KSÜ Tarım ve Doğa Derg 22(Ek Sayı 2): 388-394, 2019
KSU J. Agric Nat 22(Suppl 2): 388-394, 2019
DOI:10.18016/ksutarimdoga.vi.589216

Determination of Antioxidant Activity of Ethanol Extract of Gölevez [(*Colocasia esculenta* (L.)] Tubers

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ABSTRACT

In this study, the antioxidant activity of the ethanolic extract of *Colocasia esculenta* tubers were determined by four different antioxidant tests including DPPH and ABTS radicals scavenging activities, metal chelating activity and reducing power. The scavenging effect of extract of *C. esculenta* tubers and standards on DPPH radical at the highest concentration (600 µg mL^{-1}) decreased in the following order: Vitamin C>Trolox>*C. esculenta*>BHA and were found as 95.4, 93.6, 83.8 and 78.8 %, respectively. The scavenging effect of *C. esculenta* tuber extract and standards on ABTS radical at the highest concentration (100 µg mL^{-1}) decreased in the order: Trolox = BHA>*C. esculenta* and were found as 100, 100, 94.6 %, respectively. The metal chelating capacity of extract of *C. esculenta* tubers and standards decreased in the order of *C. esculenta*>BHA>Trolox at lowest concentration (100 µg mL^{-1}) and was found to be 78.0, 76.0, 63.5 %, respectively. Reducing power of extract of *C. esculenta* tubers and standards at the highest concentration (600 µg mL^{-1}) followed the order: BHA>Trolox>*C. esculenta*. Total phenolic compound and flavonoid amounts of *C. esculenta* tubers were designated as 2400 mg GAE/kg extract and 2050 mg QE/kg extract, respectively.

Research Article

Article History
Received : 09.07.2019
Accepted : 23.08.2019

Keywords
Colocasia esculenta
Antioxidant activity
Phenolic content
Radical scavenging

Gölevez [(*Colocasia esculenta* (L.)] Yumrularının Etanol Ekstresinin Antioksidan Aktivitesinin Belirlenmesi

ÖZET

Bu çalışmada Gölevez (*Colocasia esculenta*) yumrularının etanol ekstresinin antioksidan aktivitesi DPPH ve ABTS radikallerini giderme aktiviteleri, metal şelatlama aktivitesi ve indirgeme gücü gibi dört farklı antioksidan metotla belirlendi. *C.esculenta* yumrularının ekstreleri ve standartların en yüksek konsantrasyonda (600 µg mL^{-1}) DPPH radikali üzerindeki giderme etkisi Vitamin C >Trolox>*C.esculenta*>BHA sırasına göre azaldı ve sırasıyla % 95.4, 93.6, 83.8 ve 78.8 olarak bulundu. *C. esculenta* yumrularının ekstreleri ve standartların en yüksek konsantrasyonda (100 µg mL^{-1}) ABTS radikali üzerindeki giderme etkisi Trolox=BHA>*C.esculenta* şeklinde azaldı ve sırasıyla % 100, 100 ve 94.6 olarak bulundu. *C.esculenta* yumrularının ekstreleri ve standartların en düşük konsantrasyonda (100 µg mL^{-1}) metal şelatlama aktiviteleri *C. esculenta*>BHA>Trolox sırasına göre azaldı ve sırasıyla % 78.0, 76.0 ve 63.5 olarak bulundu. *C. esculenta* yumrularının ekstreleri ve standartların en yüksek konsantrasyonda (600 µg mL^{-1}) indirgeme güçleri BHA>Trolox>*C.esculenta* şeklinde sıralandı. *C.esculenta* yumru ekstrelerinin toplam fenolik bileşik ve flavonoid miktarları sırasıyla 2400 mg GAE/kg ekstre ve 2050 mgQE/kg ekstre olarak belirlendi.

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 09.07.2019
Kabul Tarihi : 23.08.2019

Anahtar Kelimeler
Colocasia esculenta
Antioksidan aktivite
Fenolik içerik
Radikal giderme

To Cite : Akyüz M 2019. Determination of Antioxidant Activity of Ethanol Extract of Gölevez [(*Colocasia esculenta* (L.)] Tubers. KSU J. Agric Nat 22 (Suppl 2): 388-394. DOI: 10.18016/ksutarimdoga.vi.589216

INTRODUCTION

Reactive Oxygen Species (ROS) are produced by various biochemical reactions in the human body and living organisms (Dehpour et al., 2009) during normal cellular functions (Mates, 2000). They become toxic when their levels increased (Ferreira et al., 2007). Excessive ROS and production of other radical molecules can damage biomolecules like proteins, carbohydrates, polyunsaturated fatty acids and DNA (Brighente et al., 2007). ROS also can cause many sicknesses like Parkinson's disease, cancer, diabetes, rheomatrid arthritis, aging, ischemia and liver disorders (Gowri and Madhavan, 2013). Gülçin (2010) reported that ROS play a role in more than 100 ailments related to excessive ROS. Antioxidants are often added to foods for retarding the oxidation process. These compounds can also clear free radicals and delay the advance of plenty of chronical sicknesses (Gülçin, 2012). Nowadays, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate are among the most prevalent used antioxidants in food and pharmaceutical industries (Ak and Gülçin, 2008). However, BHA and BHT are both potent antioxidants but have been reported to have carcinogenetic activity (Madsen and Bertelsen, 1995). Therefore, interest in natural and safer antioxidants from natural products has been increased (Gülçin et al., 2012).

Colocasia esculenta is commonly known as Gölevez in Turkish folk medicine (Tanker et al., 2014). Also, it is grown in Souheast Asia, Cyprus and Turkey, especially in Mersin and Anamur (Tanker et al., 2014). Moreover, it was locally cultivated and used as a vegetable in India (Yadav et al., 2017). It belongs to the family of Araceae (Tuti et al., 2015) and is a tropical perennial plant, specific to Asia and the Pacific and widely spread out in tropical regions (Li et al., 2014). *Colocasia esculenta* include dietary fiber, protein and vitamins including vitamin C, thiamine, riboflavin and niacin (Yadav et al., 2017) and minerals such as iron, potassium, sodium and zinc (Nakharekar and Berde, 2016). Therefore, it is used as a source of protein, vitamins and starch (Sheikh and Tembhre, 2016). It is also recognized for its color, flavor and therapeutic value (Nakharekar and Berde, 2016). *C. esculenta* is reported to display some biological activities including antidiabetic, anti-inflammatory (Li et al., 2014; Tuti et al., 2015; Yadav et al., 2017) antibacterial, antifungal, anthelmintic (Tuti et al., 2015), antioxidant and anticancer effects (Li et al., 2014; Yadav et al., 2017). Its edible corms and leaves are traditionally used for hepatic ailments (Nakharekar and Berde, 2016).

This study was carried out in order to determine the antioxidant activities of the ethanolic extracts of *Colocasia esculenta* (L.) tubers. The results were compared to standard antioxidants, which are Vitamin C, BHA and Trolox. At the same time, in the extract total phenolic compound and flavonoid amounts were detected.

MATERIALS and METHODS

Materials and Extraction Procedure

Colocasia esculenta (L.) tubers collected in Anamur, Mersin, and Mediterranean region of Turkey. The ethanolic extract was prepared according to Elmastaş et al., (2006). Peels of tubers thinly separated with a knife and the tubers dried in an oven at 40 ˚C. Then 25 g *Colocasia esculenta* tuber was powdered by blender and mixed with 500 mL ethanol. Residual uptake was continued until the extraction solvent had lost its color. The extracts were combined and filtered. The ethanol was taken out with rotary evaporator at 40 °C. The remaining extract transferred to a plastic flask and protected at -20 °C until used.

DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the ethanolic extract was determined according to the Blois method (1958). For this purpose, 1 mL solution of DPPH (0.1 mM) was added to 3 mL of sample extract at various concentrations (100-600 µg mL^{-1}). The mixture incubated in room condition in dark about thirty minutes. The absorbance was spectrophotometrically recorded at 517 nm. BHA, Trolox and Vitamin C were used as standard radical scavengers. Radical scavenging activity was calculated by following equation:

Radical scavenging activity (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$

ABTS radical scavenging activity

ABTS radical scavenging effect of the ethanol extract was conducted as indicated in method of Re et al. (1999) and Gülçin (2009). Firstly, 2 mM ABTS and 2.45 mM $Na_2S_2O_8$ solutions were mixed in a 1: 2 ratio and kept for 6 hours in the dark. The absorbance was read at 734 nm. To adjust the absorbance, the melange was diluted with phosphate buffer (0.1 M, pH: 7.4) when the absorbance was higher than 0.75. The sample was then placed in test tubes at different concentrations (5-100 µg mL^{-1}). Phosphate buffer was added to obtain a total volume of 3 mL. After this process, 1 mL of ABTS radicals was added to the mixture, then was vortexed and waited at room temperature for half an hour. Finally, its absorbance was spectrophotometrically recorded at 734 nm. The scavenging capability of extract and standards were calculated from equation, which given below:

ABTS scavenging effect (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$

Metal chelating activity

The chelating activity of *C. esculenta* tubers extracts and standards was determined in the light of literature (Dinis et al., 1994). Shortly, 0.4 mL of $FeCl_2$ (2 mM) and 0.4 mL of ferrozine (5 mM) solutions were added to the extracts of varied concentrations (100-600 µg mL^{-1}). The volume was completed to 4 mL with ethanol. The mixture was strongly vortexed and waited at room heat for 10 min and the absorbances were recorded by spectrophotometry at 562 nm. Metal chelating effect of extract and standards were calculated from following equation:

Metal chelating effect (%) = $[(A_{Control} - A_{Sample})/A_{Control}]\times100$

Reducing power

The reducing power effect of the extract was measured by taking the procedure proposed by Oyaizu (1986). Varied concentrations of *Colocasia esculenta* (L.) tuber extracts (100-600 µg mL^{-1}) were put into test tubes and 2.5 mL phosphate buffer (0.2 M, pH: 6.6) and 2.5 mL (1 %) potassium ferricyanide solutions were added. The mixture was thoroughly mixed, incubated at 50 ˚C for 20 min. Then 2.5 mL of TCA (10 %) solution was added to, and centrifuged for 10 min at 3000 rpm. Then 2.5 mL of supernatant and 0.5 mL of $FeCI_3$ (0.1 %) were mixed. The absorbance of the reaction mixture was read and recorded at 700 nm.

Determination of total phenolic content

Total phenolic compounds in *C. esculenta* tuber extracts were determined according to literature (Slinkard and Singleton, 1977) using gallic acid as a standard phenolic compound. Shortly, 1 mL of the extract solution was taken up in a volumetric flask and diluted with pure water (46 mL) and 1 mL Folin-Ciocalteu reagent was added and mixed well. After 3 minutes, 3 mL sodium carbonate (2 %) was added, then the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm in a spectrophotometer. The total phenolics in ethanolic extract was determined as milligram of gallic acid equivalent by using an equation that was obtained from a standard gallic acid graph.

Determination of total flavonoid amount

Total flavonoid quantification of the extracts was done as indicated in the literature (Moreno et al., 2000; Park et al., 1997) and was expressed as mg quercetin equivalent (QE). Briefly, 90 mg of extract was prepared with 15 mL of ethanol and 1 mL of was taken to a test tube. Then 0.1 mL of aluminum nitrate (10 %) and potassium acetate (1.0 M) were added and vortexed and the volume was completed with ethanol to obtain 4 mL. It was waited at room heat for forty minutes and the absorbance was read at 415 nm.

RESULTS and DISCUSSION

It has been reported that natural antioxidants show a large spectrum of biological effects such as antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory activities (Gülçin et al., 2010). Antioxidant capacity is commonly used to for characterizing foods or medicinal plants and their biologically active ingredients (Telci et al., 2009). Many antioxidant techniques and modifications are used to evaluate antioxidant capacity (Gülçin et al., 2004).

DPPH free radical scavenging activity

DPPH method is widely used to determine the antioxidant effect (Baydar, 2013). The method is based on electron-transfer that produces a purple solution in ethanol. If there is an antioxidant molecule in the ambit, the DPPH radical is reduced and leads to a colorless ethanol solution. Since the method is easy and fast, it may be useful to evaluate different products in terms of antioxidant activity at one time with spectrophotometry (Garlica et al., 2012).

C.esculenta tubers extracts scavenging effect and standards on the DPPH radical at the highest concentration (600 µg mL^{-1}) decreased in the order of Vitamin C>Trolox>*C. esculenta*>BHA and were 95.4, 93.6, 83.8 and 78.8 %, respectively and at lowest concentration (100 µg mL^{-1}) decreased in the order of Trolox>Vitamin C>BHA>*C. esculenta*> and were 88.7, 82.4, 63.4 and 24.2 %, respectively. The results were shown in figure 1.

ABTS radical scavenging activity

The ABTS radical scavenging method is often used to evaluate the antioxidant capability of foods (Fitriana et al., 2016) and biological samples (Shang et al., 2018). *C. esculenta* tubers extracts scavenging effect and standards at the lowest concentration (5 µg mL^{-1}) on ABTS radical decreased in order: Trolox>BHA> *C. esculenta* and were 100, 96.6 and 6.3 %, respectively and at highest concentration (100 µg mL^{-1}) decreased in the order of Trolox=BHA>*C. esculenta* and were 100, 100 and 94.6 %, respectively. The results were shown in figure 2.

Metal chelating activity

The metal chelating activity of an antioxidant prevents the oxidative formation and consequently oxidative detriment. Metal chelation plays an important role in antioxidant mechanisms as it reduces the transition metal concentration. These

ions are powerful catalysts and are capable of initiation lipid peroxidation particularly in cell membranes (Ruiz Ruiz et al., 2015). The metal chelating effect of *C. esculenta* tubers and standards decreased in the order of *C. esculenta*>BHA>Trolox at the lowest concentration (100 µg mL^{-1}) and were found as 78.0, 76.0 and 63.5 %, respectively and decreased in the order of Trolox>*C. esculenta*>BHA at the highest concentration (600 µg mL^{-1}) and were found as 71.5, 68.0 and 64.0 %, respectively. The results were shown in figure 3.

Reducing power

Reducing power of a compound may function as an important reflection of its potency antioxidant capacity (Benslama and Harrar, 2016). Reducing power of *C. esculenta* tubers and standards at the highest concentration (600 µg mL^{-1}) followed the order: BHA>Trolox>*C. esculenta,* respectively and at the lowest concentration (100 µg mL^{-1}) followed the order: BHA>Trolox>*C.esculenta,* respectively. The results were shown in figure 4.

Determination of total phenolic compound amount

Phenolics are the most important compounds that were found in plants (Elmastaş et al., 2006). It was emphasized that the antioxidant activity of the plants was caused by phenolic compounds (Mathangi and Prabhakaran, 2013). These particularly have strong antioxidant, antimicrobial, and antiviral effects and make strong the organisms and prevent diseases (Liaudanskas et al., 2017). Total phenolic compound amount of *C. esculenta* was determined as 2400 mg GAE/kg extract.

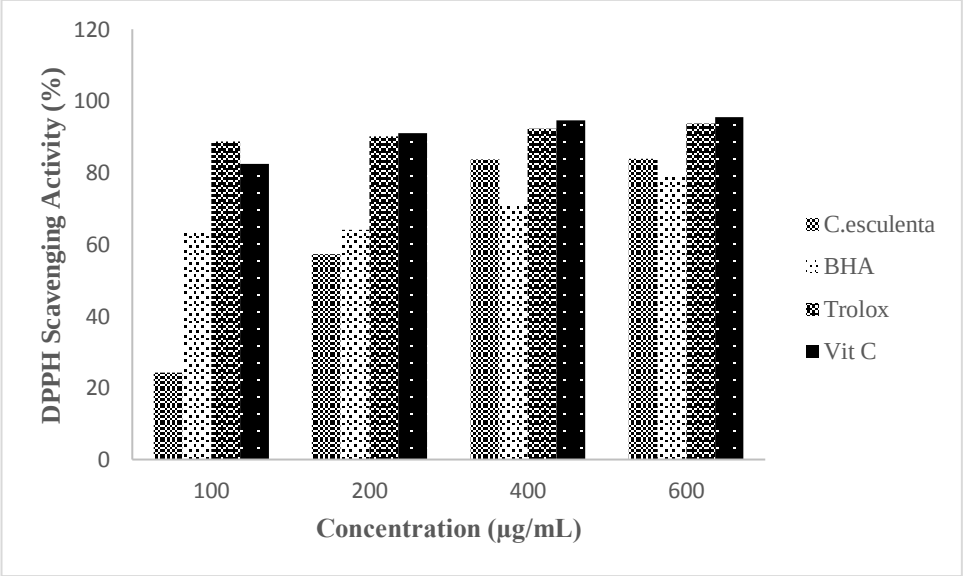

Figure 1. DPPH free radical scavenging activity of *Colocasia esculenta* tubers and standards

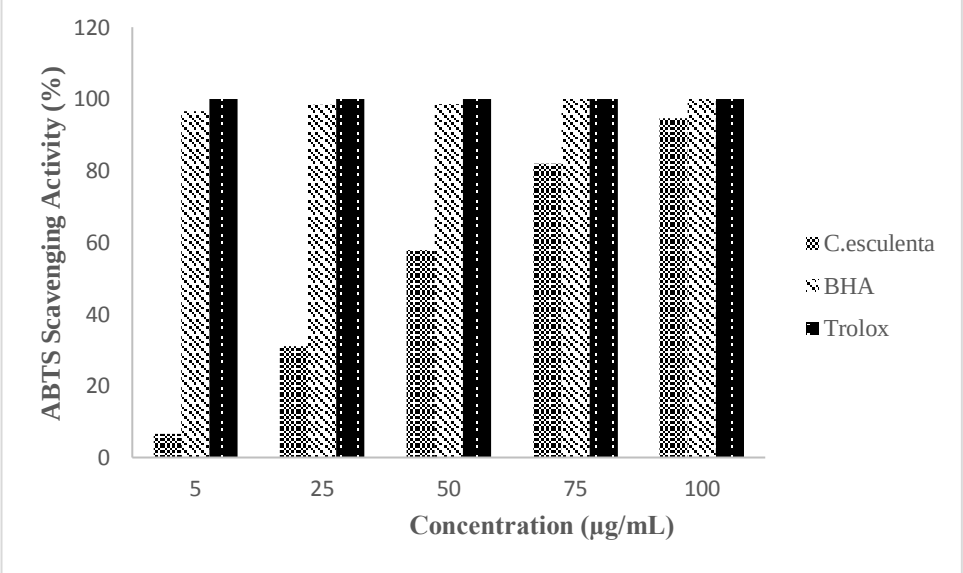

Figure 2. ABTS scavenging activity of *Colocasia esculenta* tubers and standards

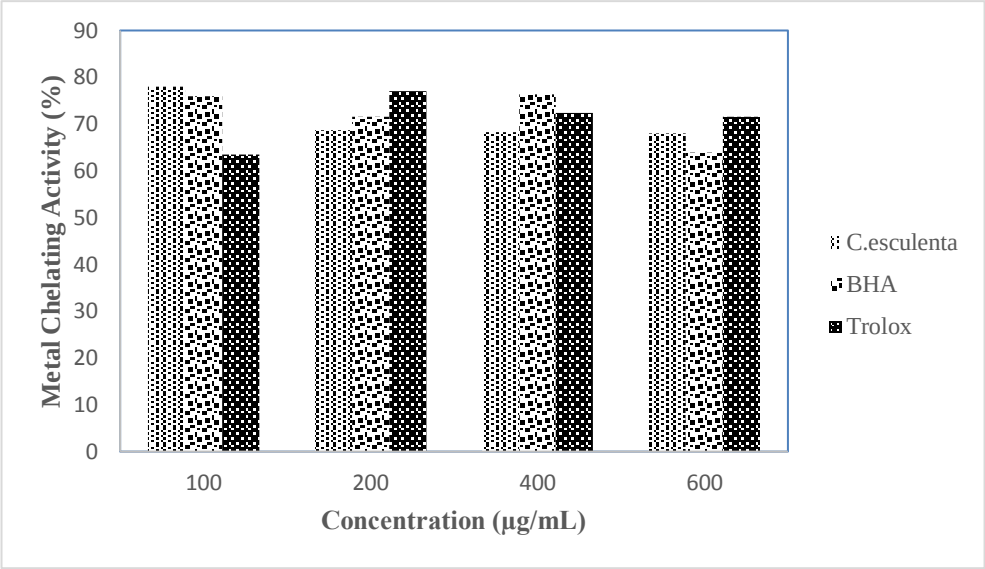

Figure 3. Metal chelating activity of *Colocasia esculenta* tubers and standards

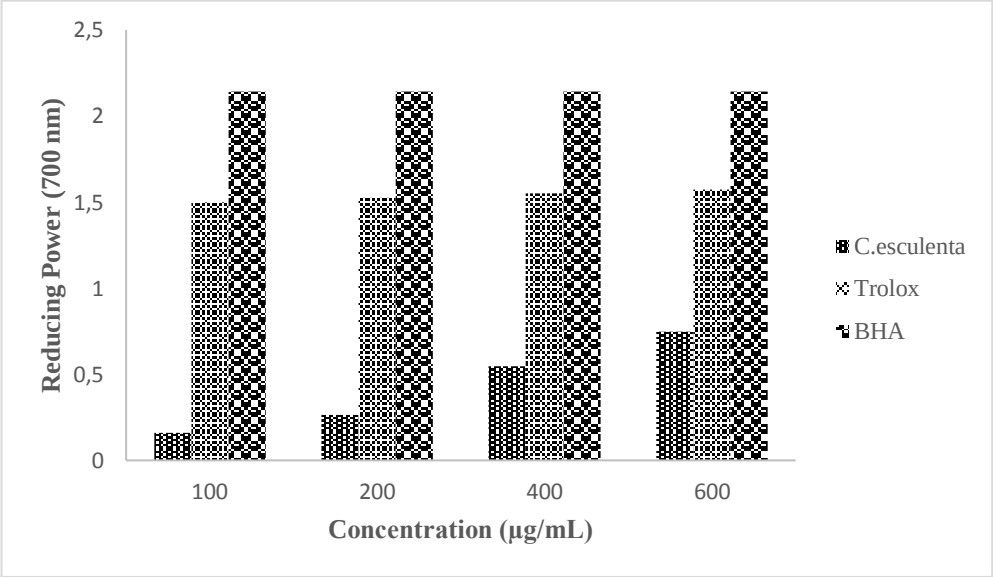

Figure 4. Reducing power activity of *Colocasia esculenta* tubers and standards

Determination of total flavonoid amount

Flavonoids are responsible for antioxidant effects and prevent the growth of bacteria and viruses and provide resistance to cancer and heart attack (Baydar, 2013). It has been reported that dietary flavonoids play a protective role against coronary heart disease (Khan et al., 2014). Total flavonoid amount of *C. esculenta* tuber was detected as 2050 mg QE/kg extract.

As a result, in this study, it was observed that the effective antioxidant activity of *C. esculenta* tuber extract was depending on its concentration. The plant has a powerful antioxidant activity to remove DPPH and ABTS radicals at the highest concentrations. Reducing power was also determined at high concentration but lower than the standards (Trolox and BHA). On the contrary at lowest concentration the plant showed higher metal chelating activity than standards (Trolox and BHA). It is thought that the radical removal activity of *C. esculenta* tuber extract was due to the flavonoid and phenolic compounds it contains. Due to radical scavenging activities and phenolic compounds, *C. esculenta* tubers may be preferred as an alternative source instead of synthetic antioxidants in food industry.

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