



Phenolic Profiles, Tyrosinase Inhibitory, and Antioxidant Effects of Green Coffee, and Turkish Traditional Coffee

Yeşil Kahve ve Geleneksel Türk Kahvesinin Fenolik Profili, Tirozinaz Enzim İnhibisyonu ve Antioksidan Etkileri

Seyda KANBOLAT¹, Merve BADEM¹, Sıla Ozlem SENER²,
Rezzan ALIYAZICIOGLU^{1*}

¹Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, Trabzon, Turkey.

seydaakkaya@ktu.edu.tr, ORCID: 0000-0001-7261-7067

ecz.mervebadem@gmail.com, ORCID: 0000-0002-1265-5616

*rezzan@ktu.edu.tr, ORCID: 0000-0003-0143-8795

²University of Health Sciences, Gulhane Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey.

silashener@hotmail.com.tr, ORCID: 0000-0001-7679-7165

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***Corresponding author /Yazışılan yazar**

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Abstract

Coffee has been drunk for millennia due to its taste and health benefits. High levels of polyphenols, and especially flavonoids and phenolic acids, are found in coffee and contribute significantly to its flavor and health-giving properties. In this study the total phenolic contents, antioxidant, and tyrosinase inhibition of green coffee, and Turkish traditional coffee extracts were evaluated. Antioxidant activities of the coffees were examined by two different methods, radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). Total phenolic contents were estimated by using Folin-Ciocalteu reagent as the gallic acid equivalent. The phenolic profiles were investigated by means of reverse phase-high performance liquid chromatography (RP-HPLC). At the same time, tyrosinase enzyme inhibition of extracts has also been worked. The extracts exhibited high levels of antioxidant activities associated with significant antioxidant compound contents. It was determined that the samples contain chlorogenic acid and benzoic acid in the RP-HPLC analysis. It was determined that green coffee extract exhibited tyrosinase enzyme inhibition as effective as kojic acid. The results show that green coffee especially from coffees can be regarded as a potential source of antioxidant compounds and tyrosinase inhibitors of significance in both the pharmaceutical and food industries.

Keywords: Green coffee, Turkish traditional coffee, Antioxidant, Phenolic compounds, Tyrosinase inhibition

Özet

Kahve, tatları ve sağlık yararları nedeniyle binlerce yıldır içilmektedir. Kahvede yüksek düzeyde polifenoller ve özellikle flavonoidler ve fenolik asitler bulunur ve bu bileşenler kahvenin lezzetine ve sağlık verici özelliklerine önemli ölçüde katkıda bulunur. Bu çalışmada, geleneksel Türk kahvesi ve yeşil kahve ekstralarının toplam fenolik içerikleri, antioksidan ve tirozinaz inhibitör aktiviteleri değerlendirilmiştir. Kahvelerin antioksidan aktiviteleri, 2,2-difenil-1-pikrilhidrazil (DPPH) ve demir indirgeyici antioksidan gücü (FRAP) olmak üzere iki farklı yöntemle incelenmiştir. Toplam fenolik içerikler, Folin-Ciocalteu reaktifi kullanılarak gallik asit eşdeğeri olarak verilmiştir. Fenolik profiller, ters faz yüksek performanslı sıvı kromatografisi (RP-HPLC) ile çalışılmıştır. Aynı zamanda ekstraların tirozinaz enzim inhibisyonu da çalışılmıştır. Ekstreler, yüksek seviyelerde antioksidan aktiviteler sergilemesi önemli antioksidan bileşenleri ile ilişkilidir. RP-HPLC ile yapılan analizde, örneklerin klorojenik asit ve benzoik asit içerdiği saptanmıştır. Yeşil kahve ekstresinin kojik asit kadar etkili tirozinaz enzim inhibisyonu sergilediği belirlenmiştir. Sonuçlar, özellikle kahvelerden elde edilen yeşil kahvenin, hem ilaç hem de gıda endüstrilerinde potansiyel antioksidan bileşikler ve önemli tirozinaz inhibitör kaynağı olarak kabul edilebileceğini göstermektedir.

Anahtar Kelimeler: Yeşil kahve, Türk geleneksel kahve, Antioksidan, Fenolik bileşikler, Tirozinaz inhibisyonu

1. INTRODUCTION

The coffee plant was first grown in the Kaffa region of Ethiopia, from where it spread to Yemen, Arabia, and Egypt and gradually became part of daily life. After water and tea, coffee is the third most popular drink worldwide (Villanueva et al., 2006). Once coffee berries have ripened, they are dried, roasted at a range of different temperatures until the desired flavor is achieved, and finally ground and brewed. The two most popular coffee berries are harvested from plant species of *Coffea robusta* L. Linden and *Coffea arabica* L.

Various studies have demonstrated an association between tea and coffee consumption and their ability to prevent disease, which has been attributed to their polyphenol contents (Klatsky et al., 2006; Nichenametla et al., 2006). Polyphenols are secondary metabolites that act as a component of the defense system against pernicious environmental factors such as ultraviolet radiation and pathogens.

Flavonoids, particularly flavanols (catechins) and phenolic acids, constitute the major polyphenols identified in coffee. The most plentiful polyphenols identified in coffee are caffeic acid and its derivative chlorogenic acid (a caffeic acid ester of quinic acid). One cup of coffee may contain 70-350 mg of chlorogenic acid (Clifford, 1999). The antioxidant activity exhibited

by coffee is associated with its chlorogenic, ferulic, caffeic, and *n*-coumaric acid contents (Nicoli et al., 1997).

Oxidative stress resulting from disequilibrium between the production and neutralization of pro-oxidants gives rise to numerous human diseases. Oxidative stress is triggered by free radicals including superoxide anions, hydrogen peroxide, nitric oxide and peroxynitrite implicated in injury to various cellular macromolecules (Oyedemi et al., 2010). Copper-containing tyrosinase is responsible for catalyzing melanin synthesis in melanocytes (Vaibhav & Lakshman, 2012). Various tyrosinase inhibitors have been discovered and described so far (Kim & Uyama, 2005; Parvez et al., 2007). Researchers are currently investigating new and potent tyrosinase inhibitors for use in foodstuffs against discoloration and as skin whitening agents.

As part of our research into medicinal plants for new enzyme inhibitors with potential capacity for use as skin whitening agents, we investigated the tyrosinase inhibition potential, phenolic composition, and antioxidant activities of green and Turkish traditional coffees from Turkey.

2. MATERIALS and METHODS

2.1. Chemicals and Instrumentation

DPPH (2,2-Diphenyl-1-picrylhydrazil) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, methanol, acetonitrile and acetic acid were purchased from Merck (Darmstadt, Germany). TPTZ (2,4,6-tripyridyl-s-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and Folin-Ciocalteu were purchased from Fluka Chemie GmbH (Buchs, Switzerland). All absorbance measurements performed in the experiments were made with the A Spectro UV-Vis Double PC-8 automated cell spectrophotometer (Labomed Inc.).

2.2. Determination of Antioxidant Capacity

Samples of green coffee, and Turkish coffee were purchased from herb markets in Trabzon, Turkey, in September 2015. The samples (1 g) were mixed with 10 mL methanol. Each mixture was macerated at room temperature. The suspension was filtrated and concentrated at 40 °C in a rotary evaporator. The samples were dissolved with methanol at a concentration of 10 mg/mL to determine the antioxidant capacity.

The total amount of phenolic substances in the extracts was determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). The Folin-Ciocalteu reagent was used

because it is sensitive to reducing compounds, including polyphenols, and gives a blue color after the reaction. This blue color can then be measured spectrophotometrically (Kolayli et al., 2012).

The ferric reducing antioxidant potency (FRAP) test, which is very preferred, was determined according to Benzie and Strain (1996). Results are given as μM Trolox equivalent of g sample.

DPPH radical scavenging activity was performed according to Molyneux (2004). The basis of this method is based on the DPPH cation radical scavenging capacity of the antioxidant. The results were expressed as SC50 (mL per mg sample), which is the concentration of the samples that caused 50% scavenging of the DPPH radical.

2.3. Determination of Phenolic Profiles by RP-HPLC

The extracts were redissolved in HPLC grade methanol and filtered through 0.45- μm membranes. p-hydroxy benzoic acid, vanillic acid, syringaldehyde, p-coumaric acid, sinapic acid, benzoic acid and quercetin as standards were used in RP-HPLC analysis. The phenolic profiles of samples were determined by validated and modified HPLC method (Korkmaz et al., 2019).

2.4. Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity (EC 1.14.1.8.1, 30 U, fungal tyrosinase, Sigma) was measured according to the method of Masuda et al. (2005). It uses different concentrations of kojic acid solutions used as standard in this method.

3. RESULTS and DISCUSSION

3.1. Antioxidant Capacity of Coffees

Polyphenols are substances commonly found in plants. Coffee is one of the main sources of polyphenols consumed daily in Turkey. The properties of coffee that make it easier to consume a lot can be attributed to its high amount of antioxidants. Humans can ingest chlorogenic acids from coffee and these are then metabolized by the intestinal flora (Manach et al., 2004; Olthof et al., 2003). High coffee consumption and its bioavailability may play a role in reducing the risk of various diseases.

In this study, total phenolic content (TPC) was determined in comparison with standard gallic acid, and the results were expressed as milligrams of gallic acid equivalents

(GAE) per gram (mg GAE/g) of extract. Measurements showed that the methanolic extract of Turkish traditional coffee had the highest total phenolic content (Table 1). TPC value increases in the order: Turkish traditional coffee > green coffee. Total phenolic contents in methanolic extract of Turkish traditional coffee, and green coffee were 13.9 ± 0.001 , and 6.6 ± 0.001 mg of GAE/g, respectively. Fukushima et al. (2009) reported that the concentration of total polyphenols in coffee, was 200 mg/100 mL (Fukushima et al., 2009). Factors such as differences in methodologies used in studies and seasonal variability may cause differences in analytical values (Hertog et al., 1992).

Table 1. The antioxidant activities of methanolic extracts of coffees

Test Compounds	TPC ¹	FRAP ²	DPPH ³
Green coffee	6.6 ± 0.001	181 ± 1.140	0.236 ± 0.009
Turkish coffee	13.9 ± 0.001	369 ± 1.000	0.190 ± 0.004
BHT			0.009 ± 0.001

¹ Total phenolic content expressed in mg of gallic acid equivalent (GAE) per gram of dry plant weight.

² Expressed as μ M trolox equivalents (TE) per gram of dry plant weight.

³ Concentration of the test sample (mg/mL) required to produce 50 % inhibition of the DPPH radical.

Coffee constitutes a valuable dietary source of antioxidants. Our study reports new data elicited by comparing the in vitro antioxidant/reducing capacities of various types of coffee. FRAP values increase in the order: Turkish traditional coffee > green coffee (Table 1). This study has shown that Turkish traditional coffee has the highest antioxidant power and green coffee has the lowest values. Total antioxidant activity as the FRAP value of Turkish traditional coffee was found 369 ± 1.000 μ mol Trolox per gram of sample in methanolic extract. Natella et al. (2002) reported that FRAP values were 96.4 mol Fe²⁺/L for coffee extract (Natella et al., 2002). Differences in results may vary with geographic regional differences in which coffee is grown, the time of year the leaves are harvested, and differences in subsequent storage conditions (Lin et al., 1996).

When antioxidants interact with DPPH, they neutralize their free radical character by donating an electron or a hydrogen atom to DPPH. The radical scavenging activity of DPPH is expressed as SC₅₀. A lower SC₅₀ value indicates higher antioxidant activity. The order of radical scavenging activity of DPPH resulted as follows: Turkish traditional coffee > green coffee. The DPPH scavenging activities of the methanolic extract of Turkish traditional coffee, expressed in terms of SC₅₀, were 0.190 ± 0.004 mg /mL (Table 1). The radical scavenging capacities of the extracts were lower than BHT (0.009 ± 0.001 mg/mL), which is used as a synthetic antioxidant in the food industry.

3.2. Phenolic Profiles by RP-HPLC

RP-HPLC of the methanolic extract was evaluated by comparison with phenolic acid standards (Figure 1).

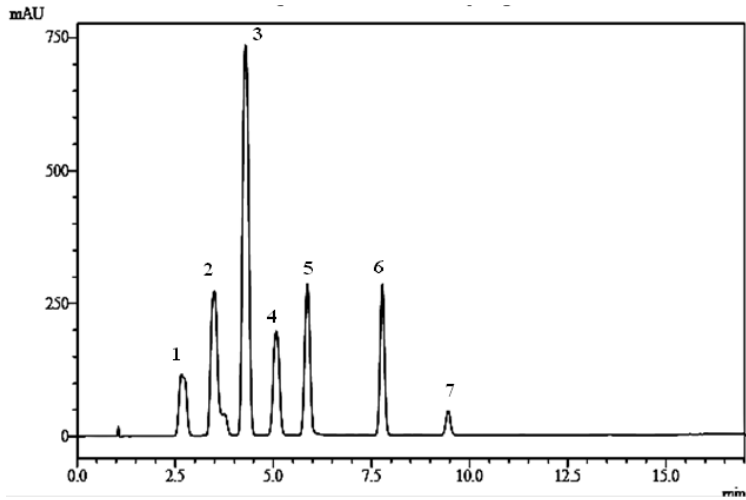


Figure 1. RP-HPLC chromatogram of phenolic standards (25 µM) searched in samples detected at 270 nm by DAD. Waters spherisorp ODS2 -C18 column (4.6 × 250 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (1) protocatechuic acid, (2) *p*-hydroxy benzoic acid, (3) chlorogenic acid, (4) caffeic acid, (5) vanillin, (6) ferulic acid, (7) benzoic acid.

The concentration of chlorogenic acid is 9.903 mg/g and 9.87 mg/g for Turkish traditional coffee and green coffee, respectively. The concentration of benzoic acid is 7.5 mg/g and 36.007 mg/g for Turkish traditional coffee and green coffee, respectively (Table 2, Figure 2, Figure 3).

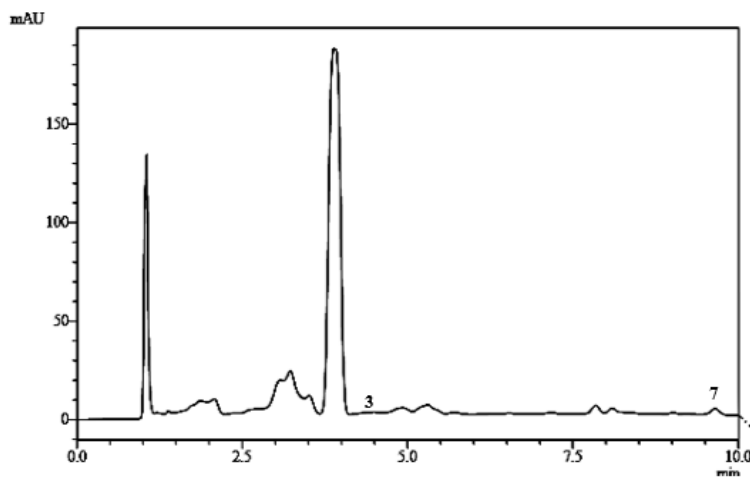


Figure 2. RP-HPLC chromatogram of phenolic standards (50 mg/mL) searched in methanolic extract of Turkish coffee detected at 270 nm by DAD. Waters spherisorp ODS2 -C18 column (4.6×250 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (3) chlorogenic acid, (7) benzoic acid.

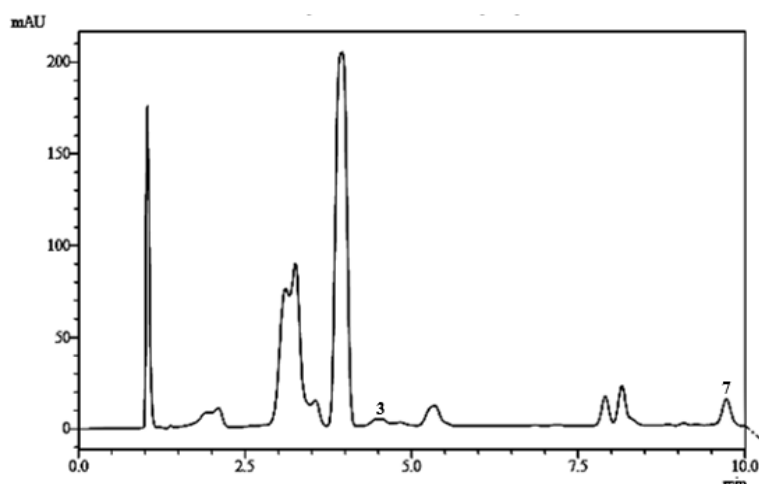


Figure 3. RP-HPLC chromatogram of phenolic standards (50 mg/mL) searched in methanolic extract of green coffee detected at 270 nm by DAD. Waters spherisorp ODS2 -C18 column (4.6×250 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water, flow rate 1.2 mL/min. Peak identification: (3) chlorogenic acid, (7) benzoic acid.

Table 2. Phenolic composition of the methanolic extract of Green Coffee and Turkish coffee

Compounds	Amount (mg/ g)	
	Green coffee	Turkish coffee
Proto-catechuic acid	-	-
<i>p</i> -hydroxy benzoic acid	-	-
Chlorogenic Acid	9.870	9.903
Caffeic acid	-	-
Vanillin	-	-
Ferulic acid	-	-
Benzoic acid	36.007	7.500

3.3. Tyrosinase Inhibitory Activity of Coffee

According to our literature survey, there are a limited number of studies on Turkish traditional coffee, green coffee (Erdem et al., 2016; Iwai et al., 2004). Iwai et al. (2004) reported that the dicaffeoylquinic acid isolated from green coffee beans also exhibited more potent (2.0-2.2-fold) tyrosinase inhibitory activities compared to caffeoylquinic acid, arbutin, and ascorbic acid (Iwai et al., 2004). Methanolic extract of green coffee was studied for enzyme inhibitory activity against tyrosinase at 25, 50, 100, and 500 µg/mL concentrations (Table 3, Figure 4). Methanolic extract of green coffee showed a high degree of inhibition against tyrosinase similar to positive control, kojic acid (Table 3).

Table 3. Tyrosinase inhibition % of the methanol extract of the green coffee and the reference (kojic acid) at 25, 50, 100, and 500 µg/mL concentrations.

Sample	Green coffee	Turkish coffee	Kojic acid ^a
I (25 µg/mL)	24.07 ± 2.95	2.56 ± 0.15	23.29 ± 0.34
II (50 µg/mL)	37.04 ± 2.83	10.25 ± 0.29	43.37 ± 0.66
III (100 µg/mL)	64.81 ± 1.85	11.20 ± 0.49	71.48 ± 0.65
IV (500 µg/mL)	92.59 ± 3.26	13.33 ± 0.33	92.77 ± 0.52

Standard error mean (S.E.M.)

^aPositive control for inhibitory activity against tyrosinase

The IC₅₀ values were determined as 63.53 µg/mL for kojic acid, 72.94 µg/mL for green coffee, and over 1000 µg/mL for Turkish coffee according to an equation of graphics (Figure 4).

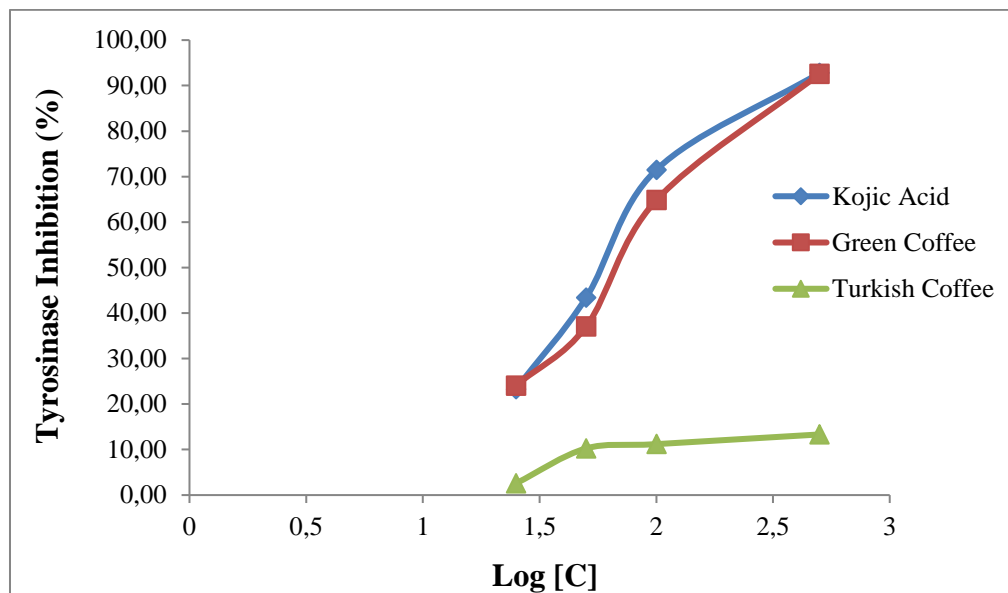


Figure 4. Tyrosinase inhibitions of the methanolic extract

The methanolic extract of green coffee extract possessed a remarkable inhibition against this enzyme (72.94 µg/mL) and was shown to contain chlorogenic acid and benzoic acid, its tyrosinase inhibitory potency might be suggested to be related to the polyphenols (Figure 3). Chlorogenic acid (an ester of caffeic acid and quinic acid) is the most abundant phenolic acid contained in coffee and has been described as a marker or characteristic compound. This has been confirmed by numerous studies investigating chlorogenic acid contents in coffee (Oliveira-Neto et al., 2004). Hence, it can be speculated that chlorogenic acid

found in Turkish traditional coffee, and green coffee may contribute to the skin-whitening effect in cosmetic through its strong antioxidant potential and moderate tyrosinase inhibitory action.

4. CONCLUSION

Our findings revealed that; extracts prepared from green coffee growing in Turkey, appear to have significant tyrosinase inhibitory and antioxidant properties, which might be possibly associated with the rich total phenol content of the green coffee. Thus, green coffee might be used as raw material by pharmaceutical industries for the preparation of natural drugs, in addition to the use in food industries.

DECLARATIONS

All authors declare that they have no conflicts of interest.

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