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Investigation of total antioxidant capacity and total poly phenols of black tea in Iranian markets

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ABSTRACT

Tea is the most widely consumed and popular non-alcoholic beverage in the world. Reactive oxygen spices may cause wide range of damages to biological systems. Purpose of this study was to determine the total antioxidant capacity and total phenolic of different black tea samples in Iran. The FRAP (Ferric reducing antioxidant power) assay was used for antioxidant activity. The total phenolic content was measured based on the Folin-Ciocalteu method. The results showed that all samples had substantial antioxidant activity and total phenolic content. Iranian tea samples had significantly the lowest 0.98 ± 0.15, 0.75 ± 0.17 µmoll^{-1} and Kenya tea samples had significantly the highest 2.67 ± 0.61, 2.10 ± 0.65 µmoll^{-1} amount of total antioxidant capacity and total phenolic, respectively. A linear positive relationship was observed between the antioxidant activity and total phenolic content of the black tea samples. These findings suggest that black tea can be considered as natural source of antioxidant and total phenolic compounds.

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1. Introduction

Tea is the most widely consumed and popular non-alcoholic beverage in the world (1). It is derived from the leaves of Camellia sinensis (the tea plant) (2). According to different production procedure, there are three various main types of teas: black, green and oolong (3), which are taken variously in different countries (4).About 76-78% of the consumers in the world prefer black tea (5). Reactive oxygen spices may cause wide range of damages to biological systems (6). The important role of oxidative stress in many chronic and degenerative diseases like cancer, diabetes mellitus and cardiovascular diseases has been proven (7,8).

Tea has antioxidant effects such as inhibiting the peroxidation of low-density lipoprotein, free radical scavenging, metal chelating ability, preventing oxidative damage of DNA and preventing the development of cancer which have been attributed to its antioxidant compounds such as flavonoid and other polyphenols (4,9,10). So the antioxidant status of the body may be improved by regular intake of tea and as a result, the risk of certain types of cancer and coronary heart disease phenolic acids are natural antioxidant compounds in different food materials.

Their potential benefits in reducing certain human health related disorders are increasing. Folin-type assays (Folin-Denis and Folin-Ciocalteu reagents) are commonly used for quantifying total phenolic compounds in fruits and vegetables. These reagents measure the ability of any mixture to reduce phosphomolybdic and phosphotungstic acids to a blue complex (11). The health benefits ascribed to the

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consumption of teas are thought to be associated with their high content of bioactive ingredients such as polyphenols (12). Polyphenols are also thought to reduce inflammation, which is thought to be the root cause of many chronic illnesses (13). Polyphenols may help fight off harmful bacteria, including *C. difficile, E. Coli*, and *Salmonella*, as well as improve symptoms of peptic ulcer disease and inflammatory bowel disease (14). Some epidemiological studies also reported the association between lower incidence of cancer and high consumption of tea (9). Some previous studies have compared the antioxidant activity of green and black teas and showed that the green tea has greater antioxidant activity (15). Few studies have evaluated the antioxidant activities of green tea samples in Iran previously (4). The objectives of this study were to determine antioxidant capacity and total phenolic content of different black tea samples in Iranian markets.

2. Materials and Methods

2.1. Materials

All solvents and chemicals used in this study were purchased from Merck Company (Darmstadt, Germany). Double-distilled, deionized water was utilized for preparing the aqueous solutions. Cintra 40 spectrophotometer was used to measure the optical absorption of samples.

2.2. Sample preparation

Tea samples (including 12 brands of Iranian black tea, 12 brands of Ceylon black tea, 10 brands of Indian black tea, 9 brands of Kenya black tea and 16 brands of tea bags) were purchased from Iran. Each sample (500 mg) was extracted for 2h with 4 ml of 50% methanol at room temperature on an orbital shaker set at 200 rpm. The mixture was then centrifuged at 6000 rpm for 10 min, and the supernatant was filtered and decanted into 250 ml volumetric flask. The extraction steps repeated twice. Supernatants were combined and diluted to 250 ml with 50% methanol. The samples were directly used for total antioxidant assay with FRAP (Ferric reducing antioxidant power assay) method without storage.

2.3. Measurement of total antioxidant activity

The FRAP procedure was performed as described by Benzie and Strain (16) previously. The principle is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous colored form in the presence of reductants (9). The FRAP reagent contained 5 ml of a (10 $mmoll^{-1}$) TPTZ (2,4,6- tripyridyl-s-triazine) solution in 40 $mmoll^{-1}$ HCl plus 5 ml of $FeCl_3$ (20 $mmoll^{-1}$) and 50 mL of acetate buffer, (0.3 $moll^{-1}$, pH=3.6).
and was prepared freshly and warmed at 37°C for 5 min. Aliquots of 50 µl samples were mixed with 1.5 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured. Spectrophotometric analysis was performed by a GBC UV-visible spectrophotometer (Cintra 40, Australia). For In vitro antioxidant activity results are expressed as ratio of ferric reducing (mmol) to gram of sample. For construction of calibration curve 6 concentrations of $FeSO_4$ $7H_2O$ (200, 400, 800, 1200, 1600, 2000 $\mu moll^{-1}$) were used and the absorbencies were measured as sample solution.

2.4. Total phenolic content

Total phenolics were determined using Folin-Ciocalteu reagent as described by Velioglu *et al*. (17) with slight modifications. The extract (200 µl) was mixed with 1.5 ml of Folin-Ciocalteu reagent (previously diluted 10 times with distilled water) and allowed to stand at room temperature for 5 min. About 1.5 ml sodium bicarbonate solution (60 gl^{-1}) was added to the mixture and after incubation for 90 min at room temperature, the absorbance level was measured at 750 nm using a UV-Visible spectrophotometer (GBC, Cintra 40). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions (25-150 μgml^{-1} in 80% methanol). The results were calculated as gallic acid equivalent (GAE) per one gram dry extract and reported as mean value ± SD.

2.5. Statistical analysis

ANOVA test with Dunnett T3 and Dunnett C post hoc were performed on samples using IBM SPSS Statistics 22 in order to compare differences between the antioxidant capacities and total phenolic content of the groups.

3. Results

The results of both post hoc were equal and showed that there is a significant difference between total

antioxidant capacity of Iranian tea samples and all other groups (p<0.05). As showed in table 1, all samples had a substantial antioxidant activity and total phenolic content. The Iranian tea samples had significantly the lowest amount of total antioxidant capacity and total phenolic content in this research 0.98 ± 0.15, 0.75±0. 17 µmoll^{-1} respectively. Ceylon tea samples, Indian tea samples and tea bags had equal antioxidant capacity and total phenolic content. The post hoc tests also showed there is a significant difference between total antioxidant capacity of Kenya tea samples and all the other groups (p<0.05). As reported in table 1, the Kenya tea samples had significantly highest antioxidant capacity and total phenolic content 2.67 ± 0.61, 2.10 ± 0.65 µmoll^{-1} respectively.

Table 1. Average of total antioxidant capacity and total phenolic in different cultivated regions

Samples	Antioxidant capacity Mean±SD (µmoll^{-1})	Total phenol (µmoll^{-1})
Iranian tea	0.98 ± 0.15	0.75±0.17
Ceylon tea	1.88 ± 0.47	1.48±0.37
Indian tea	1.73 ± 0.54	1.25±0.57
Kenya tea	2.67 ± 0.61	2.10±0.65
Tea Bags	1.45 ± 0.37	1.28±0.38

Table 2. Total antioxidant capacity and total phenolic of Iranian tea samples

Brand code	Antioxidant capacity Mean±SD (µmoll^{-1})	Total phenol (µmoll^{-1})
A1	1.03 ± 0.55	0.85±0.06
A2	1.05 ± 0.60	0.88±0.07
A3	0.85 ± 0.04	0.67±0.02
B	0.92 ± 0.01	0.73±0.01
C1	1.24 ± 0.01	0.98±0.02
D1	1.16 ± 0.01	0.87±0.01
D2	1.18 ± 0.01	0.90±0.03
E1	0.74 ± 0.03	0.42±0.02
E2	1.02 ± 0.18	0.88±0.06
E3	0.83 ± 0.18	0.65±0.06
F1	0.92 ± 0.43	0.73±0.07

Table 3. Total antioxidant capacity and total phenolic of Ceylon tea samples

Brand code	Antioxidant capacity Mean±SD (µmoll^{-1})	Total phenol (µmoll^{-1})
A4	1.64 ± 0.01	1.18±0.01
D3	1.70 ± 0.01	1.23±0.01
G1	2.25 ± 0.04	1.80±0.06
H1	2.30 ± 0.01	1.83±0.01
I1	2.52 ± 0.01	1.97±0.02
J1	2.48 ± 0.19	1.91±0.07
K1	2.05 ± 0.52	1.65±0.10
L	1.97 ± 0.07	1.46±0.03
M1	1.37 ± 0.23	1.07±0.07
N1	0.92 ± 0.19	0.72±0.07
O	2.02 ± 0.21	1.69±0.07
P	1.60 ± 0.01	1.30±0.01

Table 4. Total antioxidant capacity and total phenolic of Indian tea samples

Brand code	Antioxidant capacity Mean±SD (µmoll^{-1})	Total phenol (µmoll^{-1})
D4	2.42 ± 0.04	1.96±0.02
Q	1.38 ± 0.02	0.95±0.01
H2	1.84 ± 0.01	1.36±0.03
I2	1.010 ± 0.29	0.54±0.07
R1	2.46 ± 0.10	2.04±0.12
J2	2.44 ± 0.08	1.97±0.01
M2	1.61 ± 0.10	1.12±0.10
F2	1.96 ± 0.00	1.44±0.02
K2	1.14 ± 0.29	0.62±0.06
F3	1.08 ± 0.48	0.56±0.07

Table 5. Total antioxidant capacity and total phenolic of Kenya tea samples

Brand code	Antioxidant capacity Mean±SD (µmoll^{-1})	Total phenol (µmoll^{-1})
G2	3.34 ± 0.04	2.82±0.02
I2	3.45 ± 0.06	2.90±0.02
S	2.50 ± 0.12	1.95±0.02
T	3.50 ± 0.14	2.98±0.19
U1	2.905 ± 0.24	1.30±0.01
U2	1.89 ± 0.06	1.29±0.03
U3	1.89 ± 0.10	2.33±0.19
U4	2.36 ± 0.08	1.75±0.07
M3	2.18 ± 0.18	1.58±0.05

Table 6. Total antioxidant capacity and total phenolic of Tea bag samples

Brand code	Antioxidant capacity Mean±SD (μmoll^{-1})	Total phenol (μmoll^{-1})
V	0.87 ± 0.09	0.65±0.06
C2	1.68 ± 0.05	1.46±0.04
G3	1.55 ± 0.08	1.33±0.07
F4	1.15 ± 0.02	1.07±0.01
W	2.01 ± 0.05	1.82±0.04
H3	1.94 ± 0.07	1.78±0.06
R2	1.55 ± 0.06	1.43±0.08
G4	2.27 ± 0.07	2.02±0.07
J3	1.00 ± 0.05	0.78±0.07
H4	1.34 ± 0.05	1.09±0.07
K3	1.14 ± 0.04	0.95±0.07
I3	1.60 ± 0.06	1.50±0.07
N2	1.29 ± 0.16	1.15±0.04
K4	1.35 ± 0.13	0.95±0.07
N3	1.49± 0.24	1.40±0.02
K5	1.03 ± 0.14	0.79±0.13

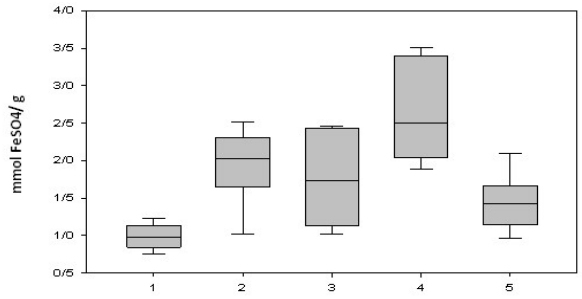

Figure 1. Average of total antioxidant capacity in different cultivated regions (1: Iranian tea, 2: Ceylon tea, 3: Indian tea, 4: Kenya tea, 5: Tea bags).

4. Discussion

Tea is the most widely consumed and popular non-alcoholic beverage in the world (18) and accounts for approximately 80% of the tea consumed in the world. Getting the total antioxidant capacity and total phenolic content are valuable in black tea samples. Previously, the antioxidant activity of green tea samples in the Iranian market had been evaluated (4) but no similar studies have been performed on black tea, so the present study was performed to determine the total antioxidant capacity and total phenol content in Black tea samples were performed. So the current study was conducted to determine the total antioxidant capacity and total phenolic content of black tea samples in Iran market by the ferric reduction antioxidant power assay and Folin-Ciocalteu method respectively. The results of the FRAP assay showed that all samples had substantial antioxidant activity ranging from 0.98 ± 0.15 (Iranian tea) to 2.59 ± 0.66 (Kenya tea). Our findings showed that Iranian tea samples had significantly the lowest and Kenya tea samples had significantly the highest amount of total antioxidant capacity in current study. The Ceylon, Indian and tea bag samples had equal antioxidant capacity. Among Iranian tea samples Brand C1 and Brand E1 had significantly the highest and the lowest antioxidant capacity and total phenolic, respectively. Among Ceylon tea samples Brand I1 and Brand N1 had significantly the highest and the lowest antioxidant capacity and total phenolic, respectively. Among Indian tea samples Brand R1 and Brand I2 had significantly the highest and the lowest antioxidant capacity and total phenolic, respectively. Among Kenya tea samples Brand T had the highest antioxidant capacity and total phenolic content Brand U3 had the lowest antioxidant capacity and Brand U2 had the lowest total phenolic capacity. Among tea bag samples Brand G4 and Brand V had significantly the highest and the lowest antioxidant capacity and total phenolic respectively. Correlation between the antioxidant capacity and the total polyphenol content was seen. The observed difference in the antioxidant activity of these different black tea samples probably reflect differences in quality, geographical regions of growth, the time of year when the leaves were picked, and storage conditions (19). The antioxidant activity of black tea has been attributed to its chemical compounds like thearubigins, phenolic acids, catechins, and theaflavins (20). There are many studies evaluating the antioxidant activity of tea samples, but the results vary according to the assay method (4). There are several methods frequently used to evaluate the antioxidant activity such as DPPH method (21), TRAP method (22), TEAC method (23) and ORAC method (24). Among them, The FRAP method was used in current study for the following reasons; other methods usually act indirectly, as they express the inhibition power of compounds against free radicals but the FRAP assay measures the antioxidant itself as a reducing agent in the presence of a color reaction. As a result, other methods have a lag phase in measuring that cause difficulties in standardizing both methods and analyses. And also cause the results occurring in wide ranges (16). India researchers in 2015 assessed the antioxidant capacity of

green and black tea samples and found that the antioxidant capacity of green tea is more than black tea. They reported that the antioxidant capacity of black tea samples (of 10 brands) was 2.034 ± 1.39 mg $AAEg^{-1}$ (1). Researchers in 1999 conducted a study on the antioxidant capacity of green, black and Oolong tea. The difference between that study and ours was in sample preparation method. For preparing the samples they boiled 5 g of dried tea in 100 ml boiling water for five minutes and after filtration, used that for FRAP assay. The used standard in that study was ascorbic acid. The results of that study showed that the black tea had the lowest and the green tea had the highest antioxidant capacity. The estimated antioxidant capacity of black tea in that study was ranged from 132 to 654 µmol $AAEg^{-1}$ that was less than our amounts. This difference can be attributed to several factors: the way of sample preparation was different, the used standard was different and the samples were from two different countries (9). Iranian researchers in 2018 conducted a study on the antioxidant and total phenol of milk in bulk milk samples, they concluded that antioxidant activity was higher than branded samples, whereas the total phenolic amounts were less than the branded ones. In the flavored milk samples, the antioxidant activity was higher than unflavored milk samples; however, the total phenolic amounts were lower than in unflavored samples (25). Researchers in 2011 compared the antioxidant capacity of black tea infusion before and after adding soy milk. The results showed that adding soy milk caused a little increase in antioxidant capacity of black tea infusion. In that research, the estimated antioxidant capacity of black tea before adding soy milk was ranged from 7796 to 10434 µmol $FeSO_4$ L^{-1} of infused tea. The difference between their findings and ours can be attributed to different way of sample preparation and also the difference between existing samples in Tehran and London. Since the report did not mention the quantity of dried tea used for preparing one liter of tea infusion, it is not possible to compare these two surveys more accurately (26).

5. Conclusions

The current study provides new data on comparing in vitro total antioxidant capacity and total phenolic of different kinds of black tea samples in Iran market. According to our findings all black tea samples had substantial antioxidant activity and among them, Iranian tea samples had significantly the lowest and Kenya tea samples had significantly the highest amount of total antioxidant capacity and total phenolic compounds. These findings suggest that black tea can be considered as a natural source of antioxidant and total phenolic compounds. There is a direct correlation between the amount of antioxidant and total phenol in tea samples.

Conflict of interest

The authors have no conflict of interest.

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