Investigation of Different Parts of Tea (*Camellia sinensis* (L.) O. Kuntze) in Terms of Polyphenol and Bioactivity

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Abstract: In this study, changes in the amount of polyphenols (C, EC, GC, EGC, EGCG, ECG) and caffeine in different parts (root, stem, leaf and apical bud) of tea plants harvested from gardens with different characteristics in three shoot periods were investigated. Analyses were performed by High-performance liquid chromatography (commonly known as HPLC). The highest amount of polyphenols were obtained from the leaf, apical bud, stem and root parts of the plant, respectively. In this study, the amount of polyphenols was generally found to be higher in the apical bud and leaf parts than in the root and stem. Polyphenols found in the apical bud and leaf of the tea plant, respectively; the highest values of EGCG were between 70.21-73.98 mg/g, caffeine 60.98-39.63 mg/g, EGC 23.83-31.43 mg/g, EC 15.04-15.86 mg/g, GC 6.95-10.92 mg/g and C 1.98-0.58 mg/g. The root and stem contain caffeine in low amounts. Polyphenols were found in the root and stem parts although less compared to the other two organs.

Keywords: Tea, polyphenol, Catechin, HPLC

Çay (*Camellia Sinensis* (L.) O. Kuntze) Bitkisinin Farklı Kısımlarının Polifenol ve Biyoaktivite Yönünden Araştırılması

Öz *Bu çalışmada, farklı özellikteki bahçelerden üç sürgün döneminde hasat edilen çay bitkisinin farklı kısımlarında (kök, sap, yaprak ve tepe tomurcuğu) bulunan polifenol (C, EC, GC, EGC, EGCG, ECG) ve kafein miktarındaki değişimler incelenmiştir. Analizler Yüksek performanslı sıvı kromatografisi (HPLC yaygın adıyla bilinir) ile yapılmıştır. En yüksek polifenol miktarı sırasıyla bitkinin yaprak, tepe tomurcuğu, sap ve kök kısmından elde edilmiştir. Bu çalışmada polifenol miktarı tep tomurcuğu ve yaprak kısmında kök ve gövdeye göre genel olarak daha yüksek bulunmuştur. Çay bitkisinin tepe tomurcuğunda ve yaprağında sırasıyla bulunan polifenoller; en yüksek EGCG'ın 70.21-73.98 mg/g, Kafein'in 60.98-39.63 mg/g, EGC'in 23.83-31.43 mg/g, EC'in 15.04-15.86 mg/g, GC'in 6.95 -10.92 mg/g ve C'in 1.98-0.58 mg/g değerleri arasında olmuştur. Kök ve sap çok fazla olmamakla beraber kafein içermektedir. Kök ve sap kısmında diğer iki organa göre kıyasla az da olsa polifenol bulunmuştur.*

Anahtar kelimeler: *Çay, polifenol, Kateşin, HPLC*

INTRODUCTION

The tea plant, which belongs to the Camellia family, is a small, evergreen, perennial, shrub-like tree that grows in humid climates. Considering the countries where the tea plant is distributed, it can be said that the tea plant is a subtropical plant based on climatic conditions (Elmas and Gezer, 2019; Kuo et al., 2005). Tea is known to have been transported to China via Assam in 2700 BC and its cultivation started in this region. Three different species of this plant are known: *Camellia assamica, Camellia cembodiensis* and *Camellia sinensis*. The tea (*C. sinensis)* is native to Southeast Asia and is now cultivated in more than 40 countries worldwide (Alikılıç, 2016). Harvesting and purchasing of new tea is done on a bud-by-bud basis, usually in three harvesting periods, but depending on the ecological structure of the Eastern Black Sea region, it can also cover four harvesting periods. The harvest season starts in May and ends in October and November each year. Periods of exile in Turkey; • 1st shoot: May- June • 2nd shoot: July - August • 3rd Shoot September - October as well as November. In the world, for example in the ecvatorial region tea harvesting lasts for 12 months thanks to ecological conditions.

Polyphenols are compounds containing more than one phenolic group in each molecule. There are more than 8000 polyphenols in plants. They are divided into four groups: phenolic acids, flavonoids, stilbenes and lignans. They are secondary metabolites of plants that offer a variety of health benefits and are often involved in defense against ultraviolet radiation or aggression by pathogens (Pandey et al., 2009).

Tea contains more than 4,000 chemicals and has the highest percentage of flavonoids by dry weight of any plant. Phenolic compounds, flavanol glycosides, coanthocyanins and theogalin are the main components of tea.

Tea plants fall into four basic categories: black tea, oolong tea, green tea and white tea (Çelik, 2006; Cabrera et al., 2006). The difference between teas is the fermentation process that takes place during production (Jung et al., 2013). Depending on the processing method, the phenolic composition of tea and therefore the amount of phenolic substances changes (Benzie and Szeto, 1999). In the European Pharmacopoeia, tea is defined as a medicinal plant due to its rich polyphenol content.

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Recent studies have shown that tea plays an important role in the prevention and treatment of cardiovascular diseases, obesity, diabetes, oxidative diseases, inflammatory diseases, bacterial diseases, viral diseases, cancer and neurological diseases due to the polyphenols it contains. Raw tea leaves in particular contain a large number of phenolic compounds. These compounds are flavonoids such as 2 catechins, flavonols, proanthocyanidins and phenolic acids (Lambert, 2013). The main flavonoids found in tea are catechin, epicatechin (EC), epigallocaetchin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG), as well as Quercetin, Kaempferol, Myricetin and their glycosides. Epigallocatechin-3-gallate constitutes 55-70% of the total catechin amount (Deka, et al., 2021; Zheng, et al., 2018).

Due to the production of black tea, oolong tea and green tea, the fermentation of catechins is carried out by the endogenous enzymes polyphenol oxidase and peroxidase. During fermentation, catechins are oxidized to dimeric and oligomeric compounds including Theaflavins, Theacitrins, Theacinensins, Theanaptoquinones and Thearubigins (Tan and Engelhardt, 2017; Liu, 2013; Rains et al.,2011).

The main catechins found in *Camellia sinensis* (L.) *O.* Kuntze are C, GC, EC, EGC, ECG and EGCG (Figure 1) and vary in amount according to different tea clones and parts (Ashihara, 2010; Rhodes, 2013; Wright, 2000)

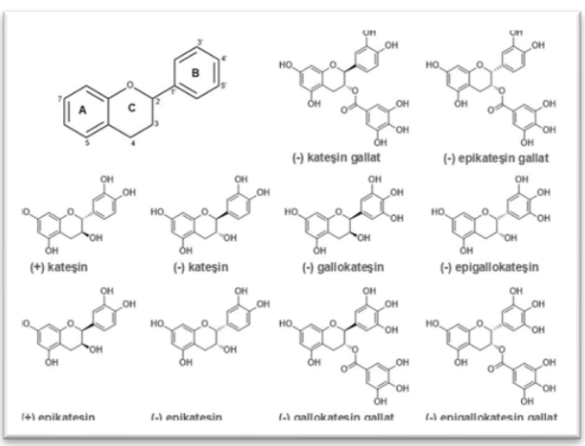

Figure 1. Chemical structure of catechins (Rashidinejad, 2021).

The amount and distribution of catechins in fresh tea leaves affects the quality of black tea. Research has shown that EC has a strong influence on black tea quality and ECG or EGCG also has an impact on tea quality (Obanda, 1997; Wright, 2000; Liu et al., 2023) . During the fermentation stage of black tea production, the amount of catechins in the tea leaf is significantly reduced, resulting in very low amounts of catechins in the final product. In black, oolong and green tea, catechins are fermented by the endogenous enzymes polyphenol oxidase and peroxidase. Catechins are oxidized during fermentation into dimeric and oligomeric compounds: Theaflavins, Theacitrins, Theacinensins, Theanaptokinones and Thearubigins (Liu, 2013; Rains, 2011; Damiani, 2014).

While catechins are more abundant in green tea, catechins are replaced by Theaflavins and Thearubigins in black tea through the fermentation process. These components also give the tea its characteristic smell and color. The most common phenolic substances in black tea are thearubigins (Lambert, 2013; Tan and Engelhardt; 2017, Liu, 2013; Rains et al., 2011).

Catechin contents of different tea cultivars are reported to vary due to many factors. Essential factors are: tea variety, harvesting season and harvesting conditions, age of the leaves, climate, cultivation practices, and drying and technological processes during tea production (Fernandez et al. 2002; Wu et al. 2012; Wei et al. 2011).

There are no detailled studies about the distribution of catechines in different plant parts of Camellia sinensis. The changes in the amount of polyphenols (C, EC, GC, EGC, EGCG, ECG) and caffeine in different parts of the tea plant (root, stem, leaf and top bud) harvested in three shoot periods from tea gardens with different characteristics are the topics of this study.

MATERIAL AND METHODS

Material

Within the scope of the planned thesis study, different tea plantations were selected as material in Rize Salarha District in 2021. The tea gardens were selected based on their pruning status; Tea garden with 5-year expansion pruning (ÇB-1), Tea Garden with 2-year expansion pruning (ÇB-2) and further a newly Established Tea Garden (ÇB-3). From these plantations, different parts of the plant (root, stem, leaf, top bud) were harvested by simple sampling in May, July and September (Figure 2). Harvested plants were dried in an oven at 30°C and stored at +4°C for analysis.

Figure 2. Outlook on sample collection from tea plantations

In this study; high performance liquid chromatography (HPLC) (Shimadzu, Japan), precision balance (Mettler Toledo, XS204, USA), water bath (Memmert, Germany), vortex (Heidolph, Germany), coffee grinder (Tefal Türkiye), deep freezer (-28 °C, Vestel, Türkiye), refrigerator (+4°C, Grundig,

Turkey), mechanical 18 shaker (Şimşek Laboratuvar), centrifuge (Nüve, Türkiye), pH meter (Sartorius, Germany) and oven (İnoksan, Türkiye) were used. The RP-HPLC column (5 µm, 250 x 4.6 mm) used in the study was purchased from Ant Teknik. C, EC, GC, EGC, EGCG, EGCG, ECG used in the analysis of phenolic compounds by HPLC were purchased from Sigma Chemical Company (St. Louis, MO, USA). Other consumables used in the study were micropipette set (Transferpette, Brand, Germany) and 0.45 µm membrane filters, sieve, plastic tubes with screw caps, tube stands, weighing cups, disposable spectrophotometer cuvettes, texture probe, Whatman No.1 filter paper and other glassware.

Method

Collection of tea samples and pre-treatment for HPLC analysis

Tea samples harvested at different shoot periods were dried in the laboratory at 30°C in an oven and ground in a coffee mill just before analysis and stored at +4°C (Figure 3).

Figure 3. View on drying and grinding of tea samples

In the preparation of Tea Samples for Caffeine and Catechin Analysis, dried and ground tea samples were subjected to different brewing processes for caffeine and catechin for HPCL readings. For this process, 3 g of ground tea plants were weighed to represent each of the 19 samples in sterile jars and placed in 100 ml sterile jars.

For caffeine measurement; 100 ml of pure water boiled at 100°C was added to all samples. The samples were steeped for 20 minutes and then filtered through a sterile filter. The samples were then filtered through 0.45 µm membrane filters into vials for reading on the HPCL device and stored at +4°C until reading on the device (Figure 4).

For catechin measurement; 50 ml of pure water boiled at 100°C and 50 ml of methanol were added to all samples and infused for 20 minutes. After the brewing process, it was filtered through a sterile filter. Afterwards, the samples were filtered through 0.45 µm membrane filters into vials for reading on the HPCL chase and stored at +4°C until reading on the device.

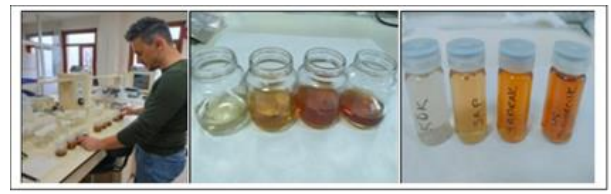

Figure 4. View of the brewing process of tea samples

In the determination of phenolic compounds by HPLC, the method described by Yurteri (2023) was used for the analysis of phenolic compounds with modification. The extract samples were filtered through a 0.45 µm membrane filter and the filtrate was injected into the HPLC column (Perkin Elmer, Flexar model). HPLC operating conditions and elution program for the analysis of phenolic compounds was as follows; HPLC operating conditions System: Shimadzu (Prominence series) Software: LCB Solution Column: Novaselect (250 x4.6 mm, ID; 5 µm; C18) Column oven: CTO 10AS VP Column Temperature: 30 °C Detector: Photodiode array (PDA) Detection wavelengths: 270 and 355 nm Pump: LC-20AD Flow Rate: 1 mL/min Injection Amount: 20 µL Elution program Time (min) Solvent A (%), Solvent B (%) 0-92, 8 10, 89-11, 57-79, 21-62, 20-80, 67-92, Solvent A: 0.1% (v/v) phosphoric acid water, Solvent B: Acetonitrile. The identification of phenolic compounds in the samples was done by comparing the retention time of the compounds on the column, UV-spectra with the time and spectra of the relevant standard substances and by adding the standard substances to the tea extract. Identification of peaks and quantification of phenolic compounds were carried out at the wavelength at which the compounds gave the maximum absorbance value. The integrated areas obtained from the HPLC chromatograms of the compounds and calibration curves prepared with intermediate stock solutions of the standard substances were used to determine the amounts of the compounds.

Statistical analyses were carried out using SPSS (SPSS statistics 23, IBM. 2015) program according to the randomized block design. The results are given as the mean ± standard deviation of 3 replicate measurements. Analysis of variance using one-way ANOVA was applied to the data obtained. Significant differences between means were determined by Duncan multiple comparative test.

RESULTS and DISCUSSION

This study was carried out to determine the polyphenols found in different parts of the plant in 3 different tea gardens, namely ÇB-1: 5-Year Expansion Pruned Tea Garden, ÇB-2: 2-Year Expansion Pruned Tea Garden and ÇB-3: Newly Established Tea Garden.. As can be seen in Table 1, a little polyphenol was observed in all plant parts..Plant parts were statistically different based on its polyphenol content.

Table.1 Average Polyphenol Quantities (mg/g) of Different Parts of Tea (*Camellia sinensis* L.) Plant Harvested from Different Tea Plantations (ÇB-1, ÇB-2 and ÇB-3) as Means of 3 Harvest Periods

			Plant Parts			
			Root*	**Handle***	**Leaf****	**Hill Bud****
Polyphenol Amounts (mg/g)	GC	Ort. ÇB-1*	0.001^{c}	0.0007^{c}	5.27^{a}	1.74^{b}
		Ort. ÇB-2*	0.0009^{c}	0.0005^{c}	7.45^{a}	6.38^{b}
		Ort. ÇB-3*	0.0008^{c}	0.001^{c}	10.92^{a}	9.95^{b}
	EGC	Ort. ÇB-1*	5.76^{d}	6.93^{c}	30.16^{a}	21.10^{b}
		Ort. ÇB-2*	5.15^{c}	4.67^{d}	29.84^{a}	21.73^{b}
		Ort. ÇB-3*	7.49^{d}	8.32^{c}	31.43^{a}	23.83^{b}
	EGCG	Ort. ÇB-1*	5.41^{d}	6.65^{c}	73.98^{a}	70.21^{b}
		Ort. ÇB-2*	3.16^{d}	5.42^{c}	73.98^{a}	61.05^{b}
		Ort. ÇB-3*	3.79^{c}	3.35^{c}	58.36^{b}	59.80^{a}
	EC	Ort. ÇB-1*	10.10^{c}	9.49^{d}	13.92^{b}	14.71^{a}
		Ort. ÇB-2*	5.77^{d}	6.82^{c}	13.46^{b}	15.04^{a}
		Ort. ÇB-3*	8.38^{d}	9.12^{c}	15.86^{a}	14.95^{b}
	C	Ort. ÇB-1*	0.14^{c}	0.11^{c}	0.58^{b}	1.52^{a}
		Ort. ÇB-2*	0.49^{b}	0.35^{c}	0.32^{d}	1.98^{a}
		Ort. ÇB-3*	0.27^{c}	0.24^{c}	0.58^{b}	0.89^{a}
	Caffein	Ort. ÇB-1*	0.73^{d}	2.00^{c}	39.45^{b}	60.98^{a}
		Ort. ÇB-2*	0.62^{d}	4.42^{c}	39.63^{b}	56.72^{a}
		Ort. ÇB-3*	0.55^{c}	0.92^{c}	39.02^{b}	39.70^{a}

(*) p<0.05, (**) p<0.01 statistically significant within error limits

Gallocatechin (GC), Epigallocatechin (EGC), Epigallocatechin Gallate (EGCG), Epigallocatechin Gallate (EGCG),Epicatechin (EC), Catechin (C), Caffein

When the polyphenol amounts in the plant parts are examined, it is possible to say that EGCG has the largest share in the catechin group in the tea plant, followed by EGC, EC, GC and C, respectively. Caffeine was found to be the lowest in the root part of the tea plant, while the highest was found in the top bud part. Considering the plant parts; the highest EGCG was found in the leaf (73.98 mg/g) and the lowest in the root (3.16 mg/g). Epigallocatechin (EGC) was highest (31.43 mg/g) in the leaf (ÇB-3) and lowest (4.67 mg/g) in the stem (ÇB-2) of the tea plant. Gallocatechin (GC) value was highest in the leaf (10.92 mg/g) part of the plants harvested from the newly established tea garden (ÇB-3) and lowest (0.0005 mg/g) in the root part of the plants harvested tea, caffeine values of 3.07-3.87%, epicatechin (EC) values of 0.45-1.11%, epigallocatechin (EGC) values of 1.64-4.39% and epigallocatechin gallate (EGCG) values of 5.94-9.26%. Karori et al. (2014) obtained EGCG values of 2.58-6.625%, EGC from the 2-year expansion pruned tea garden (ÇB-2). EC (15.86 mg/g-.77 mg/g), C (1.98 mg/g-0.11 mg/g) and caffeine (60.98 mg/g-0.55 mg/g) contents were highest in leaves and lowest in roots (Table 1). It has been reported that 60% of the total phenolic content of tea is EGCG, followed by EGC, ECG, EC, CG, CG, GC and C in descending order. (Khokhar and Magnusdottir, 2002; Balci and Özdemir, 2016; Zaveri, 2006). The findings we obtained confirm the findings of previous researches.

Our findings are in general agreement with previous studies in which tea caffeine and catechins were determined. (Burana-Osot and Yanpaisan, 2012; Chebbi, 2022). Goto et al., (1996), obtained in their researchhey conducted in green values of 1.490-6.255%, EC values of 0.845-3.280% and C values of 0.320-2.690% values in their research conducted on Kenian green tea samples. Caffeine content varied between 5.81-27.62 mg/g in dry tea samples, while

epigallocatechin gallate 5.19-58.21 mg/g, epigallocatechin 2.80-52.48 mg/g, epicatechin 0.74-11.58 mg/g, epicatechin gallate 1.01-16.45 mg/g and catechin 0.09-6.10 mg/g, in the study carried out by Burana-Osot and Yanpaisan (2012).

Further Sağlam and Türkyilmaz (2007) detected epigallocatechin 0.040-4.212%, catechin 0.000-0.115%, epigallocatechin gallate 0.096-9.154%, epicatechin 0.091-0.920% and caffeine 1.718-3.640% in tea samples in their study. In the research of Özdemir et al., (2006), while the amount of caffeine in green tea leaves was determined in the range of 1.640-2.145 g/100g, EGCG (4.510-7.310 g/100g) was determined in the highest amount among catechin compounds, followed by EGC (1.290-2.385 g/100g), EC (0.350-0.795 g/100g) and C (0.370 0.520 g/100g) compounds, respectively.

According to the analysis of young leaves, old leaves and stems, EGCG compound was determined as 2.83%, 1.02% and 0.32%, EGC compound as 1.29%, 0.84%, 0.38%, EC compound as 0.44%, 0.28%, 0.20% and C compound as 0.14%, 0.07% and 0.03%, respectively by Lin et al. (1996). Chebbi (2022) anlysed the individual phenolic compound amounts of green teas (mg/g KM) and they were were obtained as EGC 17,47 ± 1,39 EGCG 57,52 ± 0,43 ECG 8,67 ± 0,76 in fresh tea leaf, while from Green tea 1 sample; EGC 8,27 ± 0,10 EGCG 14,14 ± 0,92 ECG 1,48 ± 0,07, Green tea 2 sample; EGC 25,41 ± 1,22 EGCG 46,94 ± 2,70 ECG 3,73 ± 0,01. Khokhar (1997) found that EGC was 163 mg/l, EC 47 mg/l, EGCG 263 mg/l and ECG 44 mg/l in Chinese green tea, while EGC was 287 mg/l, EC 94 mg/l, EGCG 408 mg/l and ECG 59 mg/l in Japanese green tea. Luximon-Ramma et al., (2005) reported that fresh tea leaf buds contained catechin 2.64 µg/g, epicatechin 17.02 µg/g, epigallocatechin 15.07 µg/g and epigallocatechin gallate 25.38 µg/g. Obanda et al., (1997) detected epigallocatechin 78.10-269.30 µmol/g dm, catechin 0.03-47.20 µmol/g dm, epicatechin 36.20-57.90 µmol/g dm, epigallocatechin gallate 132.30-256.60 µmol/g dm and caffeine 27.6-45.1 g/kg dm in the samples taken to represent two leaves and one bud.

The values of caffeine and catechin compounds obtained in our study were found to be generally compatible with the ranges stated in the results of previous studies. However, in some studies, while caffeine values were not similar, catechin values were similar. In another study, there was no agreement in the EGC and EGCG values in the first of two different samples, while there was agreement in the EGC and EGCG values in the second sample The data observed in the studies and the data obtained in our study shows that caffeine and catechins in green tea vary depending on many reasons such as the type of tea, growing conditions, harvest time and storage conditions (Chebbi, 2022; Burana-Osot and Yanpaisan, 2012).

CONCLUSION

It is stated that tea buds and green tea, rich in polyphenols, are used in the treatment of many diseases. Scientific studies have shown that tea consumption has been used in the treatment of many diseases such as diet-induced obesity, reduction in cancer cells, significant reduction in the risk of ovarian cancer, antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering and thermogenic. In the study, the polyphenol content of the top bud, leaf, stem and root parts of the tea plant cultivated in Turkey was examined. The tea samples that underwent 5-year rejuvenation pruning were found to be richer in terms of polyphenol content. Based on the findings, it is possible to say that Turkish tea has a polyphenol-rich content. In light of the data, the rich polyphenol content of the plant has scientifically supported both the industrial use of the plant and its use as a medicinal plant. In addition, our study is the first to identify polyphenols found in the root and stem of the plant, apart from the consumed parts.

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