Antioxidant capacity values of ethanol callus extracts (mmol/g TEAC)

PGR combinations	Antioxidant capacity (mmol/g TEAC)/etanol
0,5B-0,25N (L)	4.145 ±0.230
0,5B-1N (B)	3.662 ±0.162
0,5B-1N (L)	4.084 ±0.145
0,5B-2N (B)	3.526 ±0.133
0,5B-2N (L)	4.404 ±0.108
0,5B-0,5D (B)	4.384 ±0.207
0,5B-0,5D (L)	3.914 ±0.156
0,5B-1D (B)	2.826 ±0.141
0,5B-2D (B)	3.302 ±0.167
0,5B-2D (L)	4.261 ±0.105
0,5K-0,25N (L)	4.349 ±0.130
0,5K-0,5N (L)	4.050 ±0.100
0,5K-1N (B)	4.424 ±0.141
0,5K-1N (L)	3.676 ±0.132
0,5K-2N (B)	4.295 ±0.096
0,5K-0,25D (L)	5.607 ±0.254
0,5K-0,5D (L)	5.736 ±0.201
0,5K-1D (B)	5.016 ±0.263
0,5K-1D (L)	3.343 ±0.188
0,5K-2D (L)	2.853 ±0.152
0,5K-0,25 I (B)	3.914 ±0.077
0,5K-1 I (B)	3.717 ±0.159
0,5K-1 I (L)	4.186 ±0.202
0,5K-2 I (B)	4.002 ±0.091
0,5K-2 I (L)	4.274 ±0.128

Table 10. Antioxidant capacity values of Methanol Callus Extracts (mmol/g TEAC)

PGR combinations	Antioxidant capacity (mmol/g TEAC)/methanol
0,5B-0,25N (B)	3.057 ±0.288
0,5B-0,5N (B)	3.227 ±0.186
0,5B-0,5N (L)	4.186 ±0.263
0,5B-1N (B)	3.744 ±0.0146
0,5B-1N (L)	3.819 ±0.133
0,5B-2N (B)	3.173 ±0.107
0,5B-0,25D (B)	3.010 ±0.088
0,5B-0,25D (L)	2.459 ±0.082
0,5B-0,5D (B)	1.942 ±0.125
0,5B-0,5D (L)	1.806 ±0.175
0,5B-1D (L)	3.683 ±0.210
0,5B-2D (B)	2.214 ±0.167
0,5B-2D (L)	2.085 ±0.132
0,5B-0,5 I (B)	4.111 ±0.211
0,5B-0,5 I (L)	3.448 ±0.153
0,5B-1 I (L)	3.785 ±0.113
0,5B-2 I (B)	1.050 ±0.211
0,5B-2 I (L)	1.970 ±0.095
0,5K-0,25N (B)	1.269 ±0.101
0,5K-0,25N (L)	1.738 ±0.79
0,5K-0,5N (B)	1.942 ±0.81
0,5K-1N (B)	3.118 ±0.143
0,5K-2N (B)	3.342 ±0.219
0,5K-2N (L)	4.180 ±0.394
0,5K-0,25D (L)	2.432 ±0.149
0,5K-0,25D (B)	2.989 ±0.104
0,5K-0,5D (L)	2.459 ±0.096
0,5K-0,5D (B)	2.500 ±0.130
0,5K-1D (B)	1.792 ±0.079
0,5K-2D (B)	1.344 ±0.085
0,5K-2D (L)	3.472 ±0.186
0,5K-1 I (B)	1.996 ±0.109
0,5K-2 I (B)	2.785 ±0.128

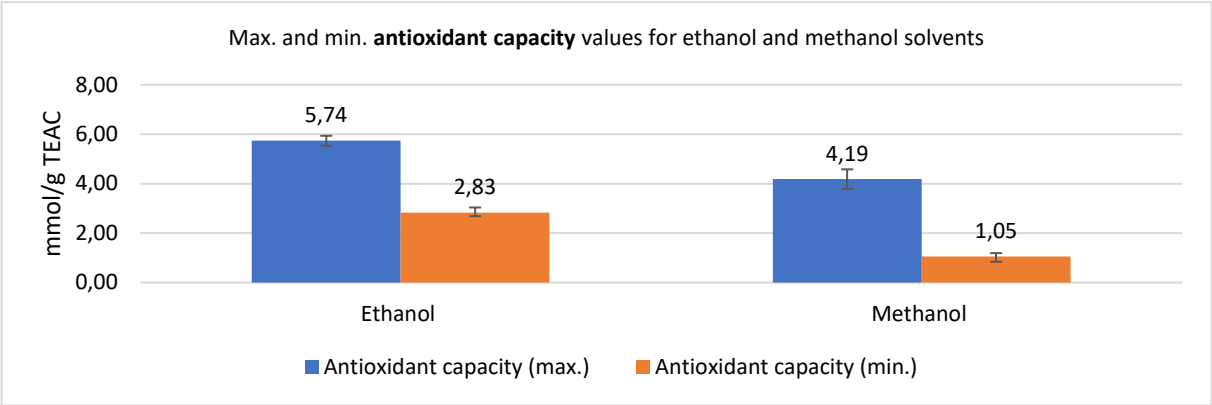

Fig. 1. Maximum and minimum total antioxidant capacity of *O. basilicum* L. callus ethanol/methanol extracts. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

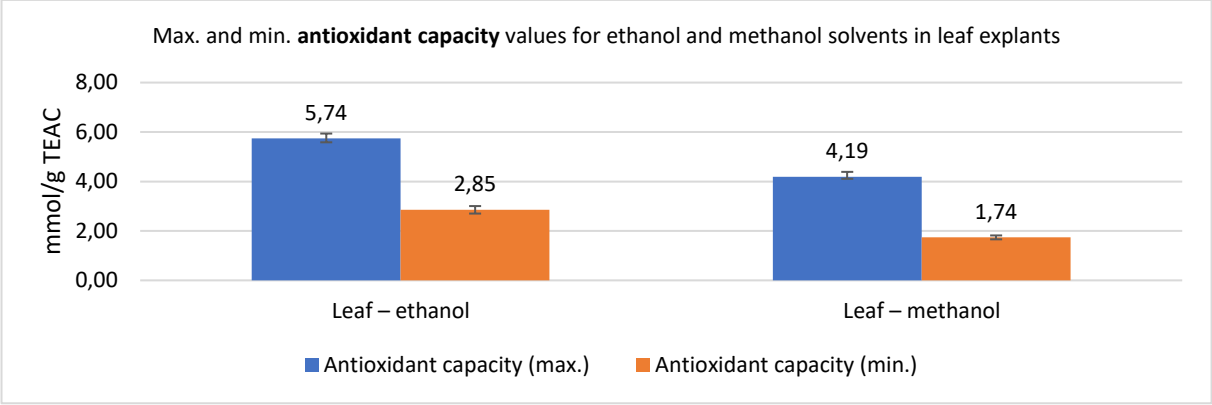

Fig. 2. Maximum and minimum total antioxidant capacity of *O. basilicum* L. leaf callus extracts. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

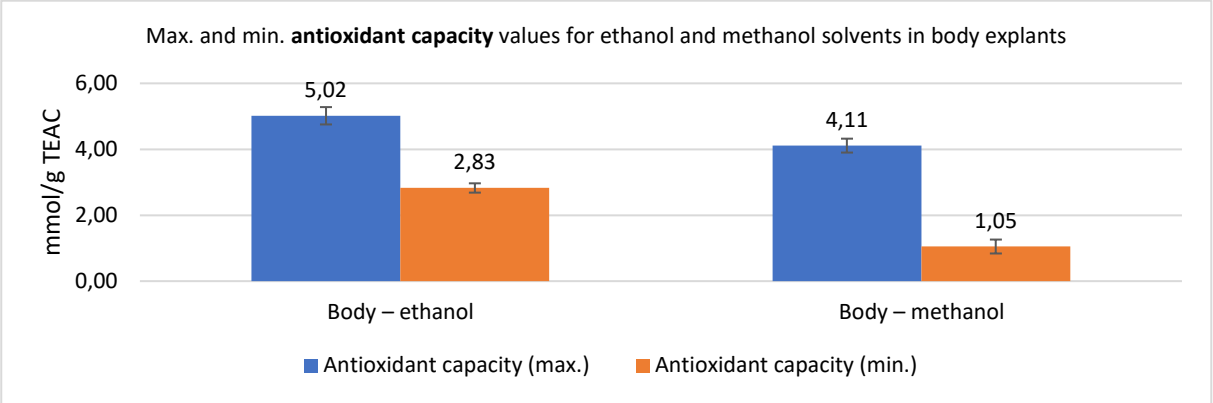

Fig. 3. Maximum and minimum total antioxidant capacity of *O. basilicum* L. body callus extracts. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

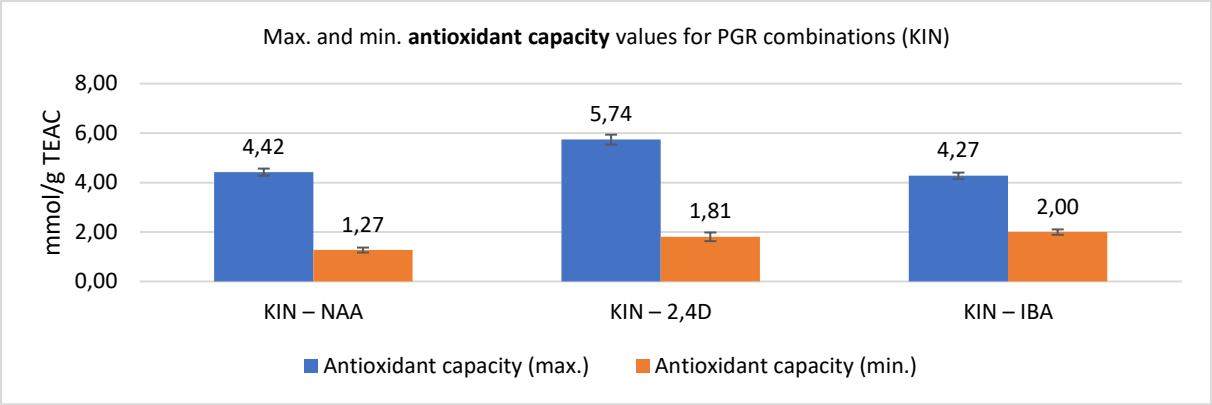

Fig. 4. Maximum and minimum total antioxidant capacity of *O. basilicum* L. callus extracts according to grown different plant growth regulators combinations. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

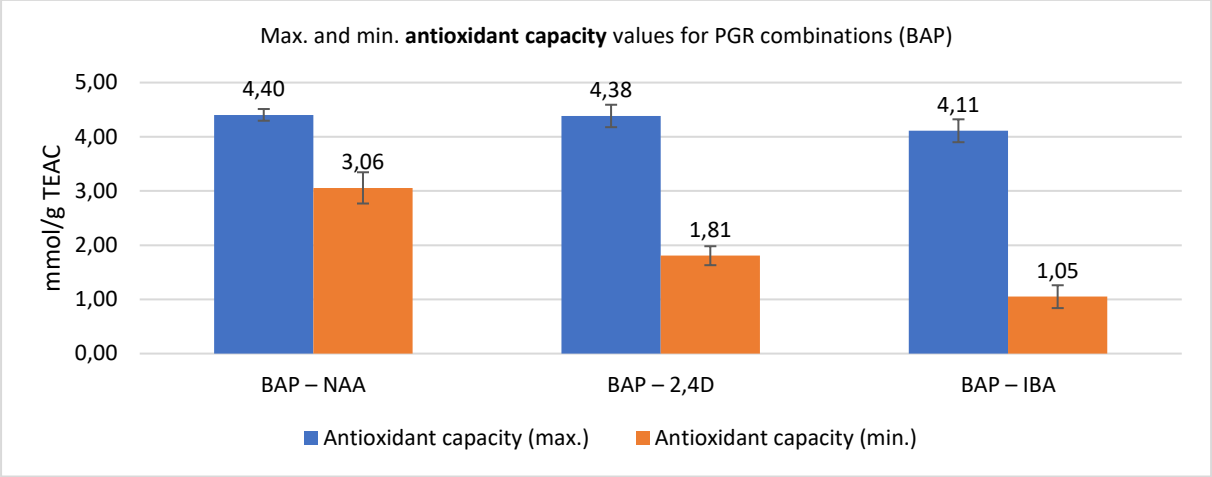

Fig. 5. Maximum and minimum total antioxidant capacity of *O. basilicum* L. callus extracts according to grown different plant growth regulators combinations. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

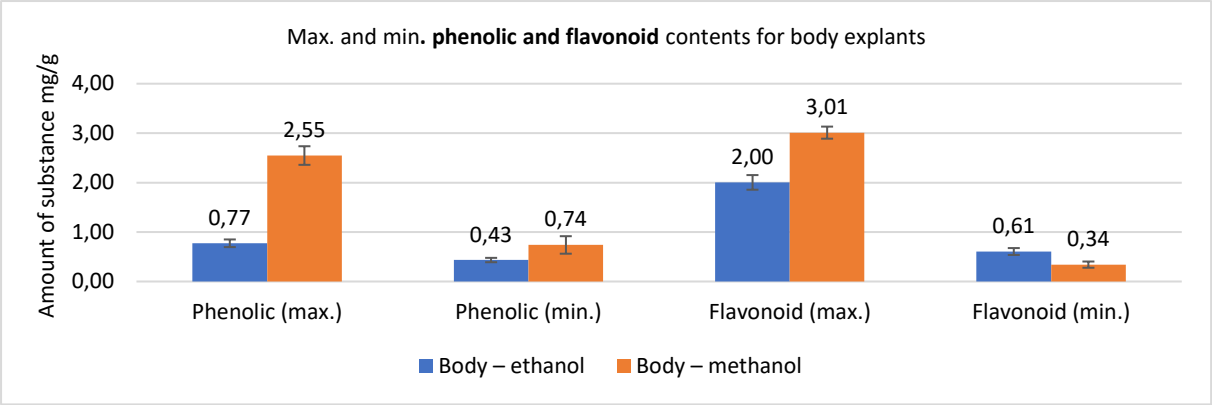

Fig. 6. Maximum and minimum total phenolic and total flavonoid content of *O. basilicum* L. body callus extracts. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

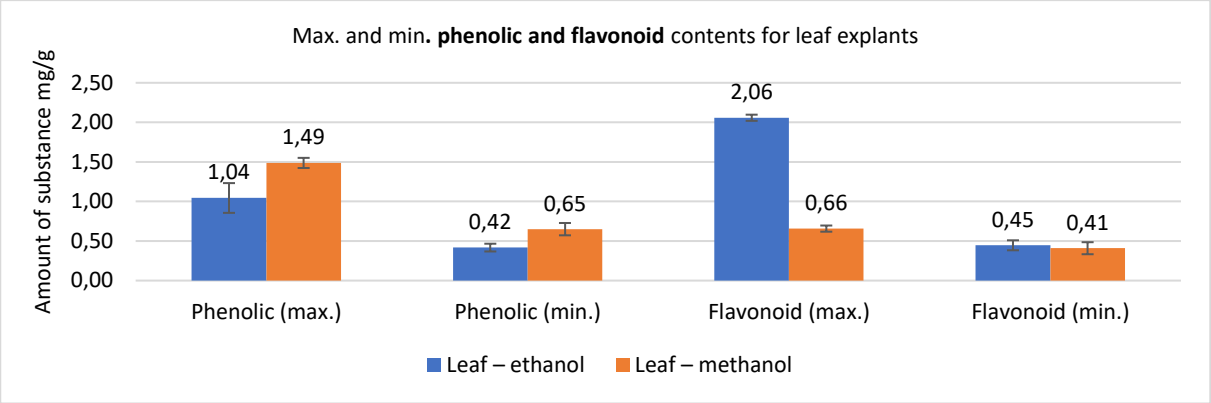

Fig. 7. Maximum and minimum total phenolic and total flavonoid content of *O. basilicum* L. leaf callus extracts. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

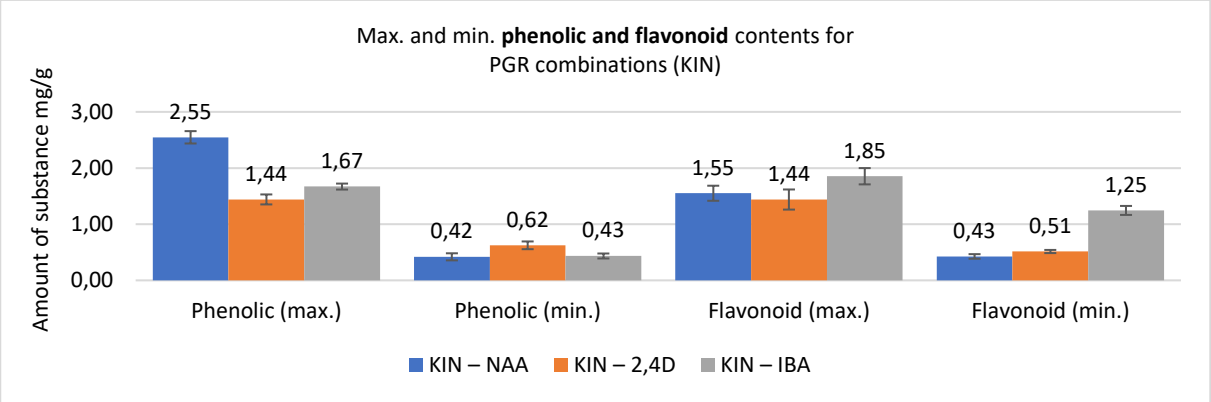

Fig. 8. Maximum and minimum total phenolic and total flavonoid content of *O. basilicum* L. callus extracts according to grown different plant growth regulators combinations. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

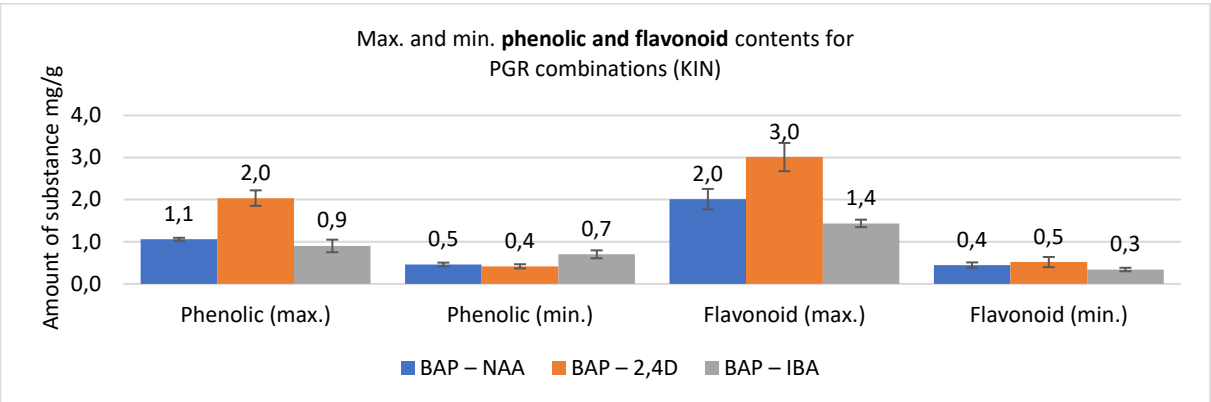

Fig. 9. Maximum and minimum total phenolic and total flavonoid content of *O. basilicum* L. callus extracts according to grown different plant growth regulators combinations. Data were presented as mean values (n = 3). The error bars represent standard deviations (±SD)

and flavonoid contents were detected to be the highest in the BAP – 2,4D combination (Fig. 9).

The minimum amount of the total flavonoid content was obtained from the PGR combination of BAP – IBA (Fig. 9).

Kaurinovic et al. [2011] in their studies on *O. basilicum* and *O. vulgare* plants, obtained total phenolic substance amounts and total flavonoid substance amounts from the water extracts of the plants. For O. basilicum, the total phenolic and total flavonoid substance amounts were 11.88 ±0.02 mg GAE/g and 26.42 ± 0.01 mg RE/g, respectively. When they performed the same analysis for *O. vulgare*, they found the total amount of phenolic and total flavonoids as 10.29 ±0.04 mg GAE/g and 25.31 ±0.07 mg RE/g, respectively.

In her thesis study, Çelebi [2010] studied the determination of phenolic substance distribution and antioxidant activity in *O. basilicum* L. plant. As per the results obtained in the study, the total phenolic and antioxidant amounts of dry basil plants of different types with each other were compared. Çelebi found the total phenolic concentration obtained from various basil plants in the range of 0.5–0.272 mg GAE/g.

In their study, Giri et al. [2012] produced calluses of Habenaria edgeworthii plant in different concentrations of benzyl adenine (BA) and methyl jasmonate (MeJA) PGR in MS medium. They found that the total phenolic content increased with increasing concentration of BA plant growth regulator and decreased with increasing concentration of MeJA PGR. They obtained the highest total phenolic content as 14.70 mg GAE/g in H. edgeworthii callus in 10 μM MeJA growth regulator supplement. In this study, the total amount of phenolic substance for *O. basilicum* L. raw leaf parts was calculated as 10.35 ±1.20 mg GAE/g.

Mabrouki et al. [2018] examined the total phenolic content of the M. officinalis subsp. officinalis plant using different solvents. As per their results, the total phenolic content was the highest in 63.00 mg GAE/g ethanolic extract and the lowest in 1.01 mg GAE/g hexane extract. In this study, because the calluses were studied more, the values obtained were lower than those obtained in the raw plant studies.

Kim et al. [2011] examined the total flavonoid content in the callus and leaves of the Stevia rebaudiana plant and obtained 1.57 mg quercetin/g in the callus of the plant. They found that the total flavonoid content in the leaves of the plant was higher than the callus value as 15.64 mg quercetin/g. In this study, the analyses' results obtained from the raw body and leaf parts of *O. basilicum* L. plant were higher than those obtained from the calluses.

It has been reported in some studies that the antioxidant potentials of callus extracts are substantially compared to in vivo materials, which can be further investigated for the natural antioxidant source. Salvia officinalis and Rosmarinus officinalis have been reported to have higher antioxidant activity in vitro than natural plant explants grown in the soil [Grzegorczyk et al. 2007, Yeşil-Celiktas et al. 2007].

CONCLUSIONS

As per the values obtained from the graphs, the optimum amount of matter for the three different analyses was obtained from the BAP – 2,4-D PGR combination. When the ethanol and methanol solvents were compared, it was found that the optimum solvent used for the analysis was methanol. A characterisation process may be applied for plant components to be specifically identified.

From the previous studies, it was observed that studies on *O. basilicum* L. were mostly conducted on the raw plant (leaf, leaf stem, stem, etc.). Because the chemical environments of these calluses growing with PGR and its combinations differed from each other, different values were obtained for each callus in the analyses performed in these studies.

Calluses were grown in the medium prepared with different PGR combinations. The analyses of total phenolic substance, total flavonoid substance and antioxidant capacity were performed. The highest and lowest values were obtained from these analyses. Calluses obtained from body and leaf explants were comparable among themselves. The PGR combinations used in this study were also compared. As per the general results, the antioxidant capacity analysis' results of the calluses obtained from the leaf explants of *O. basilicum* L. plant showed higher results for both solvents compared with the values obtained from the body explants.

In this study, four doses were prepared from each PGR combination (0.25, 0.5, 1 and 2 mg/L). The

analysis showed that as the PGR concentration increased, the analysis' values increased, decreased, or increased first and then decreased. Accordingly, the tables and figures indicate the combinations and the optimum concentrations.

As per the studies and analysis, the concentrations of the media content in which calluses are grown should be optimised in different PGR combinations with the highest values that should be applied. In this study, the values shown in the tables and graphs are expected to help many studies as per time and efficiency.

These optimisations will provide higher values in phenolic substance, flavonoid substance and total antioxidant capacity analyses using different solvents and PGRs, and increase efficiency in plant tissue culture studies

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