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DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF GREEN TEAS BY THE FERRIC REDUCING/ANTIOXIDANT POWER ASSAY

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ABSTRACT

Green tea is one of the important sources of bioactive compounds which have been used in folk medicine for many centuries. This study aimed to compare in vitro antioxidant power of different types of green tea (*Camellia sinensis*). Antioxidant activity of methanolic (50%) extracts of five green tea samples was investigated according to Ferric reducing ability power method. Total phenolic contents were analyzed using a spectrophotometric technique, based on the Folin-ciocalteau reagent, and calculated as gallic acid equivalents per gram dry weight. Total flavonoid and antocyanidin were also investigated according to aluminum chloride and vanillin colorimetric assay respectively. Total antioxidant activity varied from 0.554±0.042 in Avicen green tea sample to 3.082±0.150 mmoL Fe^{II}/g in Chinas green tea and total phenolic content ranged from the 0.030±0.001 in Avicen green tea sample to 0.196±0.012 g gallic acid per gram dry weight in Ahmad green tea. A linear positive relationship existed between the antioxidant activity, total phenolic, flavonoid and antocyanidin content of the tested green tea samples. Green tea samples possess relatively high antioxidant activity due to contribution of phenolic compounds. The present study showed that green tea samples which are more frequently consumed in Iran are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses.

Key words: Antioxidant activity, green tea, phenolic compound, flavonoid compound, antocyandin compound

INTRODUCTION

The tea plant (*Camellia sinesis* L.) is grown in about 30 countries worldwide (Graham, 1992). It grows best in tropical and subtropical areas with adequate rainfall, good drainage, and slightly acidic soil (Graham, 1999). Tea (*Camellia sinesis*) is also the most widely consumed beverage worldwide for its desirable aroma, taste and putative positive physiological functions (Zhu *et al.,* 2002; Balentine, 1992). The type and quantity of tea taken varies in different countries and races (Weisburger, 1996; Kohlmeier, 1997). Tea contains large amounts of polyphenolic compounds with antioxidant properties, and these may prevent oxidative damage of DNA (Wiseman *et al.,* 1997; Zhang and Shen, 1997).

Tea is also rich in flavonoids and other polyphenols that have been shown to possess a wide range of biological and pharmaceutical benefits, including anticarcinogenic, antioxidative, and hypolipidemic activities (Buschman, 1998; Yang, 1999). These beneficial effects are may be attributed to tea's antioxidant activity, e.g., free radical scavenging and metal chelating abilities. Studies have shown green tea has anti-inflammatory (Tipoe *et al*., 2007), cholesterol lowering (Koo and Noh, 2007), antiviral and antibacterial properties (Weber *et al.*, 2003; Friedman *et al*., 2006). Based on results mainly from animal studies, many companies are supplementing their skin care products with green tea extracts (Katiyar and Elmets, 2001). However few studies have been conducted to investigate the antioxidant activity of Iranian consumed green tea samples. The purpose of this study was to evaluate highly consumed five green tea samples

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in Iran as new potential sources of natural antioxidants and phenolic compounds. Our study also demonstrates a possible relationship between phenolic content and antioxidant activity.

MATERIALS AND METHODS

Materials

All solvents/chemicals used were of analytical grade and obtained from Merck Company (Darmstadt, Germany). Double-distilled deionized water was used for the preparation of aqueous solutions.

Sample preparation

Five samples of green tea were purchased from supermarket including: Ahmad green tea bags, a Chinas green tea which imports by behnoosh company, Armaghane tabiat green tea which is produced by medicinal and aromatics plants service center, Camellia green tea produced by golchai company and the Avicen green tea which was donated by khorraman company. All the green tea samples were expired at least one year later. 5 g of the five selected green teas were separately ground using a stainless-steel grinder. Five hundred milligrams of sample was extracted for 2h with 2 mL of 50% methanol at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 10000 g for 15 min, and the supernatant was decanted in to 50 mL volumetric flask. The pellets were extracted under identical conditions several times. Supernatants were combined and diluted to 50 mL with 50% methanol. The samples were filtered, further diluted if necessary and directly used for total phenolic, total antioxidant, total flavonoid and total anthocyanidins assay without storage.

Measurement of total antioxidant activity

The FRAP (Ferric reducing antioxidant power assay) procedure described by Benzie and Strain was followed (Benzie and Strain, 1999). The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to it's ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 mL of a (10 mmol/L) TPTZ (2, 4 ,6- tripyridyl- s- triazine) solution in 40 mmol/L HCL plus 5 mL of $FeCl_3$ (20 mmol/L) and 50 mL of acetate buffer, (0.3 mol/L, pH=3.6) and was prepared freshly and warmed at 37ºC . Aliquots of 100 µL sample were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37ºC for 10 min. For construction of calibration curve five concentrations of $FeSO_4$ $7H_2O$ (1000, 750, 500, 250, 125 µmol/L) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. Antioxidant activity was measured five times for each tea samples and the results are shown in Table 1.

Total phenolic compound analysis

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent (Velioglu *et al.,* 1998) with slight modifications. The extract (200 µL) was mixed with 1.5 mL of Folin- Ciocalteau reagent (previously diluted 10- fold with distilled water) and allowed to stand at 22ºC, for 5 min. A 1.5 mL sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 min at 22ºC, absorbance was measured at 725 nm using a UV-Visible sectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a know concentrations of galic acid standard (25-150 µg/ mL of 50% methanol). The concentrations are expressed as g of gallic acid equivalents per one g of dry weight. The total phenolic assay was measured five times for each tea samples and the results were shown on Table 2.

Total flavonoid assay

Total flvonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al*., 1999). An aliquot (1 mL) of extracts or standard solution of catechin (50, 100, 150, 200, 250 and 300 mg/L) was added to 10 mL volumetric flask containing 4 mL of double distilled water. To the flask was added 0.3 mL 5 %$NaNO_2$. After 5 min, 0.3 mL 10 % $AlCl_3$ was added At 6^{th} min, 2 mL 1 M NaOH was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed well and absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg

catechin equivalents (CE)/g dry mass. The total flavonoid assay was measured five times for each tea samples and the results were shown on Table 2.

Total anthocyanidin assay

1 mL of sample or catechin standard solutions (50-300 mg/L), 2.5 mL of 1% (w/v) vanillin in methanol, and 2.5 mL of 9.0 N HCl in methanol were added (Sun *et al*., 1998). After incubation at 30°C for 20 min, the absorbances were recorded at 500 nm. The total antocyanidin assay was measured five times for each tea samples and the results were shown on Table 2.

Statistical analyses

Six replicates of each sample were used for statistical analysis and the values are reported as mean±SD. Correlation analyses of antioxidant activity versus the total phenolic, total flavonoid and total antocyanidin content were carried out using the correlation and regression program in SPSS 11.5 and the results are shown in Fig. 1 a, b and c. Data were subjected to analysis of variance, and means were compared by Tukey posthoc multi comparison tests. Differences at $P<0.05$ were considered to be significant.

Table 1: Total antioxidant activity of different green teas

Samples	Antioxidant capacity		
	mmol Fe^{II}/g sample	g vitamin E/ g sample	mmol vitamin C/g sample
Ahmad green tea	2.876 ± 0.213	0.813 ± 0.064	1.562 ± 0.128
Avicen green tea	0.554 ± 0.042	0.156 ± 0.013	0.299 ± 0.025
Camellia green tea	1.737 ± 0.261	0.471± 0.078	0.875 ± 0.158
Chinas green tea	3.082 ± 0.150	0.874 ± 0.045	1.686 ± 0.090
Armaghane tabiat green tea	1.594 ± 0.080	0.428 ± 0.024	0.789 ± 0.048

Table 2: Total phenol, flavonoid and antocyanidin contents in different green teas

Samples	Total phenol	Total Flavonoid	Total antocyanidin ´
	g GA/g sample	mg Catechin/g sample	mg Catechin/g sample
Ahmad green tea	0.196 ± 0.012	31.152 ± 1.907	59.412 ± 0.414
Avicen green tea	0.030 ± 0.001	2.898 ± 0.412	2.820 ± 0.636
Camellia green tea	0.108 ± 0.007	19.170 ± 0.226	29.629 ± 0.591
Chinas green tea	0.160 ± 0.012	22.752 ± 1.510	44.826 ± 0.291
Armaghane tabiat green tea	0.091 ± 0.012	20.144 ± 0.694	25.767 ± 0.264

RESULTS

Antioxidant estimation

The results of the FRAP assay with methanol 50% are reported in Table 1. All extracts contained a considerable amount of antioxidant effect from minimum 0.554±0.042 to maximum 3.082±0.150 of mmol Fe^{II}/g dry plant in "Avicen green tea" and "Chinas green tea" respectively.

Total phenol estimation

The results of the Folin-Ciocalteu total phenol assay with methanol 50% are reported in Table 2. All extracts contained a considerable amount of phenolics contents from 0.030±0.001 to 0.196±0.012 g GA/g sample for green teas equivalents in Avicen green tea to Ahmad green tea samples.

Total flavonoid estimaton

The results of the aluminum chloride colorimetric assay with methanol 50% are reported in Table 2. All extracts contained a considerable amount of flavonoid contents from 2.898±0.412 to 31.152±1.907 mg Catechin/g sample for green teas equivalents in Avicen green tea to Ahmad green tea samples.

Total antocyanidin estimation

The results of the vaniline colorimetric assay with methanol 50% are reported in Table 2. All extracts contained a considerable amount of antocyanidin contents from 2.820±0.636 to 59.412±0.414 mg Catechin/g sample for green teas equivalents in Avicen green tea to Ahmad green tea samples.

Statistical results

A systematic comparison among the antioxidant activities of five different tea samples with ANOVA test and Dunnett's T3 Post Hoc were made. The results showed that there was no significant statistical differences between Chinas and Ahmad green tea, But these two samples had more potential antioxidant activity than the others

(P<0.001). Also it can be seen that Camellia and Armaghane tabiat green tea had the second rank with statistical differences (P<0.001) and the Avicen green tea had the less FRAP value with significant differences with the others. The total phenol, flavonoid and antocyanidin contents in in green tea samples were also compared with ANOVA test. The results showed that, like the FRAP value the Ahmad and Avicen green teas had the first and least rank among the teas samples respectively. The relationship between total phenolic, flavonoid and antocyanidin contents with antioxidant activity in green tea samples is shown in Fig. 1 a, b and c.

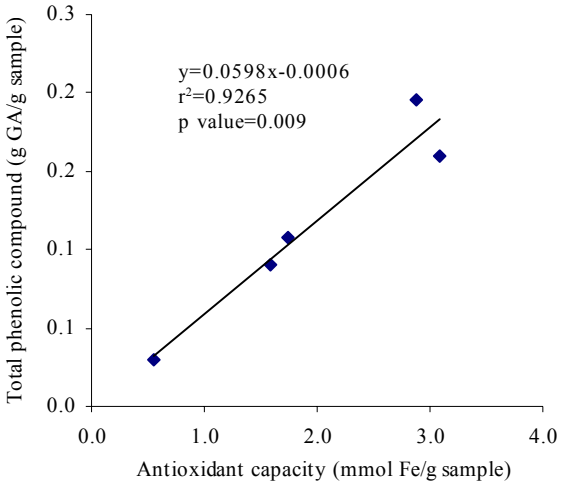

Fig. 1a: Relationship between Fe ^{II} equivalent antioxidant capacity and total phenolic contents in green tea samples

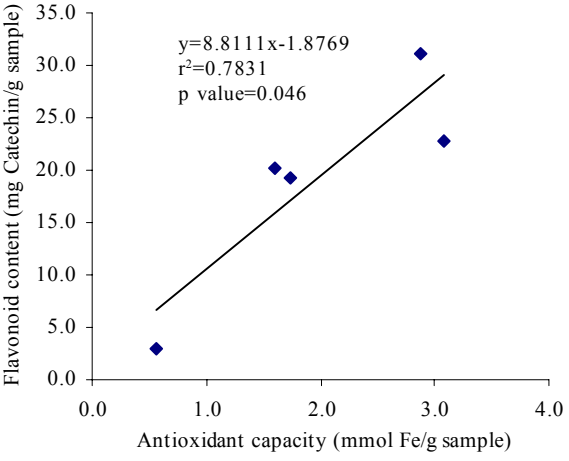

Fig. 1b: Relationship between Fe ^{II} equivalent antioxidant capacity and flavonoid content in green tea samples

The results indicate that there was a positive and highly significant (P<0.001) relationship between this effective compound with antioxidant activity indicating that total phenolics can play a major role in the antioxidant activity of green tea samples.

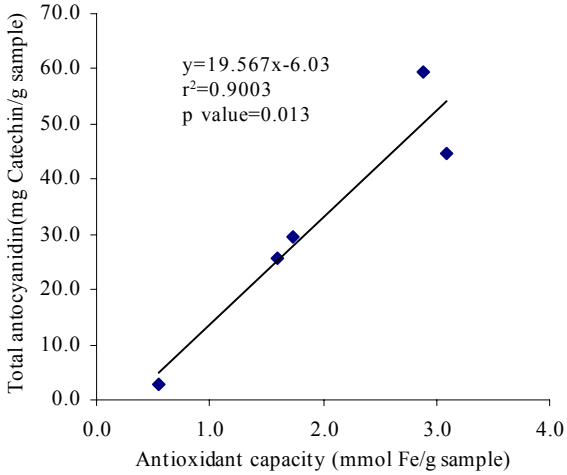

Fig. 1c: Relationship between Fe ^{II} equivalent antioxidant capacity and total antocyandins in green tea samples

DISCUSSION

In this study it is shown that different types of green teas had widely different antioxidant activities, ranging from 554 µmol Fe ^{II}/g in Avicen green tea to 2876 µmol Fe ^{II}/g in Ahmad green tea (Table 1). From these results it is estimated that one gram of green tea contains an amount of antioxidant power similar to that found in 50-275 mg of pure ascorbic acid (vitamin C), or 156-813 mg vitamin E, highlighting the enormous potential of tea as a dietary source of antioxidant power.

It is known that antioxidants inhibit lipid peroxidation by their free radical scavenging activity (Soong and Barlow, 2004).

Tea, particularly green tea, is a potentially rich dietary source of antioxidant power. In Iran, China and Southeast Asia tea is usually prepared and consumed by soaking the tea in hot water (>90 °C). The present study was undertaken to evaluate the antioxidant potential of herbal green tea extract which is usually used in Iran and to utilize it as a substitute for synthetic antioxidants. The antioxidant effects of green tea extracts have been evaluated using in vitro FRAP method in an attempt to make a systematic comparison among

their antioxidant activities and identify the samples with high antioxidant power for further studies. The study also attempts to quantify the total phenolic, flavonoid and antocyanidin compounds present in green tea extract. The results are shown in Table 1 and 2 on the basis of one gram dry weight. We tried to correlate the FRAP values obtained in this study with the data reported by others (Sun *et al*., 1998; Rechner *et al*., 2002; Pellegrini *et al.,* 2003; Ivanova *et al*., 2005; Katalinic *et al*., 2006; Kiselova *et al*., 2006; Chan *et al*., 2007; Su *et al.,* 2007). There are many reports describing the antioxidant activity of teas, but the result varies depending on the assay method. Benzie and coworkers (Benzie and Szeto, 1999) in 1999 reported the total antioxidant capacity of teas by the Ferric reducing antioxidant power assay. Their results showed that different teas had widely different in vitro antioxidant power and that the antioxidant capacity was strongly correlated (r^2=0.926) with the total phenolics content of the tea. Expressed as micromol Fe^{II} per g of dried tea leaves, values ranged 272-1144 µmol Fe^{II}/g for green teas. Therefore the tea samples screened in this paper (554- 2876 µmol Fe^{II}/g) had the more antioxidant power with highly correlation to phenolic, flavonoid and antocyanidin contents. Pellegrini and coworkers in 2003 (Pellegrini *et al.,*2003) assessed the total antioxidant capacity of plant foods, beverages and oils consumed in Italy. They reported that green tea had the antioxidant capacity equivalent to 2.25 mmol Fe^{II}/g sample, which is comparable with the result in this study, although the Ahmad green tea and Chinas green tea samples in this study had more potential antioxidant power. In summary, this study has shown that green teas more frequently consumed in Iran have the suitable antioxidant power and can be used as a favorite beverage for its antioxidant power.

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