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CATECHIC TANNIN CONTENT OF DIFFERENT TEA SAMPLES

FARKLI ÇAY ÖRNEKLERİNİN KATEŞİK TANEN İÇERİĞİ

Gülbahar Özge ALİM TORAMAN[1,2], Nur TAN[1,3,4]

[1]Istanbul University, Institute of Health Sciences, Department of Pharmacognosy, Istanbul, Turkiye
[2]Bezmialem University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkiye
[3]Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkiye
[4]Istanbul University, Drug Development Application and Research Center from Natural Resources, Istanbul, Turkiye

ORCID ID: G.Ö.A.T. 0000-0002-7816-7551; N.T. 0000-0001-7958-1917

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ABSTRACT

Objective: *Camellia sinensis* (tea) which is a member of the Theaceae family can be found in many tropical and subtropical areas with diverse cultural forms. It is grown only in the Eastern Black Sea Region of Turkey and originated from China and Japan and is defined as a small evergreen tree. White, green, and black teas are all made from the *Camellia sinensis* plant species. Scientific studies have shown that distinct types of tea have emerged because of different fermentation processes in the production stages. In this study, the macroscopic and microscopic analysis, diagnosis of tannin, and catechical tannin determination have been conducted on fifteen *Camellia sinensis* samples purchased from various places.
Material and Methods: Five grams of powdered tea samples were extracted with 50 ml of hot water and the catechical tannin determination in the extracts was made by gelatin, ferric and stiasny tests.
Results: In the macroscopical examination of A-E (white, green, and black) tea samples the researchers observed no color, odor, or any other substance. When all the samples were microscopically examined, reaserch detected the presence of idioblast which is the characteristic structure of the tea.
Conclusion: There was a positive result regarding a diagnosis of tannin and catechical tannin. Catechin substances were determined in each of the one gram samples; The **E** white tea 0,1300 g, green tea 0,1500 g, black tea 0,0693 g; the **D** white tea 0,1889 g, green tea 0,1228 g, black tea 0,0710 g; the **C** white tea 0,2118 g, green tea 0,1306 g, black tea 0,0436 g; the **A** white tea 0,2400 g, green tea 0,1972 g, black tea 0,0686 g; the **B** white tea 0,3317 g, green tea 0,1380 g, black tea 0,0517 g.

Keywords: *Camellia sinensis*, catechin, white tea, green tea, black tea

ÖZ

Amaç: Theaceae familyasının bir üyesi olan *Camellia sinensis* (çay), farklı kültürel formlarda, birçok tropikal ve subtropikal bölgede bulunabilir. Çin ve Japonya kökenli olup, Türkiye'de sadece Doğu Karadeniz Bölgesinde yetişen, yaprak dökmeyen küçük bir ağaç olarak tanımlanmaktadır. Beyaz, yeşil ve siyah çayların hepsi *Camellia sinensis* türünden elde edilir. Bilimsel çalışmalar, üretim aşamalarındaki farklı fermantasyon süreçleri nedeniyle farklı çay türlerinin ortaya çıktığını göstermektedir. Bu araştırmada beş farklı markadan satın alınan *C. sinensis* örneklerinde (A-E) makroskopik ve mikroskobik analizler, tanen tanısı ve kateşik tanen tayini yapıldı.
Gereç ve Yöntem: Beş gram toz çay numunesi 50 ml sıcak su ile ekstre edilmiş ve ekstrelerdeki kateşik tanen teşhisi jelatin, ferrik ve stiasny testleri ile analiz edilmiştir
Bulgular: A-E (her bir markadan beyaz, yeşil ve siyah çay) numunelerinin makroskopik incelemesi sırasında hiçbir renk, koku veya başka bir madde gözlenmemiştir. Örnekler mikroskobik olarak incelendiğinde tüm örneklerde çayın karakteristik yapısı olan idioblast varlığı tespit edilmiştir.
Sonuçlar: Tüm örnekler tanen ve kateşik tanen teşhisinde pozitif sonuç vermiştir. Kateşin içeriği 1 gramlık numunelerde; **E** beyaz çay 0,1300 gr, yeşil çay 0,1500 gr, siyah çay 0,0693 gr; **D** beyaz çay 0,1889 gr yeşil çay 0,1228 gr siyah çay 0,0710 gr; **C** beyaz çay 0,2118 g yeşil çay 0,1306 g siyah çay 0,0436 g; **A** beyaz çay 0,2400 g, yeşil çay 0,1972 g, siyah çay 0,0686 g; **B** beyaz çay 0,3317 gr, yeşil çay 0,1380 gr, siyah çay 0,0517 gr olarak tespit edilmiştir.

Anahtar Sözcükler: *Camellia sinensis*, kateşin, beyaz çay, yeşil çay, siyah çay

INTRODUCTION

Tea (*Camellia sinensis* L., syn. *Thea sinensis*) belongs to Theaceae family that can be found in many tropical and subtropical areas with diverse cultural forms, and it is one of the most consumed beverages around the world. It is defined as a small, evergreen tree that grows only in the Eastern Black Sea Region of Turkey and is native to China and Japan. Moreover, secondary metabolite contents of *C. sinensis* implicate especially polyphenols and alkaloids (1-3).

Corresponding Author/Sorumlu Yazar: Gülbahar Özge ALIM TORAMAN **E-mail:** galim@bezmialem.edu.tr

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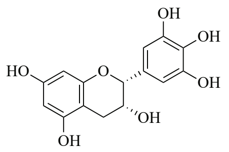
Based on the literature review, the infusions of white, green, and black teas have many benefits including antioxidant and anticancer effects (4-7). However, all kinds of teas (black, green, oolong, and white tea) possess anti-oxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic, anti-microbial, anti-viral, anti-fungal effects, and offering resistance to obesity and cardiovascular complications (3, 8-12).

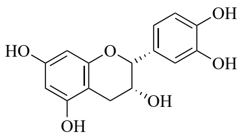
Recent studies have shown that *C. sinensis* varieties have positive effects on chronic diseases as the consumption of tea decreased the incidence of chronic diseases. The flavonoid content of *C. sinensis* is responsible for the protective effect to chronic diseases (9, 13-14).

The polyphenols in tea have many benefits for human health including preventing the recurrence and progression of many diseases (15, 16). Catechins are the main phenolic compounds of tea in the flavan-3-ol structure with biological activities (17). Teas include various catechin types of compounds, such as (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (+)-gallocatechin (GC), and (-)-catechin (C), as well as their gallate esters (EGCG, ECG, GCG and CG) (Table 1). EGCG and ECG which possess the highest biological activity among all of them (8).

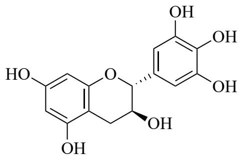
Table 1: Chemical structures of catechin derivatives



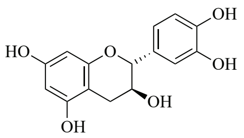
Epigallocatechin



Epicatechin



Gallocatechin



Catechin

White, green, and black teas are also the main types in Turkey. The polyphenols are obtained from the buds and leaves of *C. sinensis* according to the fermentation stages (harvesting, processing and associated degree of oxidation) in fresh tea leaves. The fermentation stages change the proportions of the catechin composition of tea infusion, which affects the benefits of tea for human health (15, 18).

Among the different processing applications, using or not using the fermentation process in tea production results in pronounced sensory properties and chemical composition. When the tea is not fermented; the oxidation of catechins is prevented by polyphenol oxidases. During fermentation, catechins are oxidized which causes a reduction of flavan-3-ol derivatives that are the main phenolic compounds in the biological activity of *C. sinensis* tea (8, 19).

White tea is an unfermented tea made from only the young tea leaves or unopened buds. Green tea is prepared from fresh tea leaves and pan-fried a little, then rolled. and dried. Black tea is prepared from slightly wilted tea leaves that are pan-fried, rolled, dried and fermented. Oolong tea is a partially fermented tea and has the taste of both green and black teas. The leaves are withered under the sunlight for one to two hours after undergoing the semi fermentation process and then oxidized, pan-fried (200^0C), and rolled, respectively. During this semi fermentation procedure, the fermentation degree of oolong tea varies from 20 to 80%. The differences in appearance, chemical content, and taste depend on the different fermentation processes. The flavonoid content is much higher in white tea because of the different production and harvesting process (3, 12, 20).

The importance of medicinal plants increases every day. Over time, the interest and confidence in herbal medicines has increased through the study of chemical content and profiles of the medicinal plants and elucidating the structures of active molecules. This study compared the catechin content of tea samples purchased from assorted brands to evaluate their chemical, macroscopic, and microscopic properties.

MATERIALS AND METHODS

Plant materials

Fifteen different types of *Camellia sinensis* were purchased from local herbalists in January 2017 in Istanbul.

Macroscopic and Microscopic Examination

A macroscopic examination was made of each tea sample in terms of appearance, color, odor, and taste. For microscopic examination, photomicrographs of idioblasts of the tea were determined by photographing the microscopic images following powdering the samples in a mortar. A SARTUR solution was used for preparation and an examination was done with a microscope (Olympus CX21FS1) using 10x and 40x sized lenses.

Phytochemical Analysis

Extraction

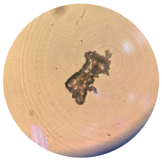
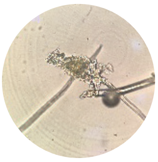
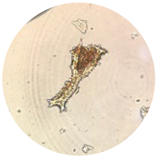
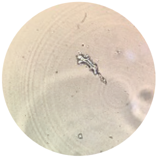
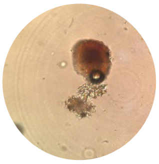
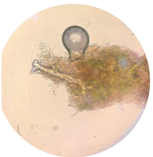
Five grams of tea leaves were infused with 50 ml boiling water for 30 minutes. The tea samples were filtered and aliquoted into 2ml and 5ml sample sizes.

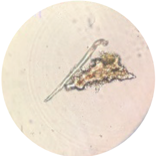
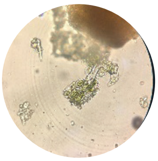
Diagnosis of tannin

Gelatine Test

Two milliliters of gelatin solution were added to an aliquot (2ml) of the infusion and tannins were detected by the gelatine test, and a cream-colored residue was observed in the mixture (1, 21).

Table 2: Macroscopic and microscopic examination and phytochemical analysis results of tea samples (A-E)

Plant Sample		Macroscopic Examination	Microscopic Examination	Gelatine Test	Ferric Test	Stiasny Test
	White			Cream Color Sediment	Olive-green	Yellowish Sediment
A	**Green**			Cream Color Sediment	Olive-green	Yellowish Sediment
	Black			Cream Color Sediment	Olive-green	Yellowish Sediment
	White			Cream Color Sediment	Olive-green	Yellowish Sediment
B	**Green**			Cream Color Sediment	Olive-green	Yellowish Sediment
	Black			Cream Color Sediment	Olive-green	Yellowish Sediment
	White			Cream Color Sediment	Olive-green	Yellowish Sediment
C	**Green**			Cream Color Sediment	Olive-green	Yellowish Sediment
	Black			Cream Color Sediment	Olive-green	Yellowish Sediment

	White			Cream Color Sediment	Olive-green	Yellowish Sediment
D	**Green**			Cream Color Sediment	Olive-green	Yellowish Sediment
	Black			Cream Color Sediment	Olive-green	Yellowish Sediment
	White			Cream Color Sediment	Olive-green	Yellowish Sediment
E	**Green**			Cream Color Sediment	Olive-green	Yellowish Sediment
	Black			Cream Color Sediment	Olive-green	Yellowish Sediment

Ferric Test

The catechin and condensed tannin analysis were detected by the Ferric Test. Three drop $FeCl_3$ solutions were added to an aliquot of the infusion (2ml) and the mixture was observed as an olive-green color in the presence of catechic tannin (1, 21).

Stiasny Test

To an aliquot of the infusion (10 ml), 5ml stiasny reagent (30% formol 100 ml + der. HCl 50 ml) was added. The mixture was left in a water bath at 80^{0}C for 30 minutes. The precipitate indicates the presence of catechic tannins in the sample (1).

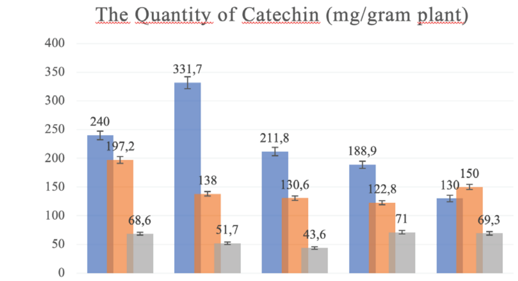
Catechic Tannin Determination

Dried one gram tea samples were put in 90ml of water (80 ^{0}C) and incubated for two hours in a water bath. Then, the tea samples were filtered and cooled to room temperature. The filtration was done using water at a volume of 100 ml and then 40ml Stiasny reagent was added to the erlenmeyers and incubated for 24 hours at room temperature. The mixtures were filtered again and dried in a drying oven (50^{0}C), and as a result, catechic tannin was obtained. The quantity of catechin was calculated (1).

RESULTS

The study demonstrates the phytotherapeutic importance of the plant by comparing the catechin contents of tea samples through examining the botanical, chemical, macroscopic, and microscopic properties.

As a result, the macroscopic and microscopic analysis, diagnosis of tannin, and catechic tannin determination were investigated on fifteen different *C. sinensis* samples. During the macroscopical examination of fifteen tea samples, no color, odor or other substance was observed. When the samples were microscopically examined, the presence of idioblast, which is the characteristic structure of the tea, was detected in all samples. Tannin and catechic tannin were determined using the gelatine and ferric tests respectively (21). All the samples gave positive results for these tests (Table 2).



	A	B	C	D	E
White Tea	240±7,5	331,7±10,5	211,8±7,37	188,9±6,27	130±5,87
Green Tea	197,2±6,05	138±3,87	130,6±3,63	122,8±3,45	150±4,27
Black Tea	68,6±2,35	51,7±2,27	43,6±2,13	71±3,14	69,3±2,94

Graphic 1: The quantity determination of catechical tannins on 15 tea samples (1 g sample each)

To investigate catechin amounts in the tea samples, a water extract was prepared with five grams of tea and analyzed using the Stiasny test. Among tea samples, B (331.7 mg/g) white tea was found with the highest percentage of catechin in the extracts, it is followed by A (240.0 mg/g), C (211.8 mg/g), and D (188.9 mg/g) white and E (150.0 mg/g) green teas. The results of catechin amounts are given in Graphic 1.

CONCLUSION

White, green, and black teas are the main tea types of *C. sinensis.* It has been observed that these tea types emerged because of the applied fermentation differences during the production process. Catechins are the major compounds in *C. sinensis,* their amount depends on the different fermentation stages and the highest catechin amount is in white tea because it is not fermented during preparation (12, 15, 22). A similar result in a study conducted by Bondarian et al. proved that the highest amount of polyphenols (catechins) was yielded in white tea (23).

Consequently, of the fifteen different tea extracts, white tea extracts were found to have a high content of catechin but the E white tea sample contains a lower-catechin amount (Graphic 1). Although the same tea types (white tea) were subjected, this lower catechin content is thought to be caused by differences in the climate, horticultural techniques, collection time, age of leaf, drying and storage conditions of E white tea. Based on the literature, when the catechin and epigallocatechin gallate contents of white and green tea were compared, it was higher in green tea (12, 24-26). Furthermore, these results that showed higher catechin proportions found in white tea could explain its high biological activities (anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-microbial, and reduced cardiovascular diseases) in comparison to green and black teas (11, 27-28). In conclusion, the literature reviews have shown that there are no detailed scientific studies on white, green, and black tea grown in Turkey and white tea has been considered as requiring clinical trials.

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