ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article DOI: 10.62425/pharmata.1565424

A Comparison of the Antioxidant Activities of Aqueous and Ethanol Extracts of Turkish Black Tea at Different Processing Stages

Meysera BAKIRCI[1]
Abdullah Al FAYSAL[1]
Ayşegül GÖLCÜ[1]

[1]Department of Chemistry, İstanbul Technical University, Faculty of Sciences and Letters, İstanbul, Türkiye

ABSTRACT

Objective: Türkiye is a country where many plants can grow due to its location and climate diversity. Rize, located in the Black Sea region, stands out notably in tea cultivation due to high rainfall and humidity. In recent years, studies in this field have acquired momentum because of interest in natural antioxidants. In this study, the antioxidant properties of the different processing stages of black tea grown in Rize were comparatively examined.

Methods: This study presents the best combinations of stirring time and solvent to obtain extracts from tea samples in the most efficient way. Five methods were used for the measurement of antioxidant activity, which are as follows: Cupric ion reducing antioxidant capacity (CUPRAC) method, Ferric reducing antioxidant potential (FRAP) method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity method, Ferrous ion chelating (FIC) method, and total phenolic content (TPC) method.

Results: The results obtained from CUPRAC, DPPH, and TPC methods strongly support each other. The other two methods also gave similar results within themselves. Sample 6 exhibited the highest antioxidant activity with results of 2873.76±4.25 mM AAE/g (CUPRAC), 133.29±8.58 µg/mL (DPPH), and 74.39±1.69 mg GAE/g (TPC). In contrast, the green leaves demonstrated the lowest antioxidant activity, with results of 763.17±2.93 mM AAE/g (CUPRAC), 463.25±4.10 µg/mL (DPPH), and 14.10±3.70 mg GAE/g (TPC).

Conclusion: The experimental study points out that black tea consumption is beneficial because it helps the body to get natural antioxidants.

Keywords: Antioxidant, Antioxidant Activity, Black Tea, *Camellia Sinensis*, Plant Extract

INTRODUCTION

The tea plant known as *Camellia sinensis* is one of the most consumed beverages in the world for reasons such as being alcohol-free and affordable.[1] In addition to these reasons, it has often been the number one drink in societies, due to its advantageous features such as warming people up on cold winter days and creating an environment for socialization. According to the results of some studies, catechins, theaflavin, and strychnine, which are tea polyphenols, prevent influenza viral infectiousness and are also effective against colds.[2] On the basis of the tea formed on the tea plant, three types of tea are produced as a result of the different levels of oxidation carried out during processing: unoxidized tea, oxidized tea, and semi-oxidized tea; in simpler terms, green tea, black tea, and oolong tea.[3] According to 2016 data, Türkiye's per capita tea consumption was approximately six times that of China, peaking at 6.96 kilograms, and according to 2019 data, Türkiye again ranks first in per capita tea consumption. Therefore, it has been a part of Turkish culture for many years and has solidified its place as an indispensable habit in daily life.

Tea production is highest in the coastal area from the city of Rize to the Georgian border, but the surrounding cities of Trabzon, Artvin, and Giresun also contribute to the country's tea harvest, and therefore most of the people in these regions earn their living from the tea harvest.[4] The tea harvested in the city of Rize is processed and consumed as black tea. Although various teas such as chamomile tea, fennel tea, linden tea, and rosehip tea are available in markets, black tea is still the most popular and consumed tea.[5]

Received 12.10.2024
Accepted 25.12.2024
Publication Date 16.03.2025

Corresponding author:
Ayşegül GÖLCÜ
E-mail:
aysgolcu@itu.edu.tr

Cite this article: Bakırcı M, Faysal AA, Gölcü A. A comparison of the antioxidant activities of aqueous and ethanol extracts of Turkish black tea at different processing stages. *Pharmata*. 2025;5(1):7-15.

After the tea is harvested and sent to the factory, it goes through withering, rolling, oxidation, drying, and sifting processes (Figure 1). A great number of components in the structure of the tea plant may change or differ in their amounts after these processes. It is stated that the main antioxidant active components of black tea are polyphenols, mainly catechins, flavins, rubigins, gallic acid, and flavonoids. The general observation throughout the processing of tea and making it available for marketing is that catechins are generally oxidized to create the flavins, the rubigins, and the brownins, and the polyphenol content diminishes. During the fermentation step to make the tea drinkable, several oxidation reactions occur and these reactions significantly change the antioxidant content in black tea.[6] An average Turkish person consumes 1250 cups of tea per year, which corresponds to approximately 4 cups of tea per day.[4] Drinking tea has been believed to be beneficial to health since ancient times and modern medical research confirms this belief due to the polyphenols in tea.[7] Moreover, it can be argued that the healing properties of black tea are not a complete myth, because research on diabetic rats has shown that black tea has powerful antioxidant and antidiabetic activity.[8]

numerous diseases in the body.[9] The function of antioxidants can fundamentally be summarized as avoiding the creation of reactive species and eliminating free radicals.[10] It is clear that antioxidants are necessary to protect against the harm of free radicals to human health.[11] With this awareness, conscious consumption of natural products such as fruits, vegetables, and tea has increased in recent years.[12] An example of the main causes of free radical formation in the body is respiration, including cellular respiration, because some of the oxygen spent during breathing contributes to radical formation.[13] Respiration is a critical action for the life of living organisms and cannot be stopped, therefore the formation of free radicals in the body remains a continuous action, which means that the struggle with free radicals will not end throughout life. The structure of free radicals, which are examined in three groups such as reactive oxygen species, reactive nitrogen species, and reactive sulfur species, consists of atoms, molecules, or ions with unpaired electrons and they are very willing to give chemical reactions with other molecules, therefore they are described as unstable and active.[9] As a result, radicals are active species that exist in the body and are formed frequently but are unwanted and harmful in their presence. In order to cope with these harmful reagents, antioxidants are needed, which we can obtain from various plants in nature.

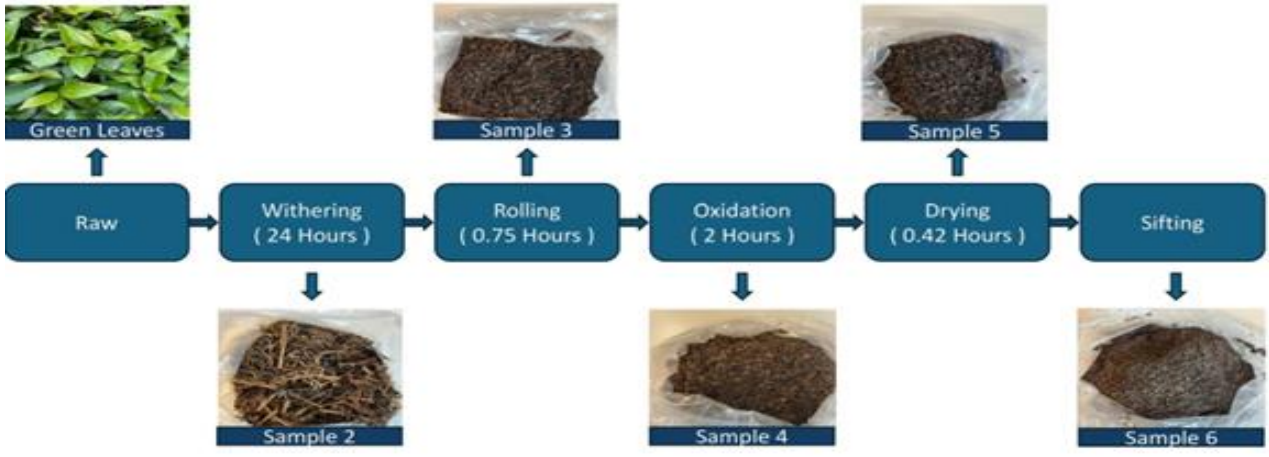

Figure 1. Sampling of the tea leaves during processing

Highly reactive radicals are known to have a significant impact on living organisms, contributing to the onset of various diseases within the human body. Living organisms require antioxidant species to cope with highly damaging radicals. When the balance that needs to be maintained between undesirable radicals and antioxidant species is achieved, the problem is eliminated. However, if this balance is not achieved and the radicals are more than the antioxidants, this situation results in the existence of

Tea is one of the most consumed natural products with antioxidant properties in daily life.[14] Nonetheless, as mentioned earlier, not every antioxidant substance found in the tea plant is found in the same density at every stage of the tea. It would not be correct to claim that all the antioxidant substances listed in the teas purchased and drunk from the market are abundant and very beneficial for health. Therefore, the issue that needs to be investigated is the antioxidant properties of the tea in its different processing stages.

In order to determine the antioxidant properties of black tea grown in Rize, black tea samples were taken from a tea factory in Rize before starting this experiment. Since there are five stages in tea processing, a sample was obtained at the end of each stage, and in addition to these, fresh green leaf samples were obtained, resulting in a total of six tea samples. After obtaining the samples, the most suitable solvent and appropriate mixing times were determined for each. The solution of each sample was prepared according to the determined data. Antioxidant properties were determined using a total of five methods. These methods are as follows: Cupric ion reducing antioxidant capacity (CUPRAC) method, Ferric reducing antioxidant potential (FRAP) method, DPPH radical scavenging activity method, Ferrous ion chelating (FIC) method, and lastly total phenolic content (TPC) method. Our objective was to assess the antioxidant activity of tea samples at various stages and determine the stage at which the antioxidant activity would be maximum.

METHODS

Materials and Preparation of Extracts

The experiment was initiated to compare the extraction efficiency of tea samples obtained from Rize and to determine the most appropriate solvent and mixing time. Initially, 0.1 gram of tea sample was weighed and 10 mL of distilled water was added. This mixture was stirred for 10 minutes with a magnetic stirrer. Subsequently, it was filtered using ordinary filter paper. 2 mL of this solution was transferred to pre-weighed watch glasses. Then, the watch glasses were placed in an oven set to 200°C and left in the oven until the samples were dried. When the solutions dried, the watch glasses were taken out of the oven and their weights were measured and noted again.

This procedure was repeated for 20 minutes, 30 minutes, 40 minutes, and 60 minutes mixing times. Similarly, since it is known that ethanol yields effective results when used for sample extraction in antioxidant activity determination, this procedure was repeated in a manner suitable for the properties of ethanol.[15]

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Method

The CUPRAC method is an effective method of expressing total antioxidant activity for many polyphenols and the chromogen used is the bis(neocuproin) copper (II) cation [$Cu(Nc)_2^{2+}$], which is reduced to the bis(neocuproin) copper (I) chelate [$Cu(Nc)_2^{2+}$], which can be read at 450 nm.[16]

Solutions of all tea samples were prepared and diluted to a concentration of 0.5 mg/mL. Then, a 1 mM concentration of ascorbic acid solution was prepared as a stock solution using distilled water. Afterwards, appropriate amounts were taken from this prepared stock solution to prepare a series at concentrations of 100, 200, 500, 800, and 1000 µM and diluted with water, and the final volume of all was adjusted to 1 mL. Subsequently, 10 mM Copper (II) chloride solution was prepared with water. An aqueous solution of ammonium acetate was prepared as a buffer solution with a pH of 7. Finally, neocuproine solution with 7.5 mM concentration was prepared using ethanol as solvent. After all solutions were prepared, 25 mL of each of copper chloride, neocuproine, and ammonium acetate buffer were taken and mixed in a beaker at a ratio of 1:1:1. Then, 0.5 mL of extract and standard solutions were transferred to falcon tubes, and 3 mL of the reaction mixture was added to these tubes and then all were vortexed. To prepare the blank solution, 1 mL of neocuproine solution, 1 ml of ammonium acetate buffer, 1 mL of water and 0.5 mL of sample solution were mixed in a falcon tube. The tubes were incubated at room temperature for 30 minutes. Finally, absorbance versus prepared blank was read at 450 nm and recorded. All procedures were carried out in triplicate, and the results were expressed as mM ascorbic acid equivalent per extract.

Ferric Reducing Antioxidant Potential (FRAP) Method

Another antioxidant determination method, the FRAP method, is based on the reduction of Fe^{3+}-tripyridyltriazine, a colorless ferric complex, to Fe^{2+}-tripyridyltriazine, a blue ferrous complex, in a low pH environment and by means of electron-donating antioxidants, and this reduction is observed by measuring the absorbance at 593 nm.[17] Initially, 40 mM HCl solution was prepared using water as the solvent. Then, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution was prepared using the freshly prepared 40 mM HCl solution. Subsequently, 20 mM $FeCl_3.6H_2O$ solution was prepared using distilled water as the solvent. Afterward, 0.3 M (pH 3.6) acetate buffer was prepared using water as solvent. After all the mentioned solutions were prepared, the FRAP agent was prepared by mixing the pH 3.6 buffer, 20 mM $FeCl_3.6H_2O$ solution, and 10 mM TPTZ solution with a ratio of 10:1:1 and incubated at 37 °C for 10 minutes. 1 mM $FeSO_4.7H_2O$ solution was prepared with ethanol as the solvent. A series of solutions were prepared by diluting 1 mM $FeSO_4.7H_2O$ solution to concentrations of 100, 200, 400, 600, 800, and 1000 µM to create a calibration curve. Finally, 1000 µg/mL ascorbic acid solution was prepared using water, and tea samples were diluted to a concentration of 0.5 mg/mL.

For the blank solution, 200 µL of water was added to a falcon tube. 200 µL of sample solution, ascorbic acid, $FeSO_4.7H_2O$, and blank solution were taken and placed in falcon tubes then, 1.8 milliliters of FRAP agent was added to all and vortexed. It was incubated in a 37 °C water bath for 30 minutes and then allowed to cool to room temperature. Absorbances are read at 593 nm against the blank solution using a UV-Vis spectrophotometer. The difference between the absorbance of the sample and the blank was calculated to observe the FRAP value. The results were expressed in mM equivalents of $FeSO_4$ per milligram and compared against the standard solution which is ascorbic acid. All measurements were carried out in triplicate.

DPPH Radical Scavenging Activity Method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is based on the reaction of the DPPH radical with an antioxidant and the change in concentration, and the antioxidant activity is measured according to this change.[18] To observe the change in concentration of DPPH radical, measurements must be taken spectrophotometrically at 517 nanometers.[19]

First, 0.1 mM DPPH solution was prepared using ethanol as solvent. Then, stock tea solutions were prepared by diluting the solutions of tea samples to 0.5 mg/mL. Each tea solution was diluted with water to concentrations of 50, 100, 200, 300, and 500 µg/mL. Subsequently, 100 µg/mL ascorbic acid solution was prepared with water to be used as a control and diluted with water to concentrations of 2.5, 5, 10, 25, and 50 µg/mL. 1 mL of extract solvent was added to a falcon tube as a blank solution. Afterward, 2 mL of DPPH solution was added to every falcon tube. Each solution was vortexed for 30 seconds and then incubated in the dark for 30 minutes. Finally, the absorbance of the solutions against the blank solution was read at 517 nm in a spectrophotometer and recorded. This procedure was repeated three times. The following formula was used to calculate the percentage of free radical scavenging activity:

$$\% \text{ Free radical scavenging activity} = (1 - A_{sample} / A_{control}) \times 100$$

In this formula $A_{control}$ stands for the absorbance of the control and A_{sample} stands for the absorbance of sample solutions. Antioxidant activity of tea extracts expressed as IC_{50} which is the concentration of a substance that is necessary to neutralize 50% of the initial DPPH radical species. The results were compared with the control group, ascorbic acid.

Ferrous Ion Chelating (FIC) Method

3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine p,p'-disulfonic acid, namely ferrozine, is a red-colored structure that can chelate with Fe^{2+}, and the red color is reduced when ferrozine is chelated with other substances, such as antioxidants, and the method is based on this color change.[20] In this method, absorbance is measured spectrophotometrically at 562 nm and the results are calculated as percent inhibition.[21]

Firstly, solutions of tea samples were prepared and diluted to 0.5 mg/mL. Then, 0.5 mg/mL Na_2EDTA solution and 0.1mM $FeSO_4.7H_2O$ solution were prepared with water. 0.25 mM ferrozine solution was prepared using water as solvent. Then, 0.5 mL of extract solvent, 0.5 mL of $FeSO_4.7H_2O$ solution, and 1 mL of ferrozine solution were added to a falcon tube to prepare the blank solution.

After these preparations were completed, 0.5 mL of the extract solution (triplicate for each sample) and EDTA solution were added to the falcon tubes. 0.5 mL of 0.1 mM $FeSO_4.7H_2O$ and 1.0 mL of 0.25 mM ferrozine solution were added to each tube and vortexed. After vortexing, they were incubated for 10 minutes at room temperature in the dark. The absorbance values of the solutions in each tube were measured and recorded at 562 nm using a UV-Vis spectrophotometer. Finally, % inhibition was calculated with the formula:

$$\% \text{ inhibition} = (1 - A_{sample} / A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of samples. Higher % inhibition indicates high metal chelating ability.

Total Phenolic Content (TPC) Method

The total phenolic content (TPC) method is an antioxidant determination method that was obtained by making minor modifications to the Folin-Ciocalteu (FC) colorimetric method, using gallic acid as a standard and measuring the absorbance of the colored compound at 765 nm.[22]

Solutions of all tea samples were prepared and diluted to a concentration of 0.5 mg/mL. Then, the FC reagent was diluted with water in a ratio of 1:10. 7.5 % (w/v) Na_2CO_3 solution was prepared using water as the solvent. As the final solution preparation, 1000 µg/mL stock gallic acid solution was prepared with water and diluted to concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. 0.4 mL of distilled water was used as a blank solution.

After adding 0.4 mL of extract and standard solutions to the falcon tubes, 2 mL of FC reagent was added, vortexed, and incubated for 5 minutes. 1.6 mL of 7.5% Na_2CO_3 solution was added and vortexed again. All tubes were incubated at room temperature for 1 hour.
Absorbances against blank solution at 765 nm were measured. The procedure was repeated three times and the results were expressed as gallic acid equivalent (GAE)/g of extract.

Statistical Analysis
Statistical analysis was performed by GraphPad Prism 9.3.1.

RESULTS AND DISCUSSION

Impact effect of Mixing Times and Solvents on Extract Yields
The data depicted in Table 1 and Table 2 represent the extract yield of six different forms of the tea plant using various mixing times and solvents.

As shown in Table 1 and Table 2, the highest yield for green leaves was obtained with 10 minutes of mixing time in ethanol. For the other samples, the highest yields were achieved using water, with mixing times of 60 minutes for S2, 20 minutes for S3 and S4, 10 minutes for S5, and 30 minutes for S6.

Table 1. Extraction data of tea samples using water as solvent

	10 min		20 min		30 min		40 min		60 min	
Sample (0.1 g)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)
Green leaves	0.0005	0.4333	0.0004	0.2333	-0.0010	-0.3333	-0.0028	-2.0333	0.0007	0.4000
	0.0004		0.0001		0.0003		-0.0015		-0.0002	
	0.0004		0.0002		-0.0003		-0.0018		0.0007	
Sample 2	0.0015	1.8333	0.0028	2.5000	0.0026	2.1667	0.0023	2.4000	0.0037	3.5667
	0.0019		0.0025		0.0029		0.0025		0.0035	
	0.0021		0.0022		0.0010		0.0024		0.0035	
Sample 3	0.0036	3.8333	0.0038	4.0667	0.0028	2.9000	0.0041	3.8000	0.0039	2.6667
	0.0029		0.0047		0.0028		0.0039		0.0032	
	0.005		0.0037		0.0031		0.0034		0.0009	
Sample 4	0.0037	4.0333	0.0049	4.2000	0.0027	3.3333	0.0034	3.5333	0.0012	2.9333
	0.0043		0.0039		0.0039		0.0038		0.0039	
	0.0041		0.0038		0.0034		0.0034		0.0037	
Sample 5	0.0038	3.9333	0.0015	2.0333	0.0033	2.9333	0.0020	2.2333	0.0029	2.9000
	0.004		0.0023		0.0029		0.0021		0.0024	
	0.004		0.0023		0.0026		0.0026		0.0034	
Sample 6	0.0044	4.6667	0.0036	4.6667	0.0053	5.3333	0.0045	3.2667	0.0040	4.3333
	0.0045		0.005		0.0053		0.0002		0.0042	
	0.0051		0.0055		0.0054		0.0051		0.0048	

Antioxidant Activity
Since plants have various bioactive compounds with high antioxidant activity, studies are carried out to clarify antioxidant activities for many plant species, thus revealing new antioxidant sources.[23] Phenolic compounds with antioxidant properties absorb and neutralize radicals, break down peroxides, and have redox properties.[24] In addition, phenolic compounds have a very important place among the compounds with antioxidant properties in many plants due to their stance against oxidative stress.[25] Structurally, plant phenolics are aromatic and contain hydroxyl groups, therefore they act as weak acids.[26]

The antioxidant effect of phenolic compounds is seen as hydrogen atom transfer, single electron transfer, loss of proton consecutively, and transition metal chelation.[27]

Oxidative stress can cause various diseases such as cancer, dementia, and asthma; however, plants with antioxidant properties can be used to treat and prevent these diseases.[28]

In this study, five different methods were used to measure the antioxidant activity of tea samples, using ethanol extract of green leaves and water extracts of S2, S3, S4, S5, and S6. (Table 3). CUPRAC is the first technique among them.

After the necessary procedure was carried out and the results were expressed as mM ascorbic acid equivalent per extract, it was seen that the sample with the highest CUPRAC activity was sample 6, while the sample with the lowest activity was green leaves. Sample 6 was followed by sample 3 and there was very little difference between samples 2 and 4. Sample 5 was the sample with the lowest activity after green leaves (Table 3).

Table 2. Extraction data of tea samples using ethanol as solvent

	10 min		20 min		30 min		40 min		60 min	
Sample (0.1 g)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)	Wt. of extract (g)	Avg. (mg)
Green leaves	0.0004		0.0003		-0.0013		0.0005		-0.0028	
	0.0013	0.933	0.0002	0.2333	-0.0011	-1.0333	0.0004	0.4000	-0.0009	-1.5333
	0.0011		0.0002		-0.0007		0.0003		-0.0009	
Sample 2	-0.0006		-0.0003		-0.0014		-0.0010		0	
	0.0002	-0.133	0.0007	0.2333	-0.0001	-1.0667	-0.0012	-0.7000	0	-0.0667
	0		0.0003		-0.0017		0.0001		-0.0002	
Sample 3	-0.0007		0.0004		-0.0001		-0.0004		-0.0013	
	-0.0004	-0.500	0.0005	0.6333	-0.0005	-0.5333	-0.0005	-0.5333	-0.0008	-0.6333
	-0.0004		0.0010		-0.0010		-0.0007		0.0002	
Sample 4	0.0002		0.0004		-0.0005		-0.0009		-0.0004	
	0.0008	0.4667	0.0003	0.4000	-0.0005	-0.3000	-0.0004	-0.9667	-0.0007	-0.7000
	0.0004		0.00 05		0.0001		-0.0016		-0.0010	
Sample 5	0.0005		-0.0501		-0.0007		-0.0008		-0.0018	
	0	0.1667	-0.0004	-17.066	-0.0012	-1.2333	-0.0001	-0.3000	-0.0008	-1.1333
	0		-0.0007		-0.0018		0		-0.0008	
Sample 6	0.0001		0.0003		-0.0003		-0.0002		-0.0007	
	0.0011	0.6333	0.0004	-0.1000	-0.0004	-0.4667	-0.0002	-0.4344	-0.0017	-1.2333
	0.0007		-0.0010		-0.0007		-0.0009		-0.0013	

Table 3. Antioxidant activity data of each sample

	CUPRAC (mM_{AAE}/g)	FRAP ($mM_{Fe(II)}/g$)	DPPH, $IC_{50}(\mu g/mL)$	FIC (mg_{EDTAE}/g)	TPC (mg_{GAE} /g)
Green leaves	763.17±2.93	475.43±8.10	463.25±4.10	17.95±7.39	14.10±3.70
Sample 2	1998.35±1.53	1211.89±3.97	213.47±7.47	28.76±4.43	40.09±2.66
Sample 3	2427.85±1.02	1638.83±1.04	135.59±0.35	14.21±1.44	65.62±4.29
Sample 4	2008.90±4.50	1147.85±5.66	313.78±3.83	34.48±4.92	67.08±3.08
Sample 5	1524.32±0.54	1042.09±7.67	214.31±9.70	23.92±1.36	45.60±3.28
Sample 6	2873.76±4.25	1446.70±7.23	133.29±8.58	31.71±5.12	74.39±1.69
Ascorbic acid	-	1800.67±1.08	71.64±10.30	-	-
Na_2EDTA	-	-	-	92.05±1.68	-

GAE: Gallic Acid Equivalent, AAE: Ascorbic Acid Equivalent, and EDTAE: EDTA Equivalent

* Each value is the mean of three experiments

* Sample 2 corresponds to the state of the tea leaves after the first process, and each sample is the state after the next process. Sample 6 is the ready-to-drink dry tea

The results of the FRAP method were expressed as mM equivalents of $FeSO_4$ per milligram and compared with ascorbic acid. As with the previous method, the sample with the lowest activity is green leaves. Similarly, the samples with the highest activity are samples 3 and 6. The difference between samples 4 and 2 is again very small. Sample 5 is the sample with the lowest activity after green leaves, although it is close to 2 and 4.

The third method, the DPPH method, was carried out and the antioxidant activity of tea extracts was stated as IC_{50}. The results were compared with the control, ascorbic acid. According to the data of this method, the tea sample with the least antioxidant properties is green leaves and there is a significant difference between it and the other samples. The sample with the most antioxidant properties is sample 6, followed by sample 3. After sample 3, samples 2, 5, and 4 come in order.

The FIC method was conducted as the fourth method and the results are calculated as percent inhibition. EDTA solution was used as the control group. The sample with the lowest activity is sample 3, but the green leaves were very close to it. The sample with the highest activity is sample 4, and they have values very close to sample 6. Sample 6 is followed by samples 2 and 5.

In the last method, TPC, the results were expressed as gallic acid equivalent (GAE)/g of extract. When the samples were compared, it was seen that the sample with the lowest antioxidant activity was green leaves and there was a big difference between this sample and the other samples. The sample with the most antioxidant properties was sample 6, followed by samples 4 and 3.

According to the experimental studies, three out of five methods showed that sample 6 was the tea sample with the highest antioxidant properties. Unprocessed green leaves ranked last in terms of antioxidant properties in the majority of the methods. Throughout the production process of black tea, a significant amount of catechins is oxidized and partially polymerized due to enzymatic reactions, leading to the creation of secondary phenolic compounds such as theaflavins and thearubigins.[29] Consistent with this, the findings of this study revealed that Sample 6, representing the final stage of black tea processing, displayed the highest antioxidant activity among all samples. In terms of antioxidant activity, Sample 2 ranked third in three methods, while Sample 5 ranked fourth in three methods.

Green tea is a type of tea that demonstrates higher antioxidant activity compared to black tea.[30] In this study, the green leaves were identified as the sample with the least antioxidant activity. If green tea leaves and black tea leaves were compared, the green tea leaves would have yielded better results. However, the focus of this study was to determine the stage of black tea that exhibited the highest antioxidant activity.

Antioxidant activity was found to be highly significant ($P < .0001$) with DPPH, FRAP, and FIC techniques for all samples when compared with ascorbic acid for FRAP and DPPH and with Na2EDTA for FIC test (Figure 2). There is a significant correlation between the five methods of antioxidant activity at $P < .0001$ (Figure 3).

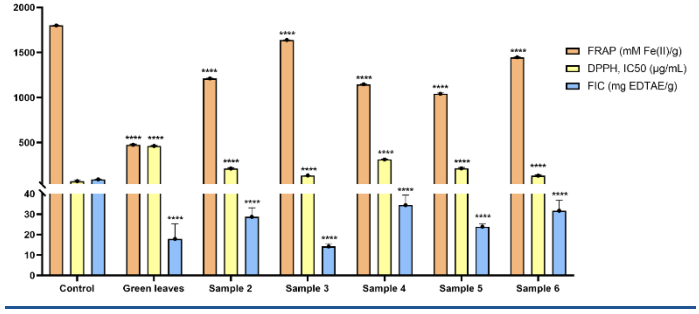

Figure 2. Comparison of antioxidant activity of Turkish black tea at various processing stages, namely green leaves, sample 2, sample 3, sample 4, sample 5, and sample 6. The antioxidant potential of the samples compared with ascorbic acid for FRAP and DPPH; and Na_2EDTA for the FIC test, data are represented as ±SE, N = 3. Statistical analysis is two-way ANOVA, ****$P < .0001$

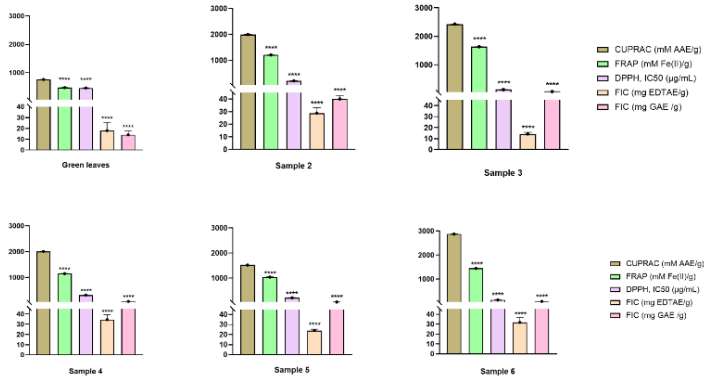

Figure 3. Comparison of antioxidant potential of Turkish black tea at various processing stages (A) green leaves, (B) sample 2, (C) sample 3, (D) sample 4, (E) sample 5, and (F) sample 6 by five different techniques, data are represented as ±SE, N = 3. Statistical analysis is one-way ANOVA ****$P < .0001$

CONCLUSION

Natural antioxidants have become the center of attention after studies emerged about the possible negative effects of consuming synthetic antioxidants. Tea is one of the three most consumed beverages in the world, and it is estimated that catechins, a type of polyphenol, provide antioxidant properties to tea and play a role in breaking down radicals such as lipid alkoxyl and peroxyl. In this study, five different spectrophotometric methods were used to measure the antioxidant activity at each processing stage of black tea. The results were largely consistent with each other. The main aim of the study was to identify the precise stage at which antioxidant activity attains its maximum level. The findings are intended to guide consumers in selecting the optimal sample for tea preparation, thereby enhancing the intake of antioxidants and promoting health benefits. The findings derived from this research indicate that, when evaluating the antioxidant properties of black tea, the samples exhibiting the most pronounced antioxidant capabilities demonstrated statistically significant results in comparison to the standards.

Nevertheless, care should be taken to use the correct amount, as excessive antioxidant intake can disrupt the redox balance in the body. For this reason, people should not only consider black tea as a natural antioxidant source but also should examine the antioxidant content of the foods they consume in their daily lives. This research will provide valuable insights for individuals who regularly drink black tea and explore herbal approaches for health maintenance, disease prevention, and overall wellness.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.

Author Contributions: Concept – M.Ş, S.G; Design - M.Ş, S.G; Supervision - M.Ş; Resources – S.G; Materials - M.Ş, S.G; Data Collection and/or Processing - S.G, İ.A.K; Analysis and/or Interpretation - M.Ş, S.G, İ.A.K; Literature Search - M.Ş, S.G; Writing Manuscript -; Critical Review - M.Ş

Declaration of Interests: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Salman S, Öz G, Felek R, Haznedar A, Turna T, Özdemir F. Effects of fermentation time on phenolic composition, antioxidant and antimicrobial activities of green, oolong, and black teas. *Food Biosci.* 2022;49:101884. [CrossRef]
2. Tominaga T, Furushima D, Yamada H. Tea Polyphenols for the Prevention of Influenza and the Common Cold. In: Tea as a Food Ingredient. *CRC Press*. 2022:127-138. [CrossRef]
3. Munim Khan MR, Islam MA, Uddin R, et al. Biochemical investigation of Bangladeshi black tea and their correlation to organoleptic quality evaluation. *Heliyon*. 2023;9(6):e16802. [CrossRef]
4. Yildirim O, Karaca OB. The consumption of tea and coffee in Turkey and emerging new trends. *Journal of Ethnic Foods*. 2022;9(1):8. [CrossRef]
5. Başgel S, Erdemoğlu SB. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Sci Total Environ.* 2006;359(1-3):82-89. [CrossRef]
6. Wang W, Le T, Wang W, et al. Effects of Key Components on the Antioxidant Activity of Black Tea. *Foods.* 2023;12(16):3134. [CrossRef]
7. Kodagoda K, Wickramasinghe I. Health benefits of green and black Tea: A Review. Int J Adv Sci Res. 2017;4(7):107-112.
8. Qu F, Zeng W, Tong X, et al. The new insight into the influence of fermentation temperature on quality and bioactivities of black tea. *LWT.* 2020;117:108646. [CrossRef]
9. Hachem K, Bokov D, Mahdavian L. Antioxidant Capacity of Caffeic Acid Phenethyl Ester in Scavenging Free Radicals by a Computational Insight. Polycycl Aromat Compd. 2023;43(3):2770-2787. [CrossRef]
10. Olszowy M. What is responsible for antioxidant properties of polyphenolic compounds from plants?. *Plant Physiol. Biochem.* 2019;144:135-143. [CrossRef]
11. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*. 2022;2(2):48-78. [CrossRef]
12. Valtueña S, Pellegrini N, Franzini L, et al. Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. *Am J Clin Nutr.* 2008;87(5):1290-1297. [CrossRef]
13. Gorbachev V, Nikulchev E, Kosenkov AN, Sokolov A, Zavalishin I, Nikitin I. Estimating the Mass of Food Components Necessary for the Utilization of Free Radical Particles in the Human Body. *Int J Environ Res Public Health*. 2022;19(23):15574. [CrossRef]
14. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J.* 2015;15(1):71. [CrossRef]
15. Ridwane Ghanimi, Ahmed Ouhammou, Yassine El Atki, Mohamed El Hassan Bouchari, Mohamed Cherkaoui. The Antioxidant Activities of Ethanolic, Methanolic, Ethyl Acetate, and Aqueous Extracts of the Endemic Species, Lavandula mairei Humbert (A Comparative Study between Cold and Hot Extraction). *Ethiop J Health Sci.* 2022;32(6). [CrossRef]
16. Bibi Sadeer N, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations. *Antioxidants.* 2020;9(8):709. [CrossRef]
17. Dudonné S, Vitrac X, Coutière P, Woillez M, Mérillon JM. Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *J Agric Food Chem.* 2009;57(5):1768-1774. [CrossRef]
18. Dawidowicz AL, Wianowska D, Olszowy M. On practical problems in estimation of antioxidant activity of compounds by DPPH method (Problems in estimation of antioxidant activity). *Food Chem*. 2012;131(3):1037-1043. [CrossRef]
19. Proestos C, Lytoudi K, Mavromelanidou O, Zoumpoulakis P, Sinanoglou V. Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants.* 2013;2(1):11-22. [CrossRef]
20. Evolutionary Diversity as a Source for Anticancer Molecules. *Elsevier.* 2021. [CrossRef]

21. Yılmaz ZT, Odabaşoğlu HY, Şenel P, et al. A novel 3-((5-methylpyridin-2-yl)amino)isobenzofuran-1(3H)-one: Molecular structure describe, X-ray diffractions and DFT calculations, antioxidant activity, DNA binding and molecular docking studies. *J Mol Struct.* 2020;1205:127585. [CrossRef]
22. Teterovska R, Sile I, Paulausks A, et al. The Antioxidant Activity of Wild-Growing Plants Containing Phenolic Compounds in Latvia. *Plants*. 2023;12(24):4108. [CrossRef]
23. Chaves N, Santiago A, Alías JC. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. *Antioxidants.* 2020;9(1):76. [CrossRef]
24. Zheng W, Wang SY. Antioxidant Activity and Phenolic Compounds in Selected Herbs. *J Agric Food Chem.* 2001;49(11):5165-5170. [CrossRef]
25. Chaves N, Santiago A, Alías JC. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. *Antioxidants.* 2020;9(1):76. [CrossRef]
26. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports.* 2019;24:e00370. [CrossRef]
27. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. *J Food Biochem*. 2020;44(9). [CrossRef]
28. Anees Ali Jafri S, Mehmood Khalid Z, Rizwan Khan M, et al. Evaluation of some essential traditional medicinal plants for their potential free scavenging and antioxidant properties. *J King Saud Univ Sci*. 2023;35(3):102562. [CrossRef]
29. Skotnicka Magdalena and Chorostowska-Wynimko J and JJ and SJE. Review paperThe black tea bioactivity: an overview. Central European Journal of Immunology. 2011;36(4):284-292. [CrossRef]
30. Mehrabi M, Amiri M, Razavi R, Najafi A, Hajian-Tilaki A. Influence of varied processing methods on the antioxidant capacity, antibacterial activity, and bioavailability of Iranian black, oolong, and green leafy teas. *Food Chem*. 2025;464:141793. [CrossRef]