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Comparative Assessment of Total Polyphenols and Antioxidant Activity of Commercial Green Tea from Tuzla Markets

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ABSTRACT

Green Tea, made from *Camellia sinensis* plant leaves, is one of the most popular drinks in the world. For the past decades, scientists have studied this plant in terms of potential health benefits. Research has shown that green tea helps prevent stroke, malignancy and infections. In this paper, antioxidant activity and total phenol content of 4 samples of green tea from local Tuzla stores were investigated, of which two were of foreign origin. The antioxidant activity of the samples was analyzed using FRAP and DPPH methods. The obtained results show that the highest content of total phenols and the largest antioxidant capacity has a sample of foreign origin. The content of total phenols in the samples ranges from 60.01 to 79.34 mg GAE/g. The highest FRAP value is 3.34 mmol/g. The antioxidant capacity was also confirmed by the DPPH method. The IC_{50} value ranges from 0.014 to 0.030 mg/mL.

Keywords-- Phenol, FRAP, DPPH, Green Tea

I. INTRODUCTION

Green tea (Camellia sinensis Theaceae) was discovered in China 3000 BC or earlier and is known to have various medical effects. It has been proven that green tea ingredients have a certain level of efficiency against cancer, obesity, bacterial and viral infections (Suzuki et al., 2012). The main components of green tea are polyphenols that are responsible for the antioxidative and other health benefits of green tea. The main polyphenols in green tea are flavonoids. Four major flavonoids are catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate. Epigallocatechin gallate is considered to be the most significant active component (Sinija and Mishra, 2009). The structures of these components are shown in Figure 1. (Hodgson et al., 2013). Several studies have noted the positive effects of green tea extract on fat metabolism during and after exercise, and after shorter and longer intake. However, in general, the literature is unconvincing. The fact is that not all the effects are related to differences in study design, bioavailability of green tea extract and variations in measurement (oxidation of fat) (Hodgson et al., 2013). Green tea acts as a cyclooxygenase inhibitor, lipoxygenase, a factor of tumor necrosis and interleukinic pathways and ultimately controls the development and progression of tumors (Rahmani et al., 2015). The chemical composition of green tea is complex. Protein content is 15-20% dry weight, amino acids 1-4% dry mass (5-N-ethylglutamin, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine and lysine), carbohydrates 7% dry weight (cellulose, pectin, glucose, fructose and sucrose), minerals and trace elements 5% (calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluoride and aluminum). The composition of certain bio components in green tea is shown in Table 1.

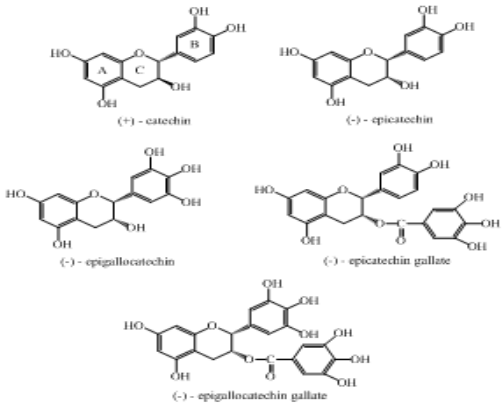

Figure 1: Chemical structures of catechin, epicatehin, epigallocatechin, epicatechin gallate and epigallocatechin gallate

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Table 1 : Content of Individual Components (%) in Green Tea and Its Solution

Component	Green Tea	Solution
Proteins	15	traces
Amino acid	4	3.5
Fibers	26	0
Carbohydrates	7	4
Lipids	7	traces
Pigments	2	traces
Minerals	5	4.5
Phenolic components	30	4.5
Oxidized phenolic components	0	4.5

II. METHODOLOGY

All chemicals were of high purity grade, purchased from Aldrich and used without further purification.

Total Phenolic Content

The content of polyphenols was determined according to the procedure previously published (Tawaha et al., 2007). Appropriate dilution was prepared for each of the extracts. Absorbance of the resulting blue colored liquids was measured at 765 nm, using a Shimadzu UV-mini-1240 UV/Vis Spectrophotometer. Quantitative analysis was performed based on the standard calibration curve of gallic acid, using the concentration range between 3 mg/mL and 150 mg/mL (y = 0.0049x + 0.0449; R^2 = 0.9982). Calibration curve of the gallic acid is shown in Figure 2.

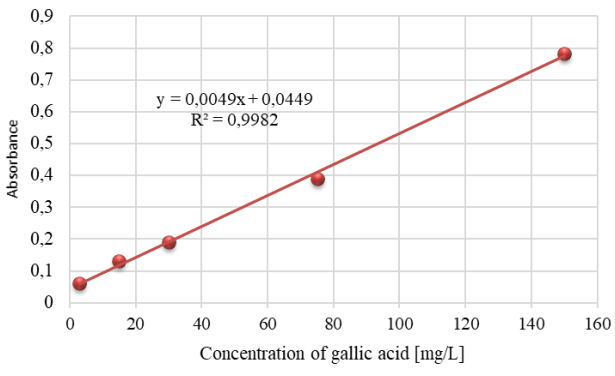

Figure 2: Calibration curve of the gallic acid

Ferric Reducing Antioxidant Power Assay (FRAP)

The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) was performed as described earlier (Jiménez-Aspee et al, 2014). To prepare the calibration curve, solutions of $FeSO_4$ x $7H_2O$ were prepared in the concentration range of 200-1000 µmol/L (y = 0.0005 + 0.0744; R^2 = 0.9981). In each tube, 0.1 mL of extract and 3 mL of FRAP reagent were added. The samples were incubated in an aqueous bath for 30 minutes at 37 °C, and the absorbance was measured at 593 nm. Obtained calibration curve of the $FeSO_4$ x $7H_2O$ is shown in Figure 3.

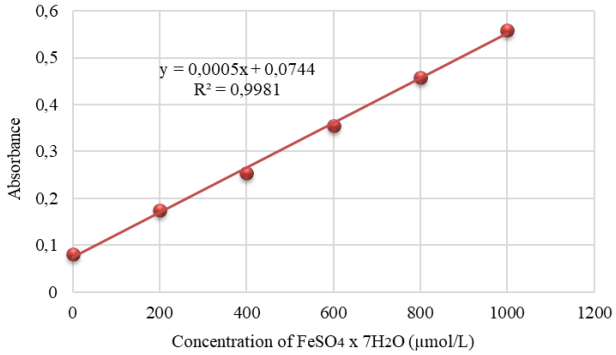

Figure 3: Calibration curve of the $FeSO_4$ x $7H_2O$

DPPH Radical Scavenging Activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to the method described earlier (Benvenuti et al, 2007). The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

[(Acontrol-Asample)/Acontrol] x 100,

where Asample is the absorbance of the solution containing the sample at 517 nm and Acontrol is the absorbance of the DPPH solution. The results are expressed as the IC50 value (mg/mL) or the concentration of extract that caused 50% neutralization of DPPH radicals.

III. RESULTS AND DISCUSSION

The content of total phenol in samples of commercial tea is shown graphically at Figure 4. Samples of foreign origin (sample 1 and 3) showed the greatest deviations in the content of total phenols. Sample 1 contains the highest polyphenol (79.34 mg GAE / g), while sample 3 is the lowest (60.01 mg GAE / g). These values are significantly higher than the results obtained by other researchers for this type of tea (Taheri et al., 2011; Nibir et al., 2017).

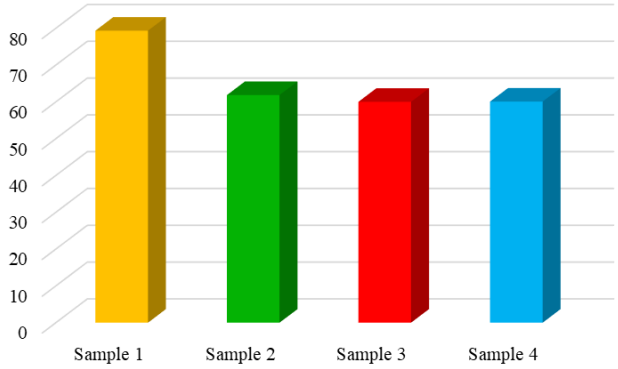

Figure 4: Content of total phenols in green tea extracts

The results of the antioxidative capacity obtained by the FRAP method are shown at Figure 5. Values ranged from 3.34 mmol/g for a sample with a maximum of 2.41 mmol/g for a sample with the lowest FRAP value. The results obtained by the DPPH method (Figure 6) confirm the results obtained by the FRAP method. The summarized results of the research are shown in Table 2.

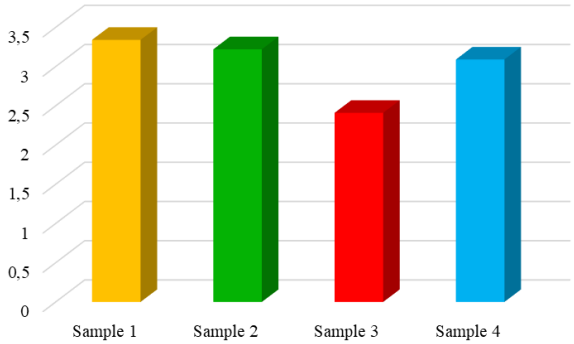

Figure 5: Results of antioxidative capacity obtained by FRAP method

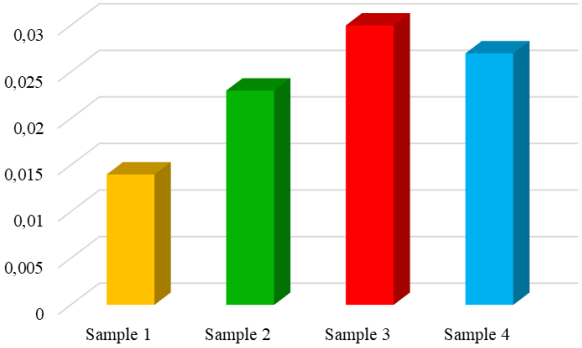

Figure 6: Results of antioxidative capacity obtained by DPPH method

Studies have shown that the conditions during tea preparation (tea extraction time, extraction temperature, solvent used) have a significant effect on the content of bioactive substances in the drink itself, such as polyphenolic compounds and methylxanthines (caffeine, theophylline, teobromine). Other factors, such as: geographic origin and conditions for processing and storing teas, have great influence on tea quality. The level of fermentation is associated with the amount of caffeine in teas, longer fermented teas have a lower overall content of polyphenols, lower catechin content, and lower antioxidant capacity (Zhao et al., 2014). The effect of the extraction of bioactive substances from green tea during the preparation of the tea beverage depends on the extraction conditions. Results have confirmed that aqueous extraction of green tea at 80 °C leads to higher antioxidative capacity compared to extraction at room temperature (Ramírez-Aristizabal et al., 2017). Studies have showed that the maximum extraction effect is achieved during water extraction at 80 °C for 5 minutes when the tea is in powder form, for 15 minutes when the tea is in the bag and for 30 minutes when the extraction is carried out from tea leaves (Kopjar et al. , 2013).

Table 2: Summarized Results of the Analysis of Total Phenol Content and Antioxidative Capacity

Sample	TPC [mg GAE/g]	FRAP [mmol/g]	DPPH [mg/mL]
1	79.34	3.34	0.014
2	61.84	3.22	0.023
3	60.01	2.41	0.030
4	60.06	3.09	0.027

IV. CONCLUSION

The results of this investigation showed that commercial green tea from Tuzla markets have high content of polyphenols and high antioxidant capacity. Probably the price is dictated by the quality in the case of tested tea samples. The displayed results of TPC, FRAP and DPPH for each sample of tea in this analysis may differ from other published values due to differences in tea variety, type of soil, height of cultivation, post harvest storage, processing conditions and ontogenetic factors.

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