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Antioxidant Properties of Some Herbal Teas (Green tea, Senna, Corn Silk, Rosemary) Brewed at Different Temperatures

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Abstract: Some non-wood forest products are brewed and consumed as tea. Among the reasons for the consumption of herbal tea, digestive problems are located in the first row. Antioxidants help to human body for arranging digestive and immune system. Herbal tea is brewed in various ways such as boiling at different durations or waiting in hot water at different temperatures etc. Type of brewing can affect to bioactive properties of herbal tea. In this study, it was investigated the bioactive properties (total phenolic content, total flavonoid content, condensed tannin content and antioxidant properties) of some herbals brewed (Green tea / *Camellia sinensis.*, senna / *Cassia* sp., corn silk / *Zea mays*, rosemary / *Rosmarinus officinalis*) at different temperature. These herbs were brewed for 10 minutes at 60ºC, 80 ºC and 100 ºC temperatures. After cooling, total phenolic, flavonoid content, total condensed tannin content and antioxidant properties of these herbs were determined. Consistently, the highest results were found in the tea brewed at 100ºC. The highest total flavonoid (0.305 ± 0.005 mg QE/g) and ferric reducing ability (670.150 ± 2.121 µmol $FeSO_47H_2O$/g**)** was in *Rosmarinus officinalis.* The highest condensed tannin (9.443 ± 0.524 mg CE/g) and the highest total phenolic content (4.872 ± 0.005 mg GAE/g) was in *Camellia sinensis* and *Cassia* sp., respectively.

Keywords: Antioxidant, corn silk, green tea, herbal tea, rosemary, senna

1. INTRODUCTION

The tendency towards natural products to live a healthy life is increasing day by day. One of the practical prepared and most consumed of these natural products is herbal teas [1]. Thanks to the climate and soil, Turkey has a very wide range of plant that can be consumed as tea [2]. Plants have many phytochemicals that are potential sources of natural antioxidants such as phenolic diterpenes, flavonoids, tannins and phenolic acids [3]. These phytochemicals were reported that have bioactive properties e.g. antioxidant, anti-inflammatory, antitumoral, anti-cancer and immuno-modulatory characteristics [4]. It has been advocated that there is a direct correlation between the increase of antioxidant-rich foods and the decrease in the number of human diseases [5].

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Natural antioxidants, especially phenolics and flavonoids, are safe and bioactive [6]. The natural antioxidants help to clear away harmful free radicals from our bodies. A free radical is any species that has the ability to independently exist, containing one or more unpaired electrons that react with the other molecule by taking or donating electrons [7]. Free radicals have been associated with an increased risk of cardiovascular disease, cancer and other chronic diseases [8]. Therefore, antioxidant research is important for the formula of healthy life.

Many people are using medicinal herbs to alleviate and cure their illnesses due to their mild characteristics and low side effects [9]. In Turkey, there are a variety of plant species brewed and consumed for various reasons, such as strengthen the immune system and digestive system. Some of these herbal teas are *Camellia sinensis* (green tea), *Zea mays* (corn silk), / *Cassia* sp. (senna), *Rosmarinus officinalis* (rosemary). The teas of herbals can be brewed at different temperatures, but the effect of temperature to antioxidant properties is not yet clearly defined. Therefore, the aim this work to compare the antioxidant activity of some herbals such as *Camellia sinensis* (green tea), Zea *mays* (corn silk), / *Cassia* sp. (senna), *Rosmarinus officinalis* (rosemary) brewed at different water temperature.

2. MATERIAL and METHODS

2.1. Plant Material

The four different commercial, pre-packaged, dry herbs were purchased from a local medicinal herbs market in Trabzon / Turkey 2016**.** Scientific name, common name of studied herbs and the months of preparation of the herbs are presented in Table 1.

Table 1. Scientific name and common name of studied herbs

Scientific name	Common name	Year	The months of preparation of the herbs
Camellia sinensis	Green tea	2016	August-November
Cassia sp.	Senna	2016	August-September
Zea mays	Corn silk	2016	August-November
Rosmarinus officinalis	Rosemary	2016	September-November

2.2. Preparation of the Extracts for Determination of Antioxidant Activity

Approximately 5 g of samples were placed into a falcon tube with additional 100 mL water. Each herbal tea was brewed at 3 different temperatures: 60 °C, 80 °C and 100°C. The mixture was stirred continuously with a shaker (Heidolph Promax 2020, Schwabach, Germany) for 10 minutes. Particles were removed using Whatman No. 4 filter paper pore size 20-25 µm. Then solutions were filtrated from hydrophilic polyvinylidene fluoride (PVDF) 0.45 µm for sterilization. The finally volume of the solution was adjusted by the level of water.

2.3. Determination of Polyphenolic Contents

The polyphenolic contents of the samples were evaluated three different ways; total phenolic contents (TPC), total flavonoids (TF) and condensed tannin (CT). For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was employed and gallic acid was used as standard [10]. Shortly, 20 µL of various concentrations of gallic acid and samples, 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water were mixed and vortexed. After 3 min incubation, 400 µL of Na_2CO_3 (10%) solution was added and vortexed. Then the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g extracts.

The concentration of total flavonoid present in the water extracts was measured using a spectrometric assay. Briefly, 0.5 mL samples, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M $NH_4.CH_3COO$ were added to a test tube and incubated at room temperature for 40 min. Then the absorbance was measured against a blank at 415 nm. Quercetin was used for the standard calibration curve. The total flavonoid concentration was expressed as mg of quercetin equivalents per g sample [11].

Condensed tannins were determined according to the method by Julkunen-Tıtto [12]. For each sample, various concentrations of 25 µL from extracts of herbs were mixed with 750 µl of 4% vanillin (prepared with MeOH) and then 375 µL of concentrated HCl was added. The well-mixed solution was incubated at room temperature in darkness for 20 mins. The absorbance against the blank read at 500 nm. (+)-Catechin was used to help make the standard curve (0.05–1 mg/ml). The results were expressed as mg catechin equivalent to (CE)/g sample.

2.4. Determination of Antioxidant Capacity

The antioxidant capacity was determined using ferric reducing antioxidant power.

2.4.1. Ferric Reducing Antioxidant Assay (FRAP)

FRAP assay was also tested to determine the total antioxidant capacity of the samples. This method is based on the reduction of tripyridyltriazine complex (Fe (TPTZ) $^{3+}$) to blue colored $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium [13]. The preparation of working FRAP reagent was carried out by mixing 25 mL of 0.3 M acetate buffer pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution. The reaction mixture consisting of 1 mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. Then, the absorbance was determined at 593 nm against blank prepared with distilled water. A calibration curve prepared with an aqueous solution of ferrous sulfate $FeSO_4.7H_2O$ in the range of 100-1000 µM was used. FRAP values were expressed in wet weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

2.5. Statiscal Analysis

All assays were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The obtained data were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

3. RESULTS and DISCUSSIONS

3.1. Total Phenolic Content

Total polyphenol content of herbal teas brewed at different temperature are presented in Fig. 1. The herbal teas are very important because of containing a high amount of phenol in human diets. It is reported that rich in phenolic compounds significantly affect human health [6]. In this study, the highest total phenolic content was determined in water extract of senna tea brewed at 100°C with 4.872±0.005 mg GAE/g (Fig 1) and the lowest in rosemary tea brewed at 60°C with 0.313±0.008 mg GAE/g. The highest total phenolic content in all variations was found in herbal samples brewed at 100°C (Fig. 1) The total phenol content of herbal teas brewed at different temperatures is statistically significantly different from each other ($p<0.05$). In a previous study, phenolic content of extracts of 27 culinary herbs and 12 medicinal herbs were reported arrange of 0.43±0.08 between 17.51±0.22 mg of GAE/g of fresh weigh [14]. Therefore, it can be said that the total amount of phenolic content may change depending on the plant species.

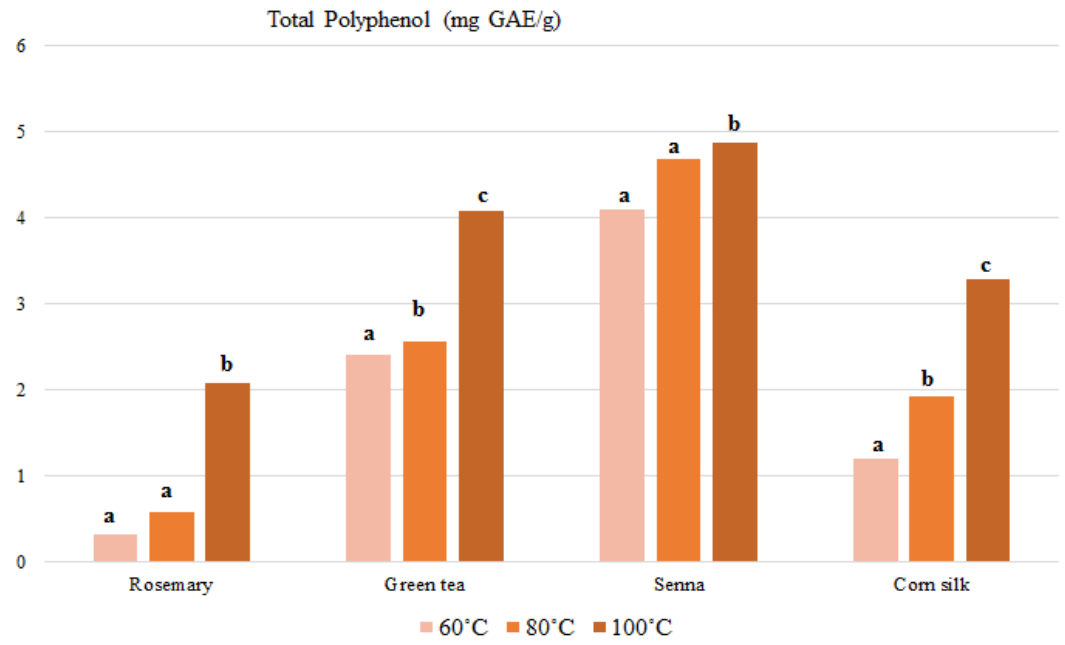

Figure 1. Total polyphenol content of herbal teas at different temperature. *Same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test; n=3.

3.2. Total Flavonoid Content

Total flavonoid content of herbal teas brewed at different temperature are presented in Fig. 2. Flavonoids, one of the leading antioxidant compounds in plants that giving color to fruit and leaves, generally show bioactive properties such as exhibiting therapeutic functions for enzyme inhibition, free radical cleaning and cofactor activity for antioxidant C vitamins [15]. The total amount of phenol in this study ranged from 0.004±0.002 to 0.305±0.005 mg QE/g (Fig 2). The highest total flavonoid content were determined in extract of rosemary tea brewed at 100°C. The flavonoid content could not be determined in rosemary tea brewed at 60°C. As can be seen in Fig 2., there is not significantly difference between senna tea brewed 60°C and 80°C (p>0.05). In generally; the total flavonid contents are quite low especially in low temperatures. In this case; it can be said that 60°C and 80°C temperatures are not sufficient degrees to achieve the full benefits from the herbal plants.

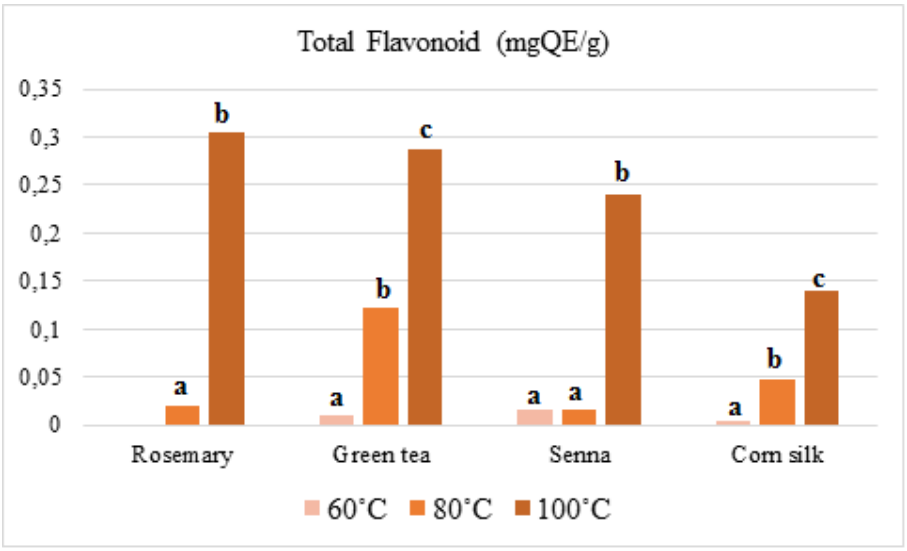

Figure 3. Total flavonoid content of herbal teas brewed at different temperature *Same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test; n=3.

Some researches were reported that total flavonoid levels of 11 herbs include rosemary and green tea methanol extracts were ranged between 23.7 and 225 mg CE/g of extract [16]. So the type of solvent also affects the total flavonoid content.

3.3. Condensed Tannin Content

The condensed tannin content of herbal teas brewed at different temperature are presented in Fig. 3. Condensed tannins are structurally more complex, and more widely spread among the plants than hydrolysable tannins [17]. Some tannin molecules (e.g., tea polyphenols) have been reported that they have anticancer or anticarcinogenic or antimutagenic activity [18, 19]. So, tannin of herbal teas is important for healthy life. In this study, condensed tannin content could not be determined in rosemary teas brewed at 60°C and 80°C temperatures. The highest and the lowest condensed tannin content was found in extract of green tea brewed at 100°C (9.443±0.524 mg CE/g) and rosemary tea brewed at 100°C (0.105±0.006 mg CE/g), respectively. The amount of condensed tannins in the green tea was found to be 9 times higher from rosemary especially in the treatment at 100°C. In a previous study, condensed tannin content could not be determined rosemary tea, too. Also, the condensed tannin content of balm, mint, black tea, sage and common verbena was reported between 0.02 ± 0.00 and 2.11 ± 0.18 mg CEs/ml [20].

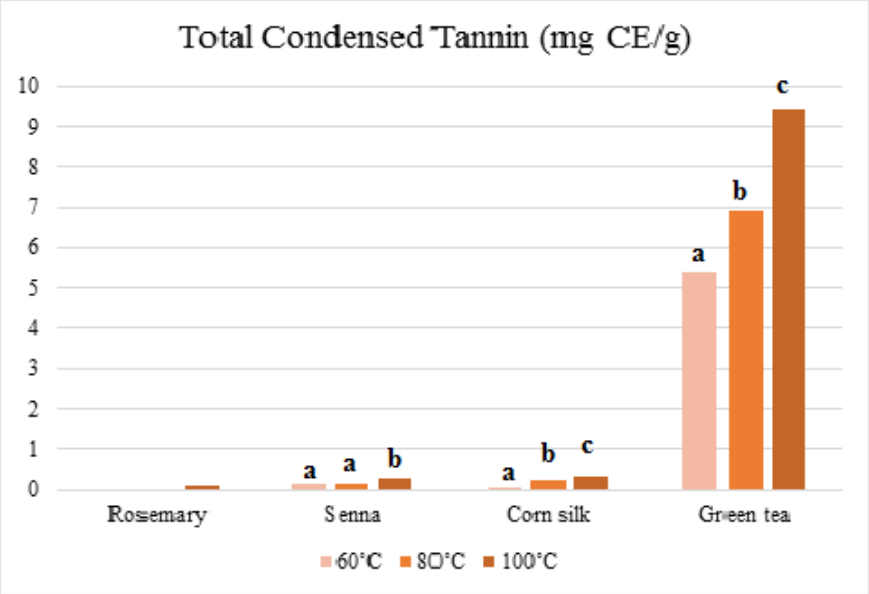

Figure 4. Total condensed tannin content of herbal teas brewed at different temperature*Same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test; n=3.

3.4. Ferric Reducing Antioxidant (FRAP) Activity

Ferric reducing antioxidant (FRAP) activity of herbal teas brewed at different temperatures are presented in Fig. 4. The FRAP assay (Ferric Reducing Ability of Plasma), a simple test of the total antioxidant power have been chosen to assess the presumable effects of some kind of tea and medicinal plant [21]. In this study, FRAP activities of herbal teas can be listed that rosemary > green tea > senna > corn silk. The highest FRAP activity was determined in rosemary tea brewed at 100°C with 670.150±2.121 µmol $FeSO_47H_2O$/g and this value was 77 times higher than the lowest FRAP activity (8.763±0.226 µmol $FeSO_47H_2O$/g); in corn silk herbal tea brewed at 60°C. FRAP activities of all herbal teas brewed at different temperatures were found significantly different (p<0.05) from each other by Duncan's multiple range test. In a previous study; FRAP activity of ethanol-based lyophilized hydrophilic extracts prepared

from herbal teas from Eastern Anatolia have been reported, ranging from 390.8 ± 13.5 to 1130.8 ± 48.2 µmol Fe^{2+}/g dried weight [22].

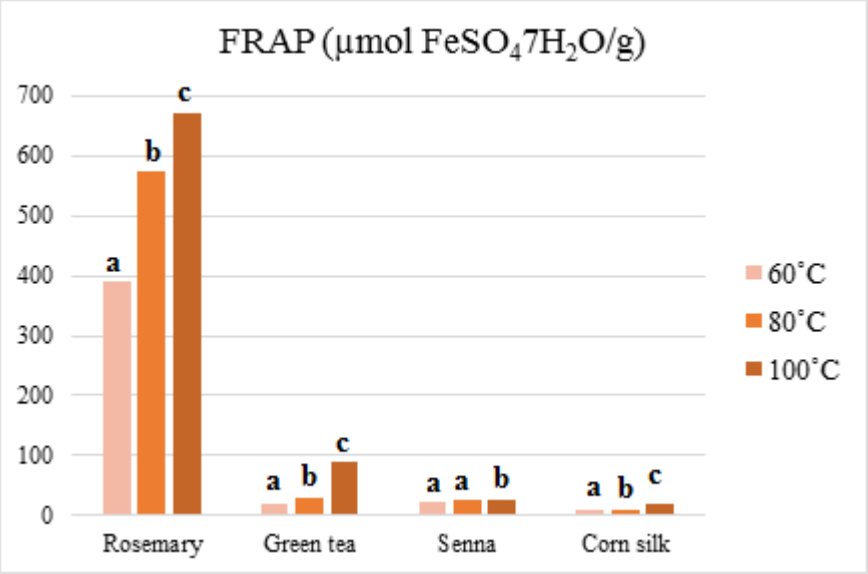

Figure 3. Ferric reducing antioxidant (FRAP) activity of herbal teas brewed at different temperatures.
*Same letter(s) are not significantly different (p>0.05) by Duncan's multiple range test; n=3

4. CONCLUSIONS

In this study, antioxidant activities of *Camellia sinensis* (green tea), Zea *mays* (corn silk), *Cassia* sp. (senna), *Rosmarinus officinalis* (rosemary) herbal teas brewed at different water temperatures (60°C, 80°C and 100°C) were determined and compared with each other. The highest total phenolic content was determined in senna tea; the highest total flavonoid content and the highest ferric reducing ability in rosemary tea, the highest condensed tannin content in green tea. The highest values were found in brewed at 100 °C temperatures in among all herbal teas. Consequently, it can be stated that plant species, solvent types, boiling temperatures can affect the total phenolic content, total flavonoid content, condensed tannin content and ferric reducing antioxidant activity. In order to make more comparison; similar analysis can be made with different herbal species and different temperatures and solvent types.

Conflict of Interests

Authors declare that there is no conflict of interests.

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