



## EVALUATION ON GALLIC ACID, EGCG CONTENTS AND ANTIRADICAL ACTIVITY OF GREEN TEA AND BLACK TEA EXTRACTS

*YEŞİL ÇAY VE SİYAH ÇAY EKSTRELERİNDEKİ GALLİK ASİT, EGCG İÇERİĞİ VE  
ANTİRADİKAL AKTİVİTESİNİN DEĞERLENDİRİLMESİ*

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### ABSTRACT

**Objective:** Tea is very well known and consumed beverage worldwide also cultivated widely. It is one of phytonutrients that has a protective role associated with its antioxidant activity. The aim of this article is to study the gallic acid, epigallocatechin gallate contents and antiradical activity of green tea and black tea extracts from *Camellia sinensis* cultivated in North Anatolia.

**Material and Method:** Gallic acid and epigallocatechin gallate contents were investigated in ethanol, methanol and water extracts of green tea and black tea by HPLC analysis and the antiradical activities were also examined for scavenging effect on DPPH and ABTS free radicals.

**Result and Discussion:** In total 6 extracts, gallic acid contents were determined in the range of 0.052-1.341 mg/100 ml and the value of EGCG (epigallocatechin gallate) were found between 0-19.54 mg/100ml. Water extract of green tea exhibited the best antiradical activity on both DPPH and ABTS radicals. Green tea could be evaluated as a good candidate for health prevention but it should be noted that the harvesting method and manufacturing process, optimum conditions on brewing time, the solvent used, chopping grade of tea leaves should also be taken into consideration during formulating both phytonutrient and pharmaceutical grade products.

**Keywords:** Antiradical activity, black tea, *camellia sinensis*, egcg, gallic acid, green tea

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**ÖZ**

**Amaç:** Çay, dünyada çok iyi bilinen ve tüketilen bir içecek olup antioksidan aktivitesine bağlı koruyucu etkileri bilinen önemli bir fitobesindir. Bu çalışmanın amacı, Kuzey Anadolu kaynaklı *Camellia sinensis* bitkisinden elde edilen yeşil çay ve siyah çaydan hareketle elde edilen ekstrelerdeki gallik asit ve epigallocateşin gallat miktarı ile ekstrelerin antiradikal aktivitesinin tespit edilmesidir.

**Gereç ve Yöntem:** Yeşil çay ve siyah çayın etanol, metanol ve sulu ekstrelerindeki gallik asit ve epigallocateşin gallat miktar tayini YPSK yöntemi ile tespit edilmiş ve ekstrelerin antiradikal aktivite tayini için DPPH ve ABTS yöntemleri kullanılmıştır.

**Sonuç ve Tartışma:** Toplam 6 ekstredeki gallik asit içeriği 0.052-1.341 mg/100 ml aralığında bulunmuş ve EGCG (epigallocateşin gallat) için bu değer aralığı 0-19.54 mg/100ml olarak tespit edilmiştir. Hem DPPH hem de ABTS radikali üzerinde en iyi etkiyi yeşil çayın sulu ekstresinin sağladığı belirlenmiştir. Yeşil çayın, koruyucu sağlıkta değerlendirilebilecek iyi bir aday olduğu ancak fitobesin ve farmasötik kalitede ürünün formülasyon çalışmalarında, bitkinin hasat yöntemi, bitkiden hareketle çay üretim prosesi, optimum karıştırma zamanı, kullanılan çözücü ve yaprakların parçalanma derecesinin kayıt altına alınması gerekmektedir.

**Anahtar Kelimeler:** Antiradikal aktivite, *camellia sinensis*, egcg, gallik asit, siyah çay, yeşil çay

**INTRODUCTION**

Polyphenols are naturally occurred in foods of plant origin and also play an important role as preventing medicine against chronic disorders. They are produced naturally in plants to protect themselves against viruses, bacteria and also linked to oxidative stability in plants. These defense mechanisms of plants could also be helpful for optimizing the human body functions [1-3].

Tea is very well known and consumed beverage worldwide, obtained from the leaves of *Camellia sinensis* (L.) O. Kuntze. According to the processing method, tea can be classified into four types; white tea, green tea, oolong tea and black tea. Fermentation is the corner stone in the process of manufacturing. White and green tea is subjected to a little or no fermentation, oolong tea is a semi-fermented final product. Black tea is the final product of full fermentation [4].

Tea is one of phytonutrients that has a protective role associated with its antioxidant activity. In addition, there are reports on its health benefits for cancer, cardiovascular disease and diabetes mellitus due to the antioxidant effect. Preventive and therapeutic effects of tea products are attributed to phenolic contents [5].

Gallic acid is a common phenolic acid, widely found in plants and tea products. Increasing scientific interest has shown that it plays an important role in the health benefits of food [6,7].

Epigallocatechin gallate (EGCG) is the major catechin and considered as the most active substance among catechins in green tea infusion. According to previous reports EGCG is a promising molecule for both prevention and treatment associated with being antioxidant, antiinflammatory, antibacterial, antiviral agent [8-11].

Especially catechins and theaflavines are major groups of polyphenols in green tea and black tea respectively. Gallic acid has also been reported to be found in both green tea and black tea products and

both epigallocatechin and gallic acid were mentioned to be major indicators of quality during standardizing pharmaceutical green tea samples [12]. There are studies to explain the chemopreventive mechanisms of EGCG, among them, target specific cell signaling pathways draw attention for regulating cellular proliferation and apoptosis [9].

The plant is used in Turkish folk medicine not only as carminative, but also tonic and diuretic. The antidote property of *C. sinensis* for alkaloid poisoning is also mentioned. The dried leaves are known to be used in the treatment of eye infections [13].

It is clear that the composition of tea is depended on geographical location, harvesting time, storage condition and manufacturing process. So, the aim of this article is to study the gallic acid and EGCG content and antiradical activity of green tea and black tea extracts from *C. sinensis* cultivated in North Anatolia and determine whether it could be defined as a source of natural antioxidants.

## MATERIAL AND METHOD

### Plant Material

All samples of green tea and black tea were supplied from commercial company in North Anatolia location in Turkey.

### Preparation of extracts for the determination of antiradical activity

The ethanol and methanol extracts was prepared from 1 g of green tea and black tea products in 100 ml of each solvent by stirring constantly at room temperature for 1 hour and then filtered. The water extracts were prepared from 5 g of each sample by adding 500 ml of boiling water and heating at 100°C temperature for 5 min and then filtered. Total 6 extracts were prepared. Each extract was coded and given in table 1.

**Table 1.** The samples and extraction solvents.

Sample	EXTRACTION SOLVENTS		
	Boiled water	Ethanol	Methanol
Green tea product	GW	GE	GM
Black tea product	BW	BE	BM

### HPLC Conditions

HPLC analysis was conducted on SSI Alliance Esence HPLC Workstation System equipped with a SSI Alliance Esence Series 4 LC pump, SSI Lab Alliance Esence UV-Vis detector. A Shimpack CLC-ODS (M) (25 cm×0.45µm) column was used for separation in this study. The wavelength was set to 270nm. A gradient elution was performed by varying the proportion of solvent A (acetonitrile) to solvent

B (0.1% phosphoric acid in water) with a flow rate of 1 ml/min. Sample quantity was 25 µl. The mobile phase composition started at 8% solvent A and 92% solvent B for 40 min. Then, the mobile phase composition was changed into 11% solvent A and 89% solvent B from 40 min to 62 min. In 62 minute, composition was bring to 18% solvent A and 82% solvent B for 18 minutes. At 80 minute the composition was changed to 23% solvent A and 77% solvent B. All prepared solutions were filtered through 0.45 µm membranes before injection onto HPLC.

The linearity was determined from the triplicate analytical curves obtained by gallic acid and EGCG standart solutions. Table 3 presents the correlation coefficient ( $r^2$ ), limits of quantification (LOQ) and limits of detection (LOD) of both gallic acid and EGCG.

### **Determination of antiradical activity**

#### ***DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test***

The solution of DPPH (0.1 mM) was prepared in methanol and 2950 µl of DPPH solution was added to 50 µl of each extract at different concentrations. The mixtures were shaken and allowed to stand in dark at room temperature for 20 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Trolox was used as the reference. Lower intense of blue color indicate the higher activity. The percentage radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated by using the following formula.

$$\% \text{ RSA: } [ (A_0 - A_t) / A_0 ] \times 100$$

$A_0$  is the absorbance of the control reaction,

$A_t$  is the absorbance in presence of all of the extract samples and reference after 20 min.

All the tests were performed in duplicate and the results were averaged. The radical-scavenging activity was expressed as antiradical activity and trolox equivalent antioxidant capacity (TEAC). The  $IC_{50}$  value (µg/ml) is the concentration required to inhibit 50% of the initial DPPH free radical, was calculated from the graph of inhibition curve. Antiradical activity ( $A_{AR}$ ) was defined as  $1/IC_{50}$ .

#### ***ABTS [2,2 '-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid] radical scavenging test***

The radical monocation of  $ABTS^+$  was generated by reacting ABTS solution (200 µmol/L) with phosphate buffer solution (pH 7.4) including 4.5 µmol/L myoglobin and also 300 µmol/L  $H_2O_2$  solution was prepared. Then the absorbance was measured at 734 nm by using a UV-VIS spectrophotometer and the change in absorbance was recorded every 30 seconds during 3 minutes to determine the level of spontanous degradation. Trolox was used as the reference. Tea extracts (and trolox solutions at different concentrations) were allowed 1 ml of ABTS solution as described above, and the absorbance was taken at 734 nm during 3 min using a spectrophotometer.

The capability of scavenging the ABTS radical was calculated by using the following formula.

$$\% \text{ RSA: } [(A_0 - A_t) / A_0] \times 100$$

$A_0$  is the absorbance of the control, namely absorbance of ABTS radical in methanol

$A_t$  is the absorbance in presence of all of the extract samples and reference at the end of 3 min.

All the tests were performed in duplicate and the results were averaged. The radical-scavenging activity was expressed as antiradical activity and trolox equivalent antioxidant capacity (TEAC). The concentration that causes a decrease in the absorbance of initial oxidants by 50% ( $IC_{50}$ ) was determined. Antiradical activity ( $A_{AR}$ ) was defined as  $1/IC_{50}$ .

## RESULT AND DISCUSSION

In this study, we tested 6 extracts prepared with water, ethanol, methanol from green tea and black tea of *Camellia sinensis* from Turkey for investigating their antiradical activity as well as gallic acid and EGCG contents in extracts.

In our study, gallic acid contents were determined in the ranged from 0.052-1.341 mg/100 ml as shown in Table 4. The highest gallic acid content was found in the water extract of green tea as 1.341 mg/100 ml, on the other hand the ethanolic extract of black tea contents the lowest gallic acid as 0.052 mg/100 ml. The amount of EGCG ranged between 0-19.54 mg/100 ml. EGCG could not detected in water and ethanolic extracts of black tea whereas, methanolic and aqueous extract of green tea samples were found close to each other in a value of 19.54 and 19.53 mg/100 ml, respectively.

The antiradical activities of ethanol, methanol and water extracts of green tea and black tea were also examined for scavenging effect on DPPH and ABTS free radicals.

In present study the water extract of green tea exhibited the best antiradical activity with 126.582 and 65.359  $A_{AR}$  value on DPPH and ABTS radicals, respectively. The antiradical activity values of methanol extracts of black tea and green tea on DPPH radical were found as 59.880 and 81.967, respectively. Methanol extracts of green tea and black tea samples showed moderate scavenging effects on ABTS radical. The  $A_{AR}$  values of methanolic extract of black tea was 56.818 and for green tea samples it was determined as 58.139. Under the same experimental condition, the  $A_{AR}$  value on ABTS radical of trolox was 114.942.

The results on antiradical activities of test samples by using DPPH and ABTS methods are given in table 2.

TEAC (Trolox equivalent antioxidant capacity) values of all potent extracts obtained by DPPH assay were higher than those obtained by ABTS assay.

**Table 2.** The results of antiradical effect of green tea and black tea extracts extracts.

Samples	DPPH method		ABTS method	
	A <sub>AR</sub> (Antiradical activity)	1mg/ml Trolox equivalent extract concentrations (mg/ml)	A <sub>AR</sub> (Antiradical activity)	1mg/ml Trolox equivalent extract concentrations (mg/ml)
GW extract	126.582	1.837	65.359	3.558
GM extract	81.967	2.791	58.139	4.000
GE extract	52.631	4.418	32.573	7.139
BW extract	62.893	3.697	54.054	4.302
BM extract	59.880	3.883	56.818	4.093
BE extract	-	-	-	-
Trolox	232.558	-----	114.942	-----

The codes of extracts were explained in Table 1

**Table 3.** Linearity results, limit of Detection (LOD) and Limit of Quantification (LOQ) of gallic and EGCG.

Compound	Equation*	r <sup>2</sup>	LOQ (µg/ml)	LOD (µg/ml)
Gallic acid	Y=11806x-5302.6	0.9993	0.03	0.010
EGCG	Y=9341,7x-9050.9	0.9997	0.005	0.017

\*Linear regression equation  $y = ax + b$ , in which x is the concentration as µg/ml and y is the peak area at the 270 nm wavelength.

**Table 4.** Gallic acid and EGCG content in terms of mg/100ml of methanol, ethanol and water extracts of green tea and black tea.

Samples	Water extracts		Methanolic extracts		Ethanol extracts	
	Gallic acid	EGCG	Gallic acid	EGCG	Gallic acid	EGCG
Green tea product	1.341	19.53	0.145	19.54	0.384	7.702
Black tea product	0.216	-	0.093	0.358	0.052	-

Chronic diseases have been increasing year by year. And it is very clear that reactive oxygen species (ROS) play a critical role in chronic diseases leading to oxidation of lipids and proteins which ultimately induces all inflammatory diseases, atherosclerosis, neurological disorder and cancer [14].

Having ability to protect the damages caused by free radicals and showing low toxicity makes natural antioxidants valuable for medicine, cosmetic and food industry. Tea products are proved to be strong antioxidants and important dietary source due to polyphenol content. On the other hand, the phytochemical profile and antioxidant activity of these products can vary strongly on the basis of different parameters.

Antioxidant activity encloses different pathways; prevention of radical formation, scavenging the radicals and repairing the damage occurred by radicals. So *in-vitro* assays on determination of scavenging radical potential is an important indicator for antioxidant activity.

In this study, we have applied two different *in-vitro* assays to test 6 extracts prepared with water, ethanol, methanol from green tea and black tea from *Camellia sinensis* growing in Turkey for their antiradical activity as well as determined the gallic acid and EGCG contents in these extracts by HPLC. As shown in table 2, the highest activity against DPPH and ABTS was exerted by water extracts of green tea with a 126.582 and 65.359  $A_{AR}$  value and 1.837 and 3.558 mg/ml of trolox equivalent concentrations, respectively. Methanolic extract of green tea exhibited moderate radical scavenging activity against DPPH and ABTS comparing with trolox. The ethanolic extract of green tea revealed a weaker antiradical activity and besides, black tea ethanol extract did not show inhibitory effect on both DPPH and ABTS radicals.

The chemical profile of tea includes polyphenols, alkaloids, amino acids, volatile compounds and minerals. It was mentioned that polyphenols are the most abundant group attributed the health benefits of tea products. EGCG is the major catechin in green tea and shown to have beneficial therapeutic effects. Potential benefits of EGCG against cancer have been demonstrated both *in-vitro* and *in-vivo*. Scientists have been studying on proving the poor bioavailability of EGCG by nanotechnology approach. Additionally, recent studies have been focused on combination therapy with other dietary (6-gingerol, curcumin, quercetin) or pharmaceutical agents (5-fluorouracil, cisplatin, docetaxel) to adopt the synergistic effects [10].

On the basis of fermentation process, catechins are inverted to polymerized products named as theaflavin. Theaflavins are another polyphenolic group responsible for antiradical activity of black tea. Previous reports mentioned the strong correlation between phenolic compounds contents in tea and leaf age, plucking time, extraction conditions and manufacturing process [15-17]. It was also reported that antiradical activity of old leaf was higher than in young leaf of the plant [16].

The antiradical activity of tea extracts have been studied widely. In general, according to previous reports, green tea extracts were found to have higher antiradical activity due to higher content of

phenolic compounds in particular flavonoids comparing with black tea samples [18, 19] and Rusak et al [17] have been reported that 40 % ethanol was the most effective solvent in the prolonged extraction of EGCG in tea leaves. In the meanwhile, Zuo et al. [20] reported that fermentation process increases the liberation of gallic acid and resulted in high levels of this acid in black tea samples. Liebert et al. [21] observed that black and green tea extracts showed increasing antiradical activity due to the increased total phenolic content with brewing time. Significant *in-vivo* antioxidant activity was also reported after ingesting 300 and 450 ml of green tea [22].

There are different reports on gallic acid content in different type of tea samples. Fernandez et al. [23] determined the gallic acid content in 45 commercial tea including non fermented and fermented samples from different location; gallic acid contents were reported in a range of 0.004-2.537% and the lower percentages belong to non fermented tea. Hilal and Engelhardt [24] analyzed teas from German market and gallic acid content in green tea and black tea was reported in a range of 0.01-0.19 g/kg and 0.16-0.60 g/kg, respectively. On the other hand, Zuo et al. [20] determined the gallic acid content of 8 types of tea including green, oolong, puerh and black tea, in the range of 0.37-5.53 mg/g and the high level of gallic acid was reported in full fermented puerh and black tea.

As EGCG is the major tea catechin, its content in different type of tea samples were also studied. Fernandez et al. [25] determined the EGCG content in 37 commercial tea including non fermented and fermented samples from different location; EGCG contents were reported in a range of 0-5.675% w/w surprisingly, in one green tea sample, EGCG was not detected. Wang et al. [26] analyzed green tea catechins and EGCG content in green tea samples was reported in a range of 0.95-32.6 mg/100 ml. On the other hand, Zuo et al. [20] analyzed total eight tea samples, four of tea samples were green tea and EGCG content was determined the in the range of 51.1-62.4 mg/g in green tea samples, in that study black tea sample was found to contain 3.79 mg/100ml EGCG. Ozturk et al studied on quality parameters of Turkish green tea and found EGCG content between 6.10-6.74 g/100g [27].

In this study, the result obtained for gallic acid content was not fully in agreement of previous reports. On the other hand, our experimental data which showed that green tea extracts contained the highest EGCG content and had the best antiradical activity complied with earlier studies. The reasons behind the variability of results could be due to environmental factors, harvesting conditons, storage, leaf age, extraction solvent, extraction time, degree of fermentation. Furthermore, earlier reports pointed out the strong influence of extraction time, drug particle size, solvent used, infusion time, leaf age and temperature factors on chemical composition of the tea products.

Using herbal products is a global trend and becoming popular for health prevention because of having protective effects against diseases. Since preventing is easier and cheaper method when compared with treatment and to hospitalise the patients, scientists have been dealing with protection.

Tea is consumed all over the world and has so many health benefits. As a natural antioxidant being cheap and supplied easily makes the tea product valuable in preventing health. But it is needed to underlined that countries have their own tea brewing and consumption culture that totally affect the ingredients of tea product. So the products obtained from tea should be designed by considering not only cultivation, manufacturing process but also the social habits.

Our results support that different location and manufacturing process form different tea products with different phytochemical profiles and also the variability of composition of tea products deeply effect the power of antiradical activity. It should be noted that besides the variations on manufacturing process, there is a correlation between brewing conditions and phenolic content of tea beverage. The harvesting method and manufacturing process, optimum conditions on brewing time, the solvent used, chopping grade of tea leaves should also be taken into consideration during formulating both phytonutrient and pharmaceutical grade products.

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