Ecological Life Sciences
ISSN: 1308 7258 (NWSAELS)
ID: 2018.13.2.5A0097

Status : Original Study
Received: September 2017
Accepted: April 2018

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DOI	http://dx.doi.org/10.12739/NWSA.2018.13.2.5A0097	
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DETERMINATION OF ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUND CONTENT OF BLACK CARROT CALLUS CULTURE

ABSTRACT

Callus culture, a method of plant tissue culture, is an alternative technique for the production of phenolic compounds that many industries need. In this study, antioxidant capacity, total phenolic, flavonoid and anthocyanin content of callus obtained from black carrot (*Daucus carota ssp. Sativus var. Atrorubens alef.*) were investigated. The antioxidant capacities of the extracts were determined by the free radical scavenging activity (DPPH) and the ferric reducing antioxidant power (FRAP) methods. The callus was derived from the MS medium (Murashige ve Skoog containing 2 mg/L 2.4-D, 0.2mg/L BAP, 30g/L sucrose and 2g/L phytagel using hypocotyl segments of plants grown in sterile conditions. The black carrot calli exhibited high DPPH free radical scavenging activity and FRAP activity in addition to high phenolic compound contents.

Keywords: Antioxidant Activity, Anthocyanin, Black Carrot, Callus Culture, Flavonoid, Phenolic Compound

SİYAH HAVUÇ KALLUS KÜLTÜRÜNÜN ANTİOKSİDAN AKTİVİTESİ VE FENOLİK BİLEŞİK İÇERİĞİNİN BELİRLENMESİ

ÖZ

Kallus kültürü bir bitki doku kültürü yöntemi olup birçok sektörün ihtiyacı olan fenolik bileşiklerin üretimi için alternatif bir tekniktir. Bu çalışmada, siyah havuç (*Daucus carota ssp. sativus var. atrorubens* Alef.) bitkisinde kallus kültürü oluşturulması ve elde edilen kallusların antioksidan kapasitesi, toplam fenolik, flavonoid ve antosiyanin içeriklerinin belirlenmesi amaçlanmıştır. Ekstraktların antioksidan kapasiteleri serbest radikal giderme aktivitesi (DPPH: 2.2-diphenyl-1-picrylhydrazyl) ve demir indirgeme antioksidan gücü (FRAP) metotlarıyla belirlenmiştir. Kallus indüksiyonu, steril koşullarda yetiştirilen bitkilerin hipokotil kısımlarının 2mg/L 2.4-D, 0.2mg/L BAP, 30g/L sükroz ve 2g/L phytagel içeren MS besi ortamında kültüre alınmasıyla (Murashige ve Skoog) sağlanmıştır. Siyah havuç kalluslarının yüksek miktarda fenolik bileşik içermesini yanında yüksek serbest radikal giderme aktivitesi (DPPH) ve demir indirgeme gücüne sahip olduğu belirlenmiştir.

Anahtar Kelimeler: Antioksidan Aktivite, Antosiyanin, Siyah Havuç, Kallus Kültürü, Flavonoid, Fenolik Bileşik

How to Cite:
Karataş, İ., Polat, F., Karataş, R., Dal, T., and Elmastaş, M., (2018). **Determination of Antioxidant Activity and Phenolic Compound Content of Black Carrot Callus Culture, Ecological Life Sciences (NWSAELS)**, 13(2):87-93. DOI: 10.12739/NWSA.2018.13.2.5A0097.

1. INTRODUCTION

Plant secondary metabolites are classified into three main groups, phenolics, terpenes and compounds containing N and S [16]. Flavonoids, which form an important part of the phenolic compounds, are composed of anthocyanins, chalcones, aurens, flavonols, flavanones, isoflavones and flavanonol [11, 13 and 26]. Anthocyanins are the most important visible pigment group after chlorophyll [5]. The red, blue and purple colors in flowers, fruits, vegetables and other plant tissues are mostly due to anthocyanins [17]. Anthocyanins have been found to have beneficial effects in human health, in addition to many functions in plants. Some of these effects are anti-allergic, anti-inflammatory, anti-viral, anti-mutagenic, anti-microbial, anti-carcinogenic, anti-diabetic and antioxidant activity [8]. Antioxidant compounds can scavenge free radicals and increase shelf life of food by retarding destruction process. Antioxidants have been widely used as food additives to provide protection against oxidative destruction of foods [10, 11 and 15].

In addition to the attractive colors of anthocyanins, water solubility allows these compounds to be used as natural colorants [2]. Due to the many negative properties of artificial colorant, demand for anthocyanins, a natural coloring agent, has increased [6]. It has been stated that anthocyanin extracts do not only impart attractive color properties to food, but also increase the oxidative stability of the foods they add [14]. The callus culture which is one of plant tissue culture methods have been found to be a viable biotechnological method for the investigation and production of valuable secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides, and food additives [20, 23, 25 and 28]. A number of strategies have been implemented to be able to produce secondary compounds in the plant cell, tissue and organ cultures [13 and 20]. The aim of this work was to determine total phenolic content, anthocyanin, total flavonoid content and antioxidant activity in black carrot callus. The antioxidant activity of callus was evaluated by measuring 1.1-diphenyl-2-picrylhydrazyl (DPPH.), ferric reduction power (FRAP).

2. RESEARCH SIGNIFICANCE

Black carrot has an attractive bluish purple in addition to the content of the five anthocyanin pigments derived from cyanide. The black carrots anthocyanins are used as a natural food colorant due to their high heat, light and pH stability. For this reason, black carrot extracts are widely used in the coloring of juices, candies, ice cream, soft drinks and other fermented drinks [13 and 18]. Production of anthocyanins using plant cell culture methods is an important technology in academic and industrial aspects. The reason for examining the production possibilities of anthocyanins by these methods is to form a process in which a large scale production can be carried out. For this purpose, studies were carried out different plant species [6 and 27]. Production of secondary metabolites using these methods is advantageous in many respects. The advantages include uniform quality and continuous supply of products in addition to independent production of environmental conditions [23].

3. EXPERIMENTAL METHOD-PROCESS

In this study, black carrot (*Daucus carota ssp. Sativus var. Atrorubens alef*) plants were used as experimental material.

3.1. Cultivation of Plants under Sterile Conditions

The plants used in the present experiment were grown in sterile conditions. The medium used for germinating the seeds and cultivating sterile seedlings was prepared by adding 30g/L sucrose and 2g/L phytagel to 4.4g/L MS medium (Murashigeand and Skoog) [19]. The pH of the medium was adjusted to 5.8 using 1M NaOH or 1M HCl and then autoclaved at 121°C, 1.2 atmospheres pressure for 20 minutes. The seeds were subjected to surface sterilization before germination. The surface sterilization of the seeds was achieved by incubation in 70% ethanol for 2 minutes and in 2% sodium hypochlorite solution containing twenn 20 for 30 minutes. The plants were raised at 25±2°C, 16 hours light and 8 hours in dark photoperiod [12].

3.2. Induction of Callus

Hypocotyl portions of one month old plants were used as explant source in callus development. The explants were cultured in a medium prepared by adding 2mg/L 2.4-dichlorophenoxy acetic acid (2.4-D), 0.2mg/L benzylaminopurine (BAP), 30g/L sucrose and 2g/L phytagel to the basal MS medium (Murashige and Skoog). After 45 days, the callus samples were harvested to make the expressed analyzes and was dried at 45°C in incubator [12].

3.3. Preparation of Extracts for Chemical Analysis

One gram of dry callus samples was extracted in a 10mL methanol/dichloromethane mixture (4:1). The extract was vortexed and then placed in an ultrasonic bath for 20 minutes. Extracts were used for total phenolic compound, flavonoid and antioxidant activity analyzes.

3.4. Determination of Total Phenolic Content

The amount of total phenolic contents was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [24]. 0.1mL of the extract solution was mixed with water (4.6mL). 0.1 ml of Folin-Ciocalteu reagent was added and the content of the flask mixed. After 3 min, 0.3mL of Na_2CO_3 (2%) was added and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760nm in a spectrophotometer. The amount of total phenolic compounds determined as microgram of gallic acid equivalent (GAE) using an equation that was obtained from calibration curve of gallic acid graph.

3.5. Flavonoid Analysis

The flavonoid content was determined according to formation of aluminium-flavonoid complex. The reaction mixture was formed from extract (0.1mL), ethanol (1.5mL), $AlCl_3$ (0.1mL, %10) and CH_3COONa (0.1mL, 1M). The final volume of the mixture was 5mL with water and then incubated for 30 min in room temperature. The absorbance was measured at 427nm in a spectrophotometer. The amount of flavonoid determined as microgram of quercetin equivalent (QUE) using an equation that was obtained from calibration curve of quercetin graph [22].

3.6. Anthocyanin Content

The content of anthocyanin was determined by making minor changes in the method described by Giusti and Wrolstad (2001). The calluses were extracted in a mixture of 1% HCl and methanol and then filtered with a 0.22µm diameter filter. The absorbance was measured at 530nm in a spectrophotometer. The anthocyanin content of the samples was calculated using the molar absorbance coefficient of cyanidin 3-O-glucoside (ε:30200, MA:449.2) [9].

3.7. DPPH• Radical Scavenging Activity

The DPPH radical scavenging activity was determined according to method Blois with minor modifications [4]. DPPH solution (0.135mM) in ethanol (1.0mL) was added the samples solution at different concentrations. The ethanol was added for final solution to be 4.0mL. After shaking vigorously, the mixture was incubated at room temperature in dark for 30 min. The absorbance was recorded at 517 nm on a spectrophotometer. The results of DPPH activity were expressed as IC_{50} was described as the sample concentration that caused a decline in the initial DPPH concentration by 50%.

3.8. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

The ferric ions (Fe^{3+}) reducing antioxidant power of callus extract were determined by the method of Oyaizu (1986) with slight modification. Different amounts of extract were mixed with sodium phosphate buffer (1.25mL, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1.25mL, 1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 1.25mL) and $FeCl_3$ (0.25mL, 0.1%) were added to the mixture, respectively. Absorbance of these mixtures was measured at 700nm using a UV spectrophotometer (Hitachi U-2900). Results were given as µmol trolox equivalent/g DW [21].

4. FINDINGS AND DISCUSSIONS

The callus obtained from the plants grown on sterile conditions were dried and extracted in methanol-dichloromethane for the analysis of total phenolic and flavonoid content and antioxidant activity. The results of the metabolite content in callus were shown in Figure 1. These results were obtained from callus samples dried at 45°C in incubator.

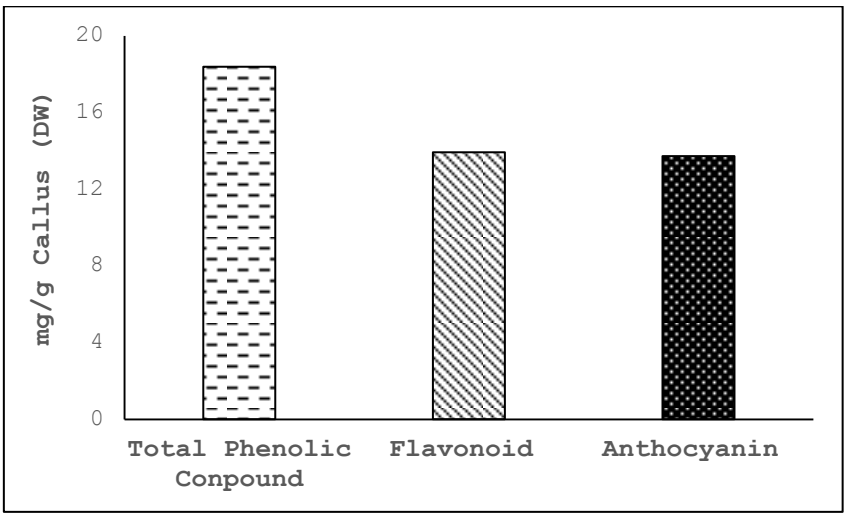

Figure 1. Contents of total phenolic, flavonoid and anthocyanin of black carrot callus

Total phenolic content was determined by Folin-Ciocalteu method and the results were given as gallic acid equivalent. Total phenolic compound contents were present at levels of 18.38±1.57mg GAE/g DW in callus. Montilla et al. (2011) reported that the total content of phenolic compounds varied between 17.9±1.4 and 97.9±3.2mg GAE/100g FW in different black carrot cultivars [18]. In another study, it was stated that the total phenol contents in Antonina and Purple Haze cultivars of black carrot were 187.8±9.0 and 492.0±63mg GAE/100g FW, respectively [1]. The content of anthocyanin was determined to be

13.72±0.62mg/g DW. These results show that 74.64% of the total phenolic compound content of dry callus samples is anthocyanins. Algarra et al. (2014) expressed that the anthocyanins constituted 25%-50% of the total phenolic compound content in different black carrot varieties [1]. It is stated that the content of total anthocyanin of black carrot was 10.8±0.6g/kg db [3]. In the light of these results, the anthocyanin content of the black carrot callus was considerably higher than that of the black carrot. In addition, the ratio of anthocyanins in the total phenolic compound is higher in calluses than in black carrot.

The antioxidant capacities of the callus extracts were determined by the free radical scavenging activity (DPPH: 2.2-diphenyl-1-picrylhydrazyl) and the ferric reducing antioxidant power (FRAP) methods. DPPH· scavenging assay method is based on the determination of the reducing ability of antioxidants from DPPH· to DPPH-H which is non-radical form. Reducing power of compounds indicates the electron-donating capacity [11]. According to FRAP method, the reduction of Fe^{3+} to Fe^{2+} was determined by measuring absorbance of the Perl's Prussian blue complex which has a strong absorbance at 700 nm [7 and 11]. In this study, the DPPH radical scavenging activity and the ferric reducing antioxidant power of black carrot callus were found to be IC_{50}=100.14±1.04µg/mL and 258.63±25.19µmol TE/g DW, respectively. Bilek at al. studied DPPH radical scavenging activity of black carrot (raw material) and gave results as a EC_{50} which was described as the sample concentration that caused a decline in the initial DPPH concentration by 50%. According to their report, the inhibition concentration (EC_{50}) was determined to be 2.75±0.12g/mL db. [3]. Compared with these results, callus extracts have higher DPPH radical scavenging activity than black carrots.

5. CONCLUSION AND RECOMMENDATIONS

In the light of these data, it is thought that the black carrot callus culture is a suitable technique to produce anthocyanins for many sectors because of the high antioxidant activity and high amount of anthocyanin content of callus. In this context, there is a need to establish bioreactor systems that will produce large scale production and to determine mass production possibilities.

NOTE

This study was presented as an oral presentation at the I. International Scientific and Vocational Studies Congress (BILMES 2017) in Nevşehir/Ürgüp between 5-8 October 2017.

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