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Investigation of the Effects of Biological Activity and Steeping Time of Four Different Black Teas Commercially Valuable in Turkey

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ABSTRACT

Objective: The aim of this study was to compare the phenolic, tannins contents and biological activities (antioxidant, anticholinesterase) of four different black teas commercially valuable in Turkey. Subsequently, to examine the effect of the steeping time on the chemical contents (phenolic and tannins contents) and biological activities of the samples.

Methods: The antioxidant activities of samples were examined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC) and ferric reducing/antioxidant power (FRAP) methods. The amount of total phenolic and tannins contents contained in the samples was determined using the Folin-Ciocalteu reagent (FCR) method. In addition, the anticholinesterase activities of different samples were evaluated by Ellman method.

Results: According to the results obtained, filtered tea bag (FT) sample obtained after 5 min steeping exhibited stronger DPPH radical scavenging activity than other samples. In addition, it was found that powder ceylon tea (PCT) samples obtained after 60 min and 10 min steeping had the highest FRAP and CUPRAC values respectively. In this study, it was also determined that the PCT samples obtained after 5 min, 10 min, 15 min, and 30 min steeping contained higher phenolic and tannins contents than the other samples. In anticholinesterase experiment, powder tea (PT) sample obtained after 5 min steeping was found to have the strongest anticholinesterase activity.

Conclusion: As a result, it was determined that the amount of phenolic contents generally increased with the infusion time. It was also found that the antioxidant, anticholinesterase activities and tannins content of the tea samples did not show significant change due to the time of the infusion.
Keywords: Black teas, antioxidant, anticholinesterase, steeping time

1.INTRODUCTION

Tea growing and consumption have been based on thousands of years ago. On a global level, tea was the second most commonly consumed drink which was prepared from systematically processed dried tea (*Camellia sinensis* L.) leaves by boiling in water (milk and/or sugar are added sometimes). Teas have been used in many countries for the treatment of infections and diseases. Tea leaves are rich in secondary metabolites such as phenolic antioxidants, proteins, amino acids, lipids, sugars, vitamin, fiber and minerals (1).

Polyphenolic compounds found in plants are important because they were responsible for biological activity. These compounds were known as potent antioxidants. Besides antioxidant activity, these compounds have antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating effects. In order to better define the potential health benefits of tea, it was important to determine the phenolic compounds in tea and the influences of infusion time on biological activity (2 – 4). Steeping time was usually controlled in an experimental environment; however, it is generally known that brands recommend steeping time for 5-10 minutes or less but the Turkish people were prepared tea in different time (4).

Therefore, the aim of this study was to compare the phenolic, tannins contents and biological activities (antioxidant, anticholinesterase) of four different teas commercially valuable in Turkey. Subsequently, to examine the effect of the infusion time on the chemical contents (phenolic and tannins contents) and biological activities of the samples. This included Powder Tea (PT), Filtered Tea Bag (FT), Powder Ceylon Tea (PCT) and Goran Tee (Mevlana) (GT). Steeping times of 5–60 min were selected to represent realistic durations used by consumers in Turkey.

2.METHODS

2.1. Samples and preparation of samples

PT (Çaykur, Rize Turkey), FT (Doğuş, Rize, Turkey), PCT (Istikan Brand, Turkey), and GT (Goran TEE, Luxus-Mischung, Germany) samples were purchased from markets in Turkey (Table 1). Tea samples (6 g) were extracted with 150 mL of

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H_2O at different time (5-60 min.) using infusion method. After certain steeping time (5, 10, 15, 30 and 60 min.) the extracts were filtrated then were concentrated by water bath (GFL, Germany).

Table 1. *Characteristics of tea samples*

Sample identification	Common Name	Scientific Name	Type
PT	Powder Tea	*C. sinensis*	Black Tea
FT	Filtered tea bag	*C. sinensis*	Black Tea
PCT	Powder Ceylon Tea	*C. sinensis*	Black Tea
GT	Goran tee	*C. sinensis*	Black Tea

2.2. Quantification of total phenolic contents

The total phenolic contents of the samples were determined using the Folin-Ciocalteu reagent (FCR) method. Briefly, 5 µL extract was taken in the plate and 225 µL of water was added. Then 5 µL of Folin-Ciocalteu reagent (diluted 1/3 with distilled water) and 15 µL of 2% sodium carbonate solution were added to the mixture. The mixture was allowed to stand at room temperature for 2 hours, and then absorbance was measured at 760 nm against the reference. The total phenolic contents in the extracts were given as mg gallic acid equivalents/g extract (5).

2.3. Determination of tannins content

The amount of tannin contained in the samples was determined by the Folin – Ciocalteu method(6). Briefly, 0.1 mL of the extracts was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent, 1 mL of 5 % Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Then absorbance of mixture was measured against the blank at 725 nm. The tannins content was expressed as mg tannic acid equivalents in milligram per gram of extract (mgTAE/ g extract).

2.4. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity

The ability of free radical scavenging of samples was determined using the DPPH method. Briefly, 240 µL of DPPH solution (0.1 mM) was added to 10 µL of extracts prepared at different concentrations (5 mg/mL-0.5 mg/mL). Then the mixture was allowed to stand at room temperature for 30 min. The absorbance of the mixture was measured against the reference using a micro plate reader at 517 nm. The experiment was repeated three times and the results obtained in the experiment were given as percent inhibition (%) (7).

2.5. Ferric reducing/antioxidant power (FRAP) assay

The ability of ferric reducing of samples was evaluated using the FRAP method. Briefly, 190 µL of FRAP reagent was mixed with 10 µL of extract and after 4 min the absorbance of the mixture was measured against the reference at 593 nm. The standard curve was prepared using $FeSO_4.7H_2O$ and FRAP values of the extracts were expressed as a mM Fe^{2+}/mg extract (8).

2.6. Cupric reducing antioxidant capacity (CUPRAC) assay

Cupric reducing antioxidant capacity (CUPRAC) method was used for evaluate the antioxidant capacity of samples. Briefly, 60 µL each of Cu(II) (1.10-2 M), neocuproine ethanolic solution (7.3.10-3 M) and 1 M NH4Ac buffer solution were mixed in plate. Extracts 60 µL and 10 µL pure EtOH were added to the initial mixture so as to make the final volume: 250 µL. The mixture was vortexed for 10 s and absorbance measurement was performed exactly after 60 min at 450 nm against a reagent blank. The CUPRAC values of samples were reported as trolox equivalents (mM trolox/mg extract) (9).

2.7. Anticholinesterase activity of samples

Inhibition of cholinesterases was evaluated using a 96-well microplate reader based on the method of Ellman et al., (1961) with some modifications. All reagent solutions (daily) were prepared using Tris-HCl buffer (50 mM, pH 8.0). Briefly, 20 µL of acetylcholinesterase (AChE) solution were mixed with 20 µL of the sample and 40 µL of Tris-HCl buffer and the mixture was left at room temperature (25 °C) for 10 minutes. Then, 20 µl of acetylcholine iodide (ATChI) (50 mM) was added the mixture and the mixture was incubated for 5 min at 25 °C. Then, 100 µL of 20 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (containing 1M NaCl and 0.2 M $MgCl_2.6H_2O$) was added in the mixture and the absorbance of mixture was read at 412 nm against the reference. The experiments were performed in triplicate in each case. Galantamine was used as reference (10).

2.8. Statistical analysis

The all experiments were done in triplicates and all data were shown as mean ± SD. The data were analyzed by Graphpad Prism 5 program (Harvey Motulsky, La Jolla, USA). Statistical differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Mean values were considered statistically significant when $p< 0.05$. **Patient consent**: Not required patient consent for this study.

3.RESULTS

3.1. Quantitative phytochemical analysis of samples

Total tannins and total phenolic contents: The total phenolic, and tannins contents of samples were analyzed

and presented in Table 2. When the amounts of phenolic and tannins content in the samples were compared, it was found that the PCT sample obtained after 5 min, 10 min, 15 min, and 30 min steeping contained higher phenolic and tannins contents than the other samples. It was determined that the amount of phenolic contents in FT, GT and PT samples obtained after 60 min steeping increased with respect to the other minute but the amount of phenolic contents in the PCT sample did not change. In this study, it was found that steeping time did not cause a significant difference in the amount of tannins contained in samples.

Table 2. *Total tannins and phenolic contents of tea samples*

Codes of samples	Total phenolic (mgGAE/g extract)	Tannins (mgTA E/g extract)
After 5 min. steeping		
FT	75±0.023^{a}	151±0.013^{a}
PCT	118±0.018^{b}	267±0.002^{b}
GT	95±0.005^{c}	250±0.003^{c}
PT	55±0.002^{d}	161±0.004^{d}
After 10 min. steeping		
FT	60±0.002^{a}	124±0.015^{a}
PCT	100±0.005^{b}	269±0.038^{b}
GT	93±0.006^{c}	206±0.025^{c}
PT	34±0.052^{d}	180±0.009^{d}
After 15 min. steeping		
FT	56±0.008^{a}	139±0.025^{a}
PCT	110±0.014^{b}	293±0.014^{b}
GT	107±0.012^{c}	195±0.003^{c}
PT	72±0.014^{d}	115±0.008^{d}
After 30 min. steeping		
FT	73±0.002^{a}	118±0.004^{a}
PCT	107±0.005^{b}	271±0.027^{b}
GT	103±0.002^{c}	247±0.009^{c}
PT	87±0.019^{d}	207±0.006^{d}
After 60 min. steeping		
FT	118±0.008^{a}	131±0.025^{a}
PCT	111±0.015^{b}	240±0.011^{b}
GT	126±0.005^{c}	275±0.019^{c}
PT	113±0.019^{d}	151±0.013^{d}

PT: Powder tea; FT: Filtered tea bag; PCT: Powder Ceylon tea; GT: Goran tee (Mevlana)
TAE–tannic acid equivalents, GAE–Gallic acid equivalents. Means with different superscripts (a-d) are significantly different, p<0.05

3.2. In vitro evaluation of antioxidant assays

FT [5 min (84.90%) and 15 min (83.22%)], PCT [30 min (83.33%) and 60 min (83%)] and GT [10 min (81.66%)] samples exhibited stronger free radical scavenging activity than other samples. When the DPPH radical scavenging activities of samples were examined at 200 µg/mL concentrations, it was determined that all samples prepared at different times showed very close free radical scavenging activity.

In this study, according to obtained FRAP values, PCT (0.19 mM Fe^{+2}/mg extract) and FT (0.18 mM Fe^{+2}/mg extract) samples obtained after 60 min steeping showed the strongest ferric reducing/antioxidant power activity. It was also found that the FRAP values of GT and PT samples did not change depending on the infusion time of the study.

According to the results obtained from the CUPRAC experiment, PCT and GT obtained after 5 min-60 min steeping were found to have higher cupric reducing antioxidant activity than the other samples. When the CUPRAC values of all samples were compared, it was found that all the samples had very close each other cupric reducing antioxidant activity. In this study, findings suggest that the steeping time did not effective on cupric reducing antioxidant activity of samples.

Table 3. *Effects of steeping time on the antioxidant activity of tea samples*

Codes of samples	FRAP(mM Fe^{+2}/mg extract)	CUPRAC (mM trolox /mg extract	DPPH (%) (200 µg/mL)
After 5 min. steeping			
FT	0.03±0.01^{a}	1.07±0.04^{a}	84.90±0.95^{a}
PCT	0.05±0.02^{b,a}	1.12±0.04^{b}	83.33±0195^{b}
GT	0.05±0.01^{c,a,b}	1.12±0.02^{c,b}	84.45±0.77^{c,a,b}
PT	0.05±0.01^{d,a,b,c}	1.04±0.12^{d}	79.70±0.01^{d}
BHA			83.22±0.7^{e,b,c}
After 10 min. steeping			
FT	0.06±0.03^{a}	1.07±0.01^{a}	77.96±0.77^{a}
PCT	0.11±0.11^{b,a}	1.13±0.01^{b}	79.64±2.18^{b,a}
GT	0.01±0.01^{c,a,b}	1.10±0.03^{c}	81.66±0.19^{c}
PT	0.03±0.01^{d,a,b,c}	1.07±0.02^{d,a}	80.76±0.70^{d,b,c}
BHA			83.22±0.7^{e}
After 15 min. steeping			
FT	0.05±0.01^{a}	1.05±0.01^{a}	83.22±0.34^{a}
PCT	0.09±0.01^{b,a}	1.09±0.01^{b}	80.76±2.18^{b}
GT	0.04±0.02^{c,a,b}	1.08±0.01^{c,b}	80.76±1.03^{c,b}
PT	0.09±0.05^{d,a,b,c}	1.04±0.01^{d,a}	82.44±1.08^{d,a}
BHA			83.22±0.7^{e,a,d}
After 30 min. steeping			
FT	0.05±0.02^{a}	1.10±0.02^{a}	78.19±2.14^{a}
PCT	0.09±0.01^{b,a}	1.10±0.02^{b}	83.33±0.70^{b}
GT	0.09±0.02^{c,a,b}	1.09±0.03^{c,b}	82.21±0.34^{c,b}
PT	0.06±0.01^{d,a,b,c}	1.06±0.05^{d,a}	79.53±1.7^{d,a}
BHA			83.22±0.7^{e,b,c}
After 60 min. steeping			
FT	0.18±0.01^{a}	1.06±0.01^{a}	76.51±1.37^{a}
PCT	0.19±0.21^{b,a}	1.09±0.02^{b}	83±0.51^{b}
GT	0.06±0.01^{c}	1.09±0.01^{c,b}	81.54±1.95^{c}
PT	0.06±0.01^{d,c}	1.07±0.03^{d,a}	82.66±1.27^{d,b,c}
BHA			83.22±0.7^{e,b,d}
BHT	1.1±0.12^{e}	5.78±0.07^{e}	

PT: Powder tea; FT: Filtered tea bag; PCT: Powder Ceylon tea; GT: Goran tee (Mevlana)
Means with different superscripts (a-e) are significantly different, p<0.05. FRAP:ferric reducing/antioxidant power; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; CUPRAC: Cupric reducing antioxidant capacity; BHA:Butylated hyroxyanisole; BHT:Butylated hyroxytoluene

3.3. In vitro evaluation of anticholinesterase activity

The results for the assessment of cholinesterase inhibitory activity of samples (500 µg/mL) were shown in Table 4. According to obtained results, PT [5 min (68.58%), 10 min (66.58%), and 60 min (62.66%)] and FT [15 min (66.21%) and 30 min (66.21%)] samples showed stronger anticholinesterase activity than other samples. When the values of enzyme inhibition of all samples were compared, it was found that all the samples showed very close each other anticholinesterase activity. In this study, findings suggest that the steeping time is no significant effective on anticholinesterase activity of samples.

Table 4. *Effects of steeping time on the anticholinesterase activity of tea samples*

Codes of samples	Enzyme inhibition (%) (500 µg/mL)
After 5 min. steeping	
FT	62.22±2.6^{a}
PCT	48.57±0.26^{b}
GT	58.29±0.97^{c}
PT	68.58±0.35^{d}
After 10 min. steeping	
FT	55.86±1.09^{a}
PCT	56.86±0.33^{b,a}
GT	60.60±0.18^{c}
PT	66.58±2.29^{d}
After 15 min. steeping	
FT	66.21±0.62^{a}
PCT	58.65±0.95^{b}
GT	63.78±0.44^{c}
PT	63.47±0.35^{d,c}
After 30 min. steeping	
FT	66.21±1.31^{a}
PCT	59.16±0.26^{b}
GT	60.47±0.18^{c,b}
PT	57.23±0.18^{d}
After 60 min. steeping	
FT	58.48±0.87^{a}
PCT	54.68±1.67^{b}
GT	59.41±2.02^{c,a}
PT	62.66±0.79^{d}
Galantamine (200 µg/mL)	98.12±0.001^{e}

PT: Powder tea; FT: Filtered tea bag; PCT: Powder Ceylon tea; GT: Goran tee (Mevlana)
Means with different superscripts (a-e) are significantly different, p<0.05

4.DISCUSSION

Because of antioxidants, products have preventive potential against a large number of diseases; antioxidants in food, beverages and herbal extracts have been extensively studied. Many studies have shown that the biological activity of tea may be due to polyphenols (11-13). Polyphenol compounds are secondary metabolites found in medicinal plants, vegetables, fruits and various beverages such as fruit juices, wine and tea (14-17). In this context, there are many reports describing the biological activity and polyphenol content of black teas. Studies have shown that black tea contains high amounts of polyphenol compounds and has strong antioxidant activity (18-20). In this study, it was confirmed that four different black teas commercially valuable in Turkey is a good source of these components. Previous studies have shown that antioxidant capacity and total polyphenol content in green tea extracts are related to extraction time (21-23). However, there are limited studies on the effect of infusion time on the extraction of phenolic compounds in tea and on the biological activities of tea.

Therefore, we have studied the effects of the time of infusion on phenolic content and biological activities of the samples obtained from different teas. It has been found that the time of the infusion was important in the amount of phenolic contents, since the TPC values of some teas (FT, GT and PT) change at different times. However, it was determined that the increase in TPC with infusion time was not linear. The amount of tannin present in the samples did not change significantly depending on the infusion time.

It has been determined in the literature that longer infusion time leads to an increase in TPC values (3, 4). In our study, the TPC values of FT, GT and PT samples increased in parallel with the literature information, but there was no significant change in the TPC value of PCT sample. The antioxidant (DPPH, CUPRAC) and anticholinesterase activities of the tea samples did not show significant change due to the time of the infusion.

Theoretically, if the health benefits associated with tea consumption are due to the presence of total phenols, then it would be beneficial to have a larger quantity and would support the longer brewing tea assumption to extract more phenols (4). In our study, in parallel with the this literature, it was determined that the amount of phenolic contents generally increased with the infusion time. According to the results obtained from this study, it was determined that four different tea have phenolic, tannins contents and biological activity very close to each other.

5.CONCLUSION

According to the results obtained from this study, it was determined that four different tea samples showed close biological activity each other. When we compare all the tea samples among themselves, FT sample obtained after 5 min steeping exhibited stronger DPPH radical scavenging activity than other samples. In addition, it was found that PCT samples obtained after 60 min and 10 min steeping had the highest FRAP and CUPRAC values respectively. In this study, it was found that the PCT samples obtained after 5 min, 10 min, 15 min, and 30 min steeping contained higher phenolic, and tannins contents than the other samples. PT sample obtained after 5 min steeping showed the strongest anticholinesterase activity. As a results, it was determined that the amount of phenolic contents generally increased with the infusion time. It was also found that the antioxidant

(DPPH, CUPRAC), anticholinesterase activities and tannins content of the tea samples did not show significant change due to the time of the infusion.

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