ORIGINAL ARTICLE

THE EFFECT OF PLANT GROWTH REGULATORS ON CAFFEIC ACID AND BETA CAROTENE QUANTITY IN THE CELL SUSPENSION CULTURES OF *CALENDULA OFFICINALIS* L. AND *CALENDULA ARVENSIS* L.

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ABSTRACT

Background: Calendula officinalis (pot marigold) L., known for its ornamental plant characteristics, is a medicinal plant which belongs to the Asteraceae (Compositae) family. **Methods**: The cell suspension cultures of *Calendula officinalis* and *C. arvensis* species with pharmacological importance were created in the four different MS media which were supplemented with different concentrations of NAA:BAP (1:1, 0.5:5 mg/l) and IAA:BAP (1:1, 0.5:5 mg/l) under sterile conditions. Caffeic acid and beta carotene quantity were researched at the end of every 30 days in the cell suspension cultures (stationary phase) during a total of 120 days. Statistical analysis was performed with Tukey Multiple Comparison Test. Maximum caffeic acid and beta carotene quantity were obtained from the cell suspension culture of *C. officinalis* species developing in MS1 nutrient medium (268,59 µg/g dry weight). **Results**: It was found that all nutrient media for the cell suspension culture (MS1, MS3, MS4, MS6) of *C. officinalis* species had more caffeic acid and beta carotene quantity than *C. officinalis* species. Caffeic acid and beta carotene quantity accumulating in the cell suspension culture of *C. officinalis* and *C. arvensis* species was more than is found in the leaves of Calendula species in the literature.

Keywords: Cell suspension culture, caffeic acid, beta carotene, *Calendula officinalis*, *Calendula arvensis*.

1. INTRODUCTION

Calendula officinalis (pot marigold) L., known for its ornamental plant characteristics, is a medicinal plant which belongs to the Asteraceae (Compositae) family. The species grows from 20 to 40 cm height and has 20 varieties. Its flower is yellow (Davis 1982). Its chemical constituents include triterpene glycosides, triterpene alcohols, flavanol glycosides, essential oil, polysaccharides and fatty oil (Gantait and Chattopadhyay 2005). Many studies have reported that the plant has pharmacological effects such as anti-cancer (Jimenez-Medina et al. 2006; Mazzio and Soliman 2009; Matić et al. 2013; Teiten et al. 2013), anti-microbial (Dumenil et al. 1980; Modesto et al. 2000; Efstratiou et al. 2012; Farjana et al. 2014; Vieira et al. 2014), anti-leishmanial (Nabi et al. 2012; Nikmehr et al., 2014), anti-HIV (Kalvatchev et al. 1997), antioxidant (Çetkovic et al. 2004; Erçetin et al. 2012; Babaee et al. 2013), cytotoxic, anti-tumor (Boucaud-Maitre et al. 1988; Jimenez-Medina et al. 2006; Ukiya et al. 2006), anti-viral (De Tommasi et al. 1991), anti-inflammatory (Hamburger et al. 2003; Ukiya et al. 2006), edema diuretic (Eglseer-Zitterl et al. 1997), hypoglycemic (Marukami et al. 2001), uterotonic (Shipochliev 1981) and lymphocyte activator effect (Jimenez-Medina et al. 2006) and is used in venous ulcer treatment (Duran et al. 2005) and for biligenic function (Ugulu et al. 2009).

Calendula genus contains 25 species. The most common of these are *C. officinalis*, *C. arvensis*, *C. suffruticosa, C. stellata*, *C. tripterocarpa*, and *C. alata* (Goncearіuc 2003). *C. officinalis* species are more effective than *C. arvensis* species in terms of pharmacological activity *C. arvensis* species is important with regards to pharmacological activity after *C. officinalis* species in the Calendula genus. The leaves and flowers of *C. officinalis* and *C. arvensis* species are used in medical treatment (Kemper 1999).

Plant cell cultures are important *in vitro* cultures used in the accumulation and production of secondary metabolites. Accumulation of secondary metabolites is induced by various stress factors (DiCosmo and Misawa 1985; Ebel and Mitho¨fer 1998; Dat et al. 2000; Lin et al. 2001). These cultures are important because they ensure the accumulation of secondary metabolites with pharmacological importance under controlled conditions (Grąbkowska et al. 2014). Cell suspension cultures are of great importance because homogeneous and high purity of secondary metabolites are important criteria (Zhang and Furusaki 1999; Sajc et al. 2000; Ramachandra Rao and Ravishankar 2002; Qu et al. 2006). Various interventions have been made to plant cell suspension cultures using chemicals or applications alone or in combination to increase the synthesis of secondary compounds in *in vitro* cultures. One of these interventions is the addition of plant growth regulators to *in vitro* cultures. In addition, growth of cells in the cell suspension cultures can be achieved more rapidly when compared with plants grown in the environment and under laboratory conditions. With the development of commercial production methods, some secondary metabolites and some products may or will be coverable. Culture conditions in the cell suspensions can be easily controlled (Misawa 1994).

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Caffeic acid has antioxidant (Vieira et al. 1998), anti-tumor (Tanaka et al. 1993), and anti-inflammatory (Fernandez et al. 1998) activity and the ability to inhibit HIV-replication (Kashiwada et al. 1995; Fesen et al. 1993). Some medical benefits have been identified. It was identified to be an effective subsidiary for the threat of erythropoietic protoporphyria and is used to reduce the risk of age-related macular degeneration and the risk of breast cancer in pre-menopausal women (Gandini et al. 2000; Seddon et al. 1994; Thomsen et al. 1979).

It was determined that beta carotene has chemical properties such as being an immunomodulator at low partial oxygen pressure, single deoxygenator and inhibitor of peroxy free radical reactions (Sies and Stahl 1995; Wang et al. 1999). Beta carotene was indicated to induce hepatic enzymes which detoxify carcinogens (Edes et al. 1989). Epidemiological studies have shown that high uptake of beta carotene reduces the risk of various diseases such as cancer and heart disease (Van Poppel and Goldbohm 1995; Ziegler et al. 1996).

The aim of this research was to investigate and compare the quantity of secondary metabolites (caffeic acid, beta carotene) produced in the cell suspension culture of *C. officinalis* and *C. arvensis* species.

2. MATERIALS AND METHODS

2.1. Plant Material

The cell aggregates developing in the cell suspension cultures were supplemented with MS1 (1 mg/l NAA+1 mg/l BAP), MS3 (0.5 mg/l NAA+5 mg/l BAP), MS4 (1 mg/l IAA+1 mg/l BAP), MS6 (0.5 mg/l IAA+5 mg/l BAP) nutrient media, with *C. officinalis* and *C. arvensis* species used as plant samples (Kaya, 2019). HPLC analysis was carried out for cell aggregates in the stationary phase of the cell suspension culture (0, 30^{th}, 60^{th}, 90^{th}, 120^{th} day).

2.2. Preparation of Standard Secondary Metabolites for HPLC Analysis

In order to analyze secondary metabolites, caffeic acid and beta carotene quantity were researched with HPLC in the cell suspension cultures. For this purpose, firstly extracts from samples were prepared in parallel with the solvents prepared in solutions with the appropriate concentrations of the standards for these secondary metabolites

Each stock solutions of standard secondary metabolite were prepared separately. For this purpose, while the caffeic acid standard was dissolved in methanol (CH_3OH); the beta-carotene standard was dissolved in n-hexane (C_6H_{14}), acetone (C_3H_6O) and ethanol (C_2H_6O) to prepare a separate stock solution for each standard. The solvents providing the best peaks for standards, the best peaks and the best amount of secondary metabolites for samples in HPLC were chosen for further study.

2.2.1. Preparation of Standard Caffeic Acid

The stock solution of standard caffeic acid (Roth,> 98%, Art.-Nr. 5869.3, 5 g) was prepared in a 100 ml flask at a concentration of 10 ppm with methanol (www.glsciences.com/technical/technicalnote/064/, June, 2016). This stock solution was diluted with methanol to concentrations of 8, 6, 4 and 2 ppm.

2.2.2. Preparation of Standard Beta Carotene

The stock solution of the beta carotene standard (97%, AB 139265, 1 g) was prepared in a 100 ml flask at a concentration of 50 ppm using acetone (LiChrosolv, for HPLC) and used for HPLC. The procedures used by Chen and Yang (1992) and Bhatnagar-Panwar (2015) were modified during standard preparation. This stock solution was diluted with acetone to 25, 15, 5 and 1 ppm concentrations.

2.3. Preparation of Sample Extracts for HPLC Analysis

Since sufficient callus could not be obtained from the MS0 nutrient medium, secondary metabolite analysis was performed from *C. officinalis* and *C. arvensis* species grown in the cell suspension culture containing MS1, MS3, MS4, and MS6 nutrient media. During the four cultures, cell aggregates obtained at the end of each culture (0, 30^{th}, 60^{th}, 90^{th}, 120^{th} day) from 0 day in the cell suspension culture were used for caffeic acid and beta carotene analysis.

Extraction of caffeic acid compounds from the samples was carried out by adapting the method applied by Rigane et al. (2013) and Riedel et al. (2010). According to this method, 1) 0.5 g of each cell aggregate is weighed. Extraction with pestle was carried out in a cold mortar by adding 2 ml of methanol, 2) The extracted sample is centrifuged at 4500 rpm for 5 min at + 4 °C by transferring to an Eppendorf, 3) The supernatant collected on the top was taken and transferred to a new Eppendorf, 4) The supernatants were concentrated in a water bath at 55 °C and flow MeOH was provided. The remaining residue was then dissolved in methanol.

To determine beta-carotene compound in the samples, the method applied by Bhatnagar-Panwar et al (2015) was altered. Basically, the method used to identify phenolic compounds is used in this method. Only acetone was used to extract the samples and to dissolve the residue remaining after the water bath.

Samples prepared by extraction and standard secondary metabolite solutions were passed through a membrane filter (non-pyrogenic, sartorius stedim biotech) with a diameter of 0.20 µm and injected into HPLC at 20 µl volume. A C18 column (GL Sciences Inc., Intertsil ODS-3, 5 µl molecular diameter, 4.6x150 mm size, S/N. 1A5151685, C/N. 5020-01731) was used.

For caffeic acid analysis, standards were analyzed using methanol (CH3OH): acetonitrile (CH3CN) (Sigma Aldrich 34851, for HPLC, gradient grade): 5% acetic acid (CH3COOH) (Sigma Aldrich 27225) (prepared with ultra-pure distilled water) [20: 20: 60] as the mobile phase (pH: 2.75), 1 ml/min at flow rate on HPLC equipment (Thermo Scientific, Dionex Ultimate 3000). The samples were researched during 10 min on HPLC. The temperature of the HPLC column was set at 40 °C (www.glsciences.com/technical/technicalnote/064/, June, 2016). Each sample was analyzed at 280 nm (www.glsciences.com/ technical/ technicalnote/064/, June, 2016), 330 nm (Rigane et al. 2013), 370 nm (Palacio et al. 2012), 450 nm (Dumbrava et al. 2013) and 475 nm (Radu et al. 2012; Linga Rao and Savithramma 2014) wavelengths during 10 min on HPLC.

For beta carotene analysis, methanol: acetonitrile: chloroform (Li Chrosolv, for liquid chromatography) (50:40:10) as solvent A mobile phase and methanol: acetonitrile: chloroform (35:35:30) as solvent B mobile phase (pH: 10.26) were used. The mobile phase was applied to the HPLC equipment at a flow rate of 1.2 ml / min in a multistep gradient by adjusting in the first 2 min with solvent A, in the next 6 min with solvent B and then followed by 4 min of solvent A. The samples were injected into HPLC at 20 µl volume and each sample was researched on HPLC for 12 min. The temperature of the HPLC column was set at 30 °C (Bhatnagar-Panwar et al. 2015). HPLC equipment was adjusted to 450 nm (Dumbrava et al. 2013), 455 nm (Strati et al. 2012; El-Qudah 2014), 461 nm (Varzakas and Kiokias 2016) and 475 nm (Olives Barba et al. 2006; Radu et al. 2012) wavelengths.

The mobile phases mentioned were initially sonicated with a sonicator (Bandelin Sonorex) for 15 min. After homogenization was completed, these mobile phases were placed in the HPLC. The measurements from plant samples were carried out in two repetitive readings on HPLC. Injections of each standard were performed on HPLC, separately.

2.4. Statistical Analysis

The Tukey Multiple Comparison Test was used to evaluate the effect of plant species, day and nutrient medium on secondary metabolite quantity.

3. RESULTS

3.1 Results of HPLC Analysis of Standard Secondary Metabolite Solutions

The peak retention time and peak area of each secondary metabolite standard were determined as a result reading in the HPLC. The caffeic acid standard peaked at 330 nm; while the beta carotene standard peaked at 450, 455, 461 and 475 nm. Moreover, the beta carotene standard had the best peak and retention time at 475 nm.
Quantities (µg/g) of the prepared standards and samples were calculated using the peak area.
The peak retention time, the peak area and the quantity of caffeic acid standard (10 ppm) dissolved in methanol and beta-carotene standard (50 ppm) dissolved in acetone are indicated in Table 1.

Table 1: The table prensents the HPLC results of caffeic acid and beta carotene standards.

Standard	Peak retention time (min.)	Peak area	Quantity (µg/g)
Caffeic acid	2.333	30.756	0,62
Beta carotene	9.614	165.979	3,94

3.2 Results of HPLC Analysis of the Sample Extracts

Samples were analyzed at the wavelengths (330 nm for caffeic acid; 475 nm for beta carotene) with the best peak and the best retention time on HPLC. It was determined that the maximum amount of caffeic acid and beta carotene were obtained in the MS1 nutrient medium of *C. officinalis* cell suspension culture according to caffeic acid and beta carotene peak area obtained from cell aggregates. The maximum quantity of caffeic acid was determined to be 268.585 µg/g dry weight in the MS1 nutrient medium of cell suspension of *C. officinalis* species at the end of the 4th culture (120th). The retention time for caffeic acid was established to be 2.328 min in this culture (Fig. 1).

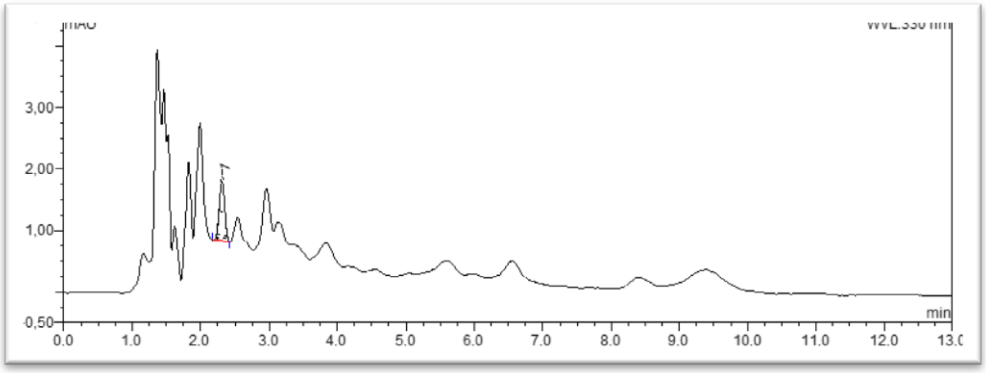

Figure 1: 1 Chromatogram of caffeic acid peak of *C. officinalis* cell suspension in MS1 nutrient medium at the end of 4th culture (120th).

The maximum caffeic acid quantity was determined to be 235.868 µg/g dry weight in the cell suspension of MS1 nutrient medium of *C. arvensis* species at the end of the 4th culture (120th). The retention time on HPLC chromatogram was confirmed to be 2.332 min (Fig. 2) in this culture.

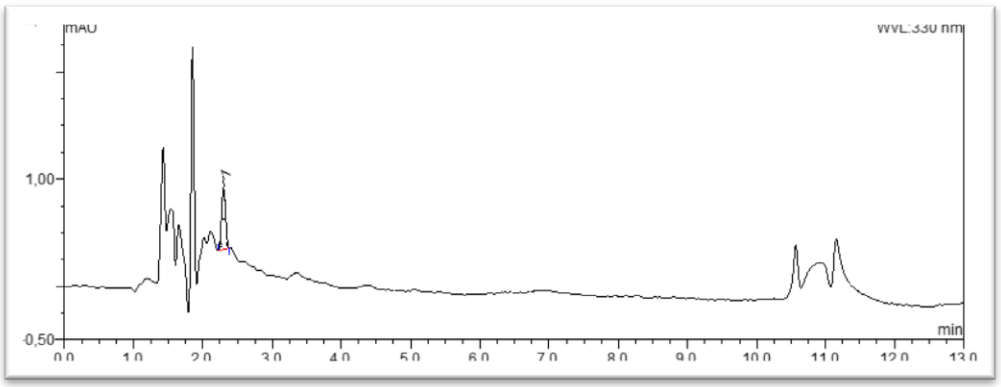

Figure 2: Chromatogram of caffeic acid peak of *C. arvensis* cell suspension in MS1 nutrient medium at the end of 4th culture (120th).

The maximum beta carotene quantity was established to be 523.685 µg/g dry weight in the cell suspension of MS1 nutrient medium of *C. officinalis* species at the end of the 4th culture. The retention time on HPLC chromatogram was determined to be 9.619 min in this culture (Fig. 3).

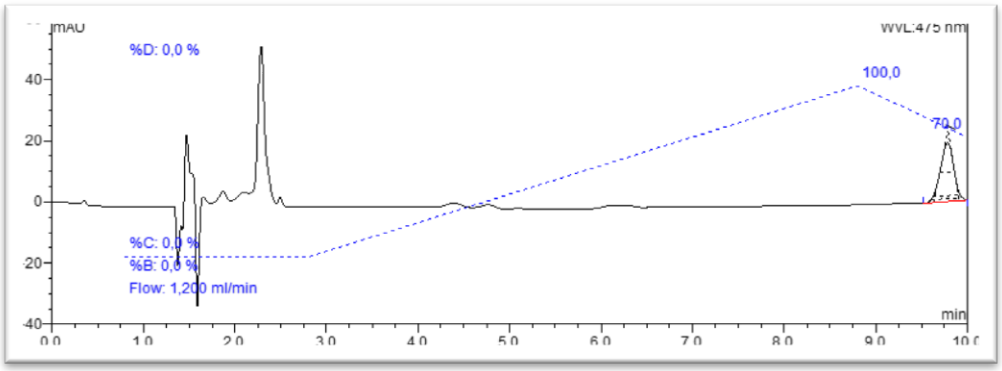

Figure 3: Chromatogram of beta carotene peak of *C. officinalis* cell suspension in MS1 nutrient medium at the end of 4th culture (120th).

The maximum beta carotene quantity was determined to be 385.115 µg/g dry weight in the cell suspension of MS1 nutrient medium of *C. arvensis* species at the end of the 4th culture (120th). The retention time on HPLC chromatogram was established to be 9.817 min in this culture (Fig. 4).

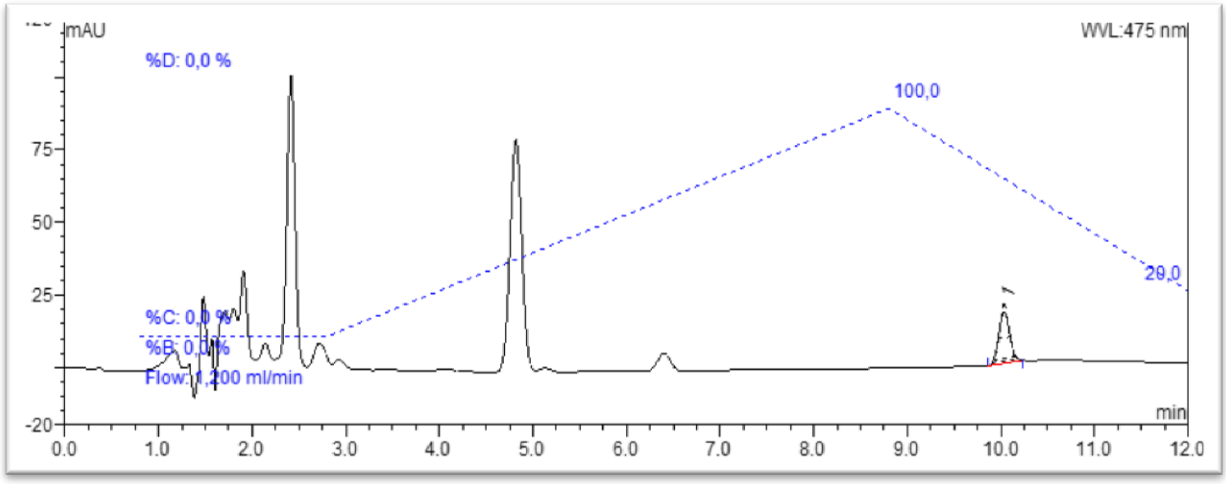

Figure 4 : Chromatogram of beta carotene peak of *C. arvensis* cell suspension in MS1 nutrient medium at the end of 4th culture (120th day).

When the amounts of caffeic acid in *C. officinalis* and *C. arvensis* were compared; the maximum caffeic acid quantity was obtained from MS1 nutrient medium of *C. officinalis* cell suspension culture (268.585 µg/g dry weight) and MS1 nutrient medium of *C. arvensis* cell suspension culture (235.865 µg/g dry weight) at the 120th day, respectively. The maximum caffeic acid quantity of *C. arvensis* species in the cell suspension culture at the 120th day was determined in MS1 and MS4 nutrient media, respectively. Beta carotene quantity on the 120th day of cell suspension culture in MS1 nutrient medium of *C. officinalis* and *C. arvensis* species was 523.685 µg/g and 385.115 µg/g dry weight, respectively.

In order to examine the effect of plant species, nutrient medium and day on the amount of caffeic acid and beta carotene, the Repeated Measured Variance Analysis Technique was used by considering day as the repeated measurement factor and the plant species and nutrient medium as normal. In order to determine which subgroup caused the difference, the Tukey Multiple Mix Test was used. Descriptive statistics and Multiple Comparison Test results are given separately in Table 2 (caffeic acid) and Table 3 (beta carotene).

As a result of repeated measurement analysis of variance, plant species X nutrition medium X day triple interaction had a statistically significant effect on both caffeic acid ($p \leq 0.05$) and beta carotene ($p \leq 0.05$). In other words, differences between nutrient media varied significantly according to plant species and day.

Table 2: Descriptive statistics and Tukey Multiple Comparison Test results according to plant species, nutrient medium and day with regard to caffeic acid quantity (µg/g dry weight).

		Day				
		0. day	**30. day**	**60. day**	**90.day**	**120. day**
Plant species	**Nutrient medium**	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
C. officinalis	**MS1**	4,06 ± 0,01 AeI	13,15 ± 0,10 AdI	27,25 ± 0,10 AcI	93,53 ± 0,23 AbI	268,59 ± 1,15 AaI
	MS3	1,17 ± 0,14 BCeI	6,49 ± 0,12 CdI	14,13 ± 0,12 CcI	49,14 ± 0,10 CbI	191,65 ± 0,20 CaI
	MS4	2,02 ± 0,10 BeI	9,03 ± 0,11 BdI	20,64 ± 0,11 BcI	73,99 ± 0,11 BbI	213,36 ± 0,10 BaI
	MS6	0,45 ± 0,10 CeI	5,35 ± 0,10 CdI	10,61 ± 0,11 DcI	34,76 ± 0,10 DbI	121,66 ± 0,20 DaI
C. arvensis	**MS1**	1,36 ± 1,31 ABeII	8,69 ± 0,02 AdII	20,68 ± 0,02 AcII	78,54 ± 0,42 AbII	235,87 ± 0,02 AaII
	MS3	0,65 ± 0,04 ABeI	2,69 ± 0,02 CdII	9,43 ± 0,02 CcII	47,23 ± 0,03 CbII	169,99 ± 0,05 CaII
	MS4	1,61 ± 0,03 AeI	5,63 ± 0,02 BdII	12,82 ± 0,02 BcII	50,82 ± 0,03 BbII	203,44 ± 0,05 BaII
	MS6	0,26 ± 0,03 BeI	3,78 ± 0,02 CdII	8,67 ± 0,02 CcII	23,17 ± 0,03 DbII	102,54 ± 0,04 DaII

Note 1. Differences between nutrient medium averages shown with different capital letters in the same plant species and same day is important ($p \leq 0.05$)
Note 2. Differences between day averages shown with different small letters in the same plant species and same nutrient medium is important ($p \leq 0.05$)
Note 3. Differences between plant species averages shown with different roman numerals in the same day and same nutrient medium ($p \leq 0.05$)

Table 3: Descriptive statistics and Tukey Multiple Comparison Test results according to plant species, nutrient medium and day with regard to beta carotene quantity (µg/g dry weight).

		Day				
		0. day	30. day	60. day	90. day	120. day
Plant species	Nutrient medium	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
C. officinalis	MS1	8,12 ± 1,72 AeI	17,12 ± 1,72 AdI	51,34 ± 5,18 AcI	184,55 ± 0,50 AbI	523,68 ± 0,04 AaI
	MS3	2,25 ± 0,63 AdI	4,56 ± 0,20 BdI	13,58 ± 0,14 CcI	35,47 ± 0,15 CbII	225,84 ± 0,10 CaII
	MS4	4,00 ± 1,05 AdI	11,00 ± 1,05 ABdI	25,30 ± 2,40 BcI	93,57 ± 6,16 BbI	325,48 ± 0,10 BaI
	MS6	1,17 ± 0,07 AdI	3,47 ± 0,11 BcdI	11,45 ± 0,20 CcI	35,87 ± 0,06 CbI	152,55 ± 0,11 DaI
C. arvensis	MS1	5,81 ± 0,57 AeI	14,81 ± 0,57 AdI	44,44 ± 1,71 AcI	177,96 ± 6,70 AbI	385,12 ± 0,10 AaII
	MS3	1,55 ± 0,07 AdI	5,21 ± 0,12 BdI	15,68 ± 1,74 BCcI	54,88 ± 6,09 CbI	236,33 ± 4,90 CaI
	MS4	2,71 ± 0,24 AdI	9,15 ± 0,79 ABdI	21,05 ± 1,83 BcI	75,22 ± 7,19 BbII	309,87 ± 0,04 BaII
	MS6	0,63 ± 0,02 AcI	2,20 ± 0,06 BcI	8,78 ± 0,22 CcI	39,50 ± 1,00 DbI	131,62 ± 2,50 DaII

Note 1. Differences between nutrient medium averages shown with different capital letters in the same plant species and same day is important (p≤0.05);
Note 2. Differences between day averages shown with different small letters in the same plant species and same nutrient medium is important (p≤0.05);
Note 3. Differences between plant species averages shown with different roman numerals in the same day and same nutrient medium is important (p≤0.05).

The findings of caffeic acid and beta carotene quantity determined in the cell suspension culture of *C. officinalis* and *C. arvensis* species are indicated on Fig. 5 and Fig. 6, respectively.

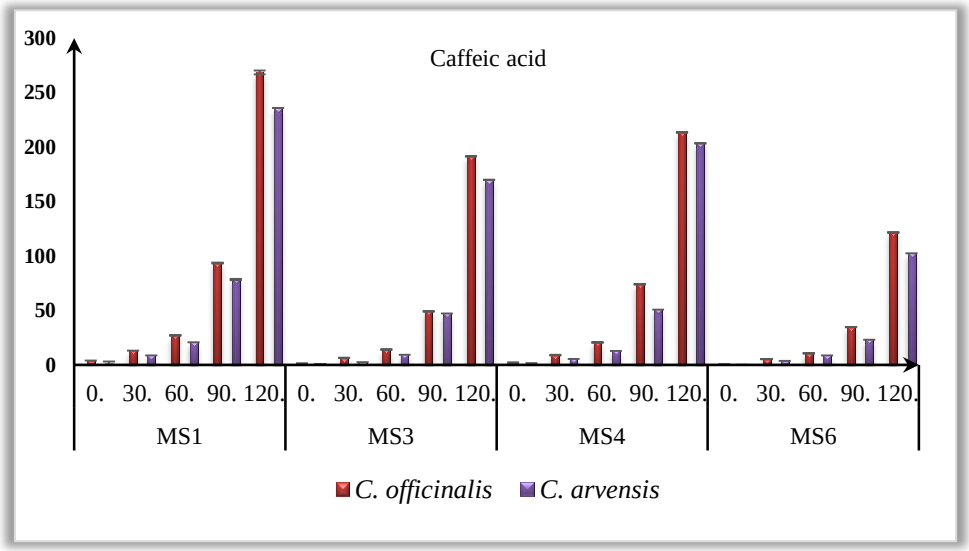

Figure 5: Caffeic acid quantity determined in the cell suspension culture of *C. officinalis* and *C. arvensis* (µg/g dry weight).

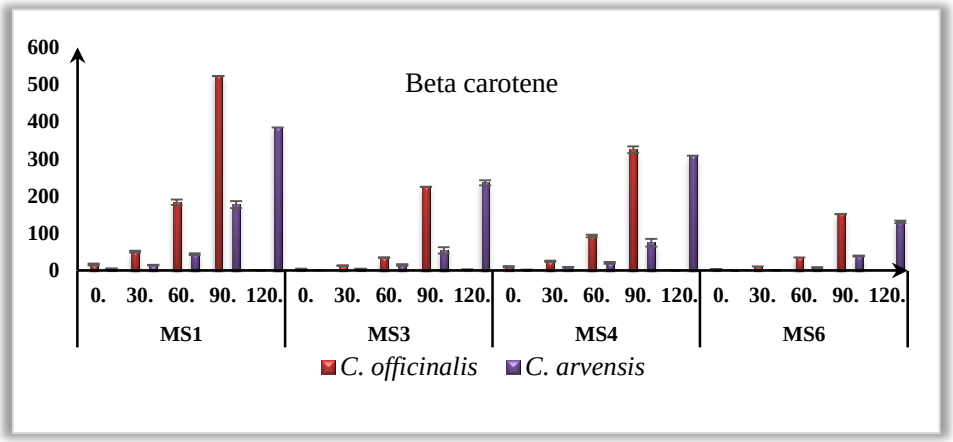

Figure 6: Beta carotene quantity determined in the cell suspension culture of *C. officinalis* and *C. arvensis* (µg/g dry weight).

4. DISCUSSION

Anca et al. (2012) found that caffeic acid quantity and the concentration of total phenolic derivatives in *C. officinalis* according to HPLC analysis were 148.3 µg/g and 2195.0 µg/g, respectively. In our research, maximum caffeic acid quantity was obtained from the cell suspension culture developing in MS1 nutrient medium of *C. officinalis* (268.59 µg/g dry weight) and *C. arvensis* species (235.87 µg/g dry weight) at 120th day.

Britton et al. (1995) determined that typical green tissues include β-carotene compound besides the various carotenoids. Bunghez and Ion (2011) found that beta carotene quantity using UV-VIS and FT-IR spectrophotometric methods was higher in the leaves of *C. officinalis* species. Bakó et al. (2002) carried out beta carotene composition analysis with HPLC in the roots, leaves, petals and pollen of *C. officinalis*. It was found that beta carotene compounds were mostly in the leaves. Nan et al. (2012) established that the percentage and quantity of β-carotene in the leaves of *Inula helenium* L. (Asteraceae) was mainly 38.7% and 18.84 µg/g, respectively. Total carotenoid quantity in the leaves was 48.7 µg/g fresh weight and beta carotene quantity in inflorescence was 1.33 µg/g. In our completed research, beta carotene quantity in *C. officinalis* species was remarkable. Dumbrava et al. (2013) determined the maximum total carotenoid quantity (1667.42 µg/g) and β-carotene quantity (145.45 µg/g) in the carotenoid extract prepared from *C. officinalis* flowers. Nan et. al. (2012) designated β-carotene quantity (1.33 µg/g) in the inflorescences of *Inula helenium* L. The maximum beta carotene quantity was determined to be 523.68 µg/g dry weight in our study. So, beta carotene quantity was found to be higher than in the literature.

Dlugosz et al. (2013) found a positive relationship between the concentration of secondary metabolites and fresh/dry weight occurring in the cell suspension culture of *C. officinalis*. In accordance with Dlugosz et al. (2013), caffeic acid and beta carotene quantity were maximum when fresh and dry weight was maximum (30th day in each culture) in our research. In contrast to our study, Heng et al (2013) stated that maximum weight was obtained on the 16th day in the cell suspension culture of Artemisia annua L. but the quantity of artemisinin secondary metabolite was not maximum on the 16th day. Dwivedi et al. (2016) reported that stevioside secondary metabolite occurred abundantly at log phase in the cell suspension culture of Stevia rabaudiana. Contrary to Dwivedi et al. (2016), caffeic acid and beta carotene quantity occurred more in the stationary phase (between 25th and 30th day) of the cell suspension culture in our research. Modarres et al (2018) was indicated that the highest amount of caffeic acid was obtained with 15th day of *Salvia leriifolia* cell suspension culture adding with 5 mg/L BAP: 5 mg/L NAA. Yang et al (2019) was stated that phenolic compounds, flavonoids, terpenoids, saponins and triterpenoids was found rich in *Helicteres angustifolia* cell suspension culture compared to wild roots. Ali et al (2013) caffeic acid quantification was carried out with HPLC in *Artemisia absinthium* cell suspension culture. This research's results was indicate that increased accumulation of phenolics can be obtained from *A. absinthium* cell suspension cultures. Naik and Al-Khayr (2018) was occurred *Phoenix dactylifera* cell suspension culture obtained from shoot tip induced calli in MS media supplemented with 1.5 mg L^{-1} 2-isopentenyladenine (2iP) and 10 mg L^{-1} NAA. It was found that highest biomass accumulation (62.9 g L^{-1} fresh weight and 7.6 g L^{-1} dry weight) and highest caffeic acid quantity (162.7 µg L^{-1}) was acquired from 11-week-old cell suspension cultures (at stationary phase). Açıkgöz et al (2018) was indiceted that the highest caffeic acid quantity obtained at 12th day of *Echinacea purpurea* cell suspension cultures supplemented with 1.0 mg/L BAP and 2.0 mg/L NAA in the light.

5. CONCLUSION

Our research is important with regards to being the first review of the accumulation of caffeic acid and beta carotene in the cell suspension culture containing different concentrations of NAA:BAP and IAA:BAP. So, the enhanced accumulation of medicinally important caffeic acid and beta carotene was carried out in the cell suspension culture of *C. officinalis* and *C. arvensis* species. It can be concluded that the cell suspension culture of *C. officinalis* L. has the potential to provide medicinally-important secondary metabolites. The cell suspension cultures were found to produce significantly higher levels of caffeic acid and beta carotene. Caffeic acid and beta carotene compounds accumulated in our research will be studied in bioreactors, which is the next step for commercial production by pharmaceutical industries, in order to further enhance the medicinally important phenolic acid and terpene compounds.

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