Antioxidant Capacity and Metal Content of *Physalis peruviana* L. Fruit Sold in Markets

Marketlerde Satılan *Physalis peruviana* L. Meyvesinin Antioksidan Kapasitesi ve Metal İçeriği

Antioxidant Activity and Metal Content

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Özet

Amaç: Antioksidan aktivite gösteren ve eser elementlerin gerekli miktarlarını içeren tıbbi gıdaların diyetle alınması, iyi bir sağlıklı yaşam sürdürmek için önemlidir. Çalışmamızda, Kayseri'de marketlerde satılan Altınçilek (*Physalis peruviana* L.) meyvesinin antioksidan kapasitesinin ve metal içeriğinin belirlenmesi amaçlanmıştır. Gereç ve Yöntem: *P. peruviana* meyvesinin antioksidan kapasitesi, 2,2-difenil-1-pikrilhidrazil (DPPH) serbest radikal süpürme testi ile belirlenmiştir. Meyvenin metanol (MeOH) ekstresinin DPPH serbest radikalini temizleme kapasitesi, referans olarak askorbik asit, gallik asit ve bütillendirilmiş hidroksitoluen (BHT) gibi bilinen antioksidanlarla karşılaştırılmıştır. *P. peruviana* meyvesinin metal içeriği atomik absorpsiyon spektrometresi (AAS) kullanılarak ölçülmüştür. Bulgular: *P. peruviana* meyve ekstresinin DPPH serbest radikal temizleme aktivitesinin olduğu, fakat antioksidan kapasitesinin standart maddelerden daha düşük olduğu tespit edilmiştir. *P. peruviana*, askorbik asit, gallik asit ve BHT'nin % 50 inhibitör konsantrasyon (IC_{50}) değerleri sırası ile 32 mg/ml, 3.8 mg/ml, 3.51 mg/ml ve 1.21 mg/ml olarak belirlenmiştir. AAS ile yapılan analizler sonucu, meyvenin eser element yönünden zengin içeriğe sahip iken ağır metal içeriğinin az miktarda veya hiç olmadığı gözlenmiştir. Tartışma: Bu sonuçlar, *P. peruviana* meyvesinin doğal kaynaklı potansiyel bir antioksidan ve eser element kaynağı olduğunu göstermektedir.

Anahtar Kelimeler

Eser Elementler; *Physalis*; Antioksidan Etki

Abstract

Aim: The dietary intake of medicinal food with antioxidant activity and required amounts of trace elements is important to pursue good healthy life. In our study, we aimed to determine the antioxidant capacity and metal content of goldenberry (*Physalis peruviana* L.) fruit sold in markets in Kayseri. Material and Method: The antioxidant capacity of *P. peruviana* fruit was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The DPPH radical scavenger capacity of the methanol extract of fruit was compared with known antioxidants such as ascorbic acid, gallic acid, and butylated hydroxytoluene (BHT) as references. The metal content of *P. peruviana* fruit was measured by using atomic absorption spectrometer (AAS). Results: The fruit of *P. peruviana* was found to possess DPPH free radical scavenging activity but the antioxidant capacity was lower than the standard substances. Inhibitory concentration 50% (IC_{50}) values of *P. peruviana*, ascorbic acid, gallic acid, and BHT were determined as 32 mg/ml, 3.8 mg/ml, 3.51 mg/ml, and 1.21 mg/ml, respectively. As a result of the analysis by AAS, it was observed that *P. peruviana* fruit contented plentiful trace elements and the content of heavy metal was small amount or not detected. Discussion: These observations suggest that the fruit of *P. peruviana* has a potential source of antioxidant and trace elements of natural origin.

Keywords

Trace Elements; *Physalis*; Antioxidant Effect

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Introduction

Free radicals play a crucial role in the development of some chronic diseases such as cancer, cardiovascular disease, diabetes mellitus, rheumatic disease, liver disorders, renal failure, inflammation, and neurodegenerative disease in living organisms [1, 2]. The present of antioxidants in the cellular system are known to prevent the toxicity of free radicals in the human body. Dietary intake of antioxidants from medicinal plants has been increasingly accepted as a strategy for maintaining a healthy life [3].

The fruit of *Physalis peruviana* L. are called as goldenberry, gooseberry, cape gooseberry all over the world [4, 5]. In Turkey, this fruit is known as "altın çilek or güveyfeneri" [6]. Physalis species are grown naturally and cultured in a wide range of countries. In Turkey, the fruit of *P. peruviana* is cultured in Antalya. *P. peruviana* is a medicinal plant widely used in folk medicine for treating diseases such as malaria, asthma, hepatitis, dermatitis, cancer, diuretic, rheumatism [3, 7]. The fruit of *P. peruviana* may be considered as good sources of natural antioxidants for medicinal uses [8, 9].

Fruits are valuable sources of nutritional elements such as magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), phosphorus (P), iron (Fe), zinc (Zn) [10]. Today scientists pay attention to the content of essential trace elements in medicinal plant [11]. These elements play an important role in the metabolic regulations of the human body. Increased consumption of fruit can improve the mineral regulation and reduce cardiovascular diseases and certain cancer risks [12]. Copper (Cu) is an essential trace element required as cofactor in the antioxidant enzymes. The recommended limit for dietary daily requirement for Cu is up to 10 ppm and average dietary daily requirement is 1-3 mg [13, 14]. Zn is one of the less toxic metal and is essential for proper maintenance of body functions such as immune system, proper brain functioning and is vital for the development of fetal growth. The reported limit of Zn for human uptake is up to 150 ppm and the daily intake limit is up to 15 mg [13, 14]. Fe is an essential element and is a core component of the red blood cells. The daily intake limits is 8-11 mg [12, 14]. Ca is known as nutrition for human and is necessary for the development and growth of skeletal [12]. Mg has an important role in nervous system stability, muscles contraction as activator of alkaline phosphatase. The daily recommended allowances for Mg are 320-420 g/day [12]. Nickel (Ni) has an important role as a coenzyme in different enzymes and lower content of Ni in fruits can lead to increased blood sugar level, hypertension and deficient growth in human. The acceptable range of Ni intake daily is 3-7 mg [12]. Cobalt (Co) is essential trace element that is required for the vitamin B12. The daily recommended range of Co in human is 0.005 mg. [12]. Molybdenum (Mo) is an essential element for humans, and dietary recommendation was estimated at 25 µg/day [15]. Lead (Pb) and Cadmium (Cd) are among the most abundant heavy metals and have toxic effects. The excessive amount of these harmful metals in food is associated with some diseases such as cardiovascular, kidney, nervous, and bone diseases [14].

In this study, we aimed to investigate the antioxidant activities of *P. peruviana* fruit extract by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and to evaluate the metal content of this fruit sold in local market in Kayseri.

Material and Methods

Chemicals

DPPH, butylated hydroxytoluene (BHT), gallic acid, and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol (MeOH), the element standard solutions (1000 mg/L), and nitric acid (HNO_3), hydrogen peroxide (H_2O_2), ammonium dihydrogenphosphate ($NH_4H_2PO_4$), and magnesium nitrate ($Mg(NO_3)_2$) were provided by Merck (Darmstadt, Germany). All reagents used were of analytical grade. All the plastic and glassware were cleaned by soaking them in a 10% HNO_3 solution and rinsing them with distilled water prior to use.

Sample preparation

P. peruviana fruits were purchased from the local market in Kayseri, Turkey. Fruits were washed and air-dried before the extraction. 5 g of fruits of *P. peruviana* were powdered and macerated with 100 mL of MeOH for 8 h at room temperature with magnetic stirrer and the extracts were filtered. Under the same conditions, this procedure was repeated twice with 100 mL of MeOH. The collected extracts were dried under vacuum using rotavapor at 40 °C. The dried extracts were dispersed in water and lyophilized for the antioxidant activity.

DPPH test for antioxidant activity

The free radical-scavenging activity was evaluated using an improved DPPH assay by the method of Gyamfi et al. [16]. The reaction mixture contained 100 µM DPPH in MeOH and different concentrations of the extract. Absorbance at 517 nm was measured after 30 min, at room temperature. Each experiment was performed in triplicate. Inhibitory concentration 50% (IC_{50}) values are determined from a calibration curve. Gallic acid, ascorbic acid, and BHT were used as standard compound. The percentage of the DPPH radical inhibition was calculated using the following equation [17]: % inhibition= [(Abscontrol – Abssample)/ Abscontrol] x100

Determination of metal content

Sample digestion

The dried fruit materials taken for the analysis were weighed precisely 0.50 g in the sample cup. 6.0 ml of 65% HNO_3 and 1.0 ml of 30% H_2O_2 were added to the samples and digested in microwave acid digestion unit (Milestone MLS-1200 MEGA). Digested samples were quantitatively transferred to 10.0 ml volumetric polypropylene tubes and adjusted the final volume to 10.0 ml. Microwave acid digestion program is given in Table 1.

Table 1. Microwave acid digestion program

Step	Time (min)	Power (W)	Pressure	Temp1	Temp 2
1	5	250	0	0	0
2	5	400	0	0	0
3	5	650	0	0	0
4	5	250	0	0	0

Metal analyses

Determinations of the elements were performed by the atomic absorption spectrometer (Perkin-Elmer Instruments, 800 Shel-

ton, CT, USA) equipped with longitudinal Zeeman affect background corrector [18]. The element standard solutions (1.000 mg/L) were prepared for each element depending on the linear working range, corresponding five dilutions were made and their absorbances were measured. Matrix modifiers at the concentration of 0.005 mg (5 µl, 1 % w/v) $NH_4H_2PO_4$ and 0.003 mg (5 µl, 0.06 % w/v) $Mg(NO_3)_2$ were added to 20 µl sample. All determinations were made in triplicate. The atomic absorption signal was detected in peak height mode against a calibration curve. The results are expressed as ng/g or µg/g. Parameters of graphite furnace and flame atomic absorption systems are given Table 2 and 3, respectively.

Table 2. Parameters of graphite furnace atomic absorption system

	Co	Pb	Cd	Mo	Ni
Lamp wavelength (nm)	242.5	283.3	228.8	313.3	232.0
Pyrolysis temperature (oC)	1400	850	500	1500	1100
Atomization temperature (oC)	2400	1900	1500	2450	2300
Measurement time (second)	4	3	3	7	5
Repeats	2	2	2	2	2
Sensitivity check (µg/L)	20	50	2.0	20	50

Table 3. Parameters of flame atomic absorption system

Elements	Lamp Wavelength (nm)	Gas mixture (Flow: 16.0-7.8 L/min.)
Cu	327.4	Air- acetylene
Zn	213.9	Air- acetylene
Fe	372.0	Air- acetylene
Mg	285.2	Air- acetylene
Ca	422.7	Nitrous oxide- acetylene

Results

Antioxidant capacity

The antioxidant potential of *P. peruviana* fruit was evaluated using the stable DPPH radical. The DPPH free radical scavenging activities of fruit extract and reference antioxidant substances were calculated according to their ability that causes reduction in initial absorbance of DPPH solution. DPPH free radical scavenging capacity of the extract was compared with the known antioxidants such as ascorbic acid, gallic acid, and BHT as references. The results of the DPPH test demonstrate that the extract of fruit has free radical scavenging activity but lower than the standard substances. IC_{50} values of *P. peruviana* were shown in Table 4.

Table 4. Radical scavenging activities of *P. peruviana* fruit.

Sample	IC_{50} (mg/ml)
P. peruviana	32
Ascorbic acid	3.8
Gallic acid	3.51
BHT	1.21

The metal content

According to our results, the *P. peruviana* fruit contented plentiful trace elements, Mg (1.45 mg/g), Ca (191 µg/g), Cu (7.7 µg/g), Zn (11.46 µg/g), Fe (36.11 µg/g), Co (27.46 ng/g), Mo (161.14 ng/g), Ni (not detected) which are necessary for human health. We found that the content of heavy metals was small amount; especially Cd level was 7.39 ng/g and this value was lower than permissible level recommended by World Health Organization (WHO). The toxic element Pb was not detected in the fruit. The element concentrations of *P. peruviana* fruit were shown in Table 5.

Table 5. The concentrations of elements (mg/100 g) in *P. peruviana* fruit.

Elements	Present study	Leterme et al. (2006)	Rodrigues et al. (2009)
Mg	145	19	34.7
Ca	19.1	23	9
Cu	7.7	0.64	0.28
Zn	11.4	0.28	0.49
Fe	36	0.09	1.47
Co	0,0027	not detected	0.01
Mo	1.6114	-	-
Ni	not detected	0.02	-
Cd	0.000739	-	-
Pb	not detected	-	-

Discussion

The DPPH radical scavenging assay is widely used to evaluate the ability of antioxidants to scavenge free-radicals. This method has been used extensively to predict antioxidant potency due to the relatively short time required for analysis and it is more stable than the hydroxyl and superoxide radicals [19]. In our study, the antioxidant capacity of *P. peruviana* fruit was evaluated using the stable DPPH radical.

In this study, the results of the DPPH assay demonstrate that the extract of fruit has free radical scavenging activity, but the antioxidant capacity was at low level. Demir et al. [7] also found similar results that the antioxidant activity of *P. peruviana* fruit, from a local supplier from Antalya, using the DPPH radical scavenging method determined the IC_{50} values of ascorbic acid and *P. peruviana* were calculated as 1.06 µg/ml and 430 µg/ml, respectively. Chang et al. [8] suggest that the aqueous extract of *P. peruviana* has antioxidant activity in a concentration-dependent manner. When compared with vitamin C (for the 5 µg/ml, scavenging 94.64 %), their extract concentrations 50 µg/ml, 100 µg/ml, and 300 µg/ml showed the scavenging 13.17 %, 22.04 %, 52.72 %, respectively. They mentioned that the fruit extract was as good as vitamin C in total antioxidant activity but was weaker in DPPH radical scavenging. However, there are some studies using different methods for the determining the antioxidant potency of *P. peruviana* fruit, suggesting the fruit possess good antioxidant activities [1, 3].

Recently, scientists have focused on trace elements in the environmental conditions due to the factors essential for the proper functions of living organisms. These elements are comprised in enzymes and activate them, thus in an essential way influencing biochemical process in cells [2]. Fruits are known to be excellent source of nutrients such as minerals and vitamins. Required amounts of trace elements must be in human diet to pursue good healthy life [11]. Diets high in fruits are associated with decreased risk for diseases like diabetes and cancer.

According to our results, the *P. peruviana* fruit material contained plentiful trace elements such as Mg, Ca, Cu, Zn, Fe, Co,

and Mo, which are necessary for human health. In particular, the amount of Mg found in large quantity in our *P. peruviana* fruit. Medicinal plants can be toxic because they contain heavy metals such as Pb and Cd. We found that the content of heavy metals was small amount; especially cadmium level was lower than permissible level recommended by WHO. The toxic element Pb was not detected in the fruit. Leterme et al. [20] determined that the mineral composition of *P. peruviana* was Ca (23 mg/100g), Mg (19 mg/100g), Fe (0.09 mg/100g), and Zn (0.28 mg/100g). In addition, Rodrigues et al. [21] reported that the mineral content in *P. peruviana* fruit consumed from Brazil was Ca (9.00 mg/100g), Fe (1.47 mg/100g), Mg (34.70 mg/100g), Cu (0.28 mg/100g). and Zn (0.49 mg/100g).

Conclusion

The daily intake of medicinal food with antioxidant activity and required amounts of trace elements could be important in human diet to pursue good healthy life. In our results suggest that the fruit of *P. peruviana* has a potential source of antioxidant and trace elements of natural origin. Therefore, it could be used as a source of natural antioxidant and possible mineral supplementation.

Competing interests

The authors declare that they have no competing interests.

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