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Evaluation of total phenolic compounds, flavonoids and antioxidant activity of black and green tea drink among some available brands in the Egyptian market

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ABSTRACT

The main target of the present study was the evaluation and comparison of total phenolic compounds and flavonoid contents and the antioxidant activity of black and green tea drink among some available brands in the Egyptian Market. Different brands of commercial black and green tea were prepared by two methods: infusion (Koshary) and boiling. The prepared samples were assayed to evaluate their total phenolic compounds and flavonoid contents and antioxidant activity spectrophotometrically. Folin-ciocalteus method was used for estimation of total polyphenolic content while the antioxidants activity was estimated by measuring the radical scavenging behavior of the samples on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The results showed that, there were significantly differences ($P<0.05$) in the total polyphenols content and antioxidant activity of the commercial tea samples. Koshary black tea and boiled green tea drinks had the highest levels of the total phenolic compounds while, the Koshary black and green tea drinks had the highest flavonoid content and antioxidant activity as well. There was also significant moderate correlation between the total phenolic and flavonoid contents and the antioxidant activity for boiled tea drinks. These results suggested that, Koshary black tea and green tea was the best method for maintaining the high levels of the antioxidants activity.

Keywords: Tea, Phenolic compounds, Flavonoids, Antioxidant activity, DPPH.

Introduction

Tea is the most popular non-alcoholic beverage in the world. Among tea producing countries the principal producers are China, India, Sri Lanka, Kenya and Indonesia which account for 80% of global production. There are essentially three main types of Camellia tea, which are Green, Oolong and Black tea. The difference lies are in the 'fermentation' which actually refers to oxidative and enzymatic changes in the tea leaves during processing, (Hicks, 2001). Worldwide, the consumer's preference is approximately 76-78 % of black tea followed by 20-22% for green tea and 2% for oolong tea. Black tea leaves are exposed to the highest levels of oxidization and fermentation compared to its other tea counterparts. When black tea is oxidized, the catechins are converted into theaflavins and thearubigins, which still act as antioxidants, (Ho *et al.,* 1994).

In the last decades, lots of epidemiological studies were focused on bioactive of phytochemicals such as phenolic compounds due to their beneficial effect on human health. It has been suggested that an initial cause of most chronic diseases is free radical attack on biomolecules. Thus, consumption of foods rich in phenolic compounds which are capable of scavenging the reactive species may be a mechanism of protection recommending these foods for maximum health benefits. These beneficial effects are associated mainly with antioxidant properties of tea polyphenols. Moreover, anti-mutagenic, anti-carcinogenic, hypo-cholesterolemic, antibacterial, and antiallergenic effects of tea have been reported (Cooper, 2011).

Free radicals and other small reactive molecules have emerged as important causes of many physiological and pathological processes, (Nathan and Ding, 2010). Increased levels of these short-lived reactive molecules can cause oxidative damage to biological macromolecules and disrupt the cellular reduction–oxidation (redox) balance, (Dowling and Simmons, 2009). Oxidative stress which caused by the accumulation of free radicals in the body is involved in various pathological processes including cardiovascular diseases, cancer, neurodegenerative disorders, and aging, (Yoshihara *et al.,* 2010). An antioxidant is a compound that can delay or inhibit the oxidation of lipids or other

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molecules by blocking the initiation or propagation of oxidative chain reactions, which prevents or repairs the damage done to the cells by oxygen, (Tachakittirungrod *et al.,* 2007). The consumption of natural antioxidants presents the potential health benefits, (Yoshihara *et al.,* 2010). Thus, there is considerable interest in finding new antioxidants from plant materials. Antioxidant compounds from plants, particularly polyphenols, can inhibit the propagation of free radical reactions and protect the human body from diseases, (Perron and Brumaghim, 2009; Lizcano *et al.,* 2010). The antioxidant activity of these chemical components basically depends on some intrinsic properties, such as: the reduction potential, the property to chelate metals, work as singlet oxygen scavengers, and the possibility to capture or scavenge free radicals. These properties make the phenolic compounds to act as antioxidant in both steps of initiation and propagation of the oxidation process, (Shaidi *et al.,* 1992). And give these bioactive compounds a significant presence and a potential capacity to promote health benefits. So the most literature has considered the green tea as a functional food, (Kao *et al.,* 2000) and (Lamarão and Fialho, 2009). Catechins are group of polyphenols found naturally in teas and have protective effects against cancer and cardiovascular disease (Lima *et al.,* 2009). The most catechins that are present in high quantity in tea "Camellia sinensis" are: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and catechin (Ho *et al.,* 1992; Nagle *et al.,* 2006).

In Egypt, tea considered the main hot drink for most of the people and almost found in every house. Tea drink is prepared by two methods, the first is infusion which locally called Koshary Tea and the second is boiling which considered the main way of preparation of Upper Egypt. The aim of the present study was to evaluate the polyphenols, flavonoid contents and antioxidant activity of some black and green tea brands available in the Egyptian market prepared by two methods: infusion "Koshary" and boiling compared to each other. And study the correlations between the preparation method and the stability of antioxidant potential in the tea extracts. Also for demonstration the function of natural antioxidant present in the tea extracts for nutritionists and the general consumers.

Materials and Methods

Materials:

1- Five different types of commercial black tea (*Camellia sinensis* L.) (Labeled as 1-5) and another five different types of commercial green tea (labeled as 6-10) ranged from globally famous brand to local economic one as shown in the following table were purchased from local market in Giza, Egypt.
2- All chemicals and standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA), Cairo, Egypt.

Table I: Different types of black and green tea used in the present study

Type of tea	No.	Origin	Brand Type
Black tea	1	Kenya *	Local Famous
	2	Kenya *	Global
	3	Kenya *	Local Commercial Economic
	4	Kenya + India	Local Commercial Economic
	5	Kenya *	Local Commercial Economic
Green tea	6	China	Local
	7	Kenya+ Indonesia	Global
	8	-	Local Commercial Economic
	9	Kenya *	Local Commercial Economic
	10	Kenya *	Arabian Famous

*From different packaging companies.

Methods:

Preparation of tea drinks "extracts":

The tea drinks were prepared according to the methods of Koczka *et al.* (2016) with some modifications as follow:-

2 grams of dried samples of green or black tea were weighed in dry glass beaker. The first method (Koshary tea) was prepared by pouring 100 ml of boiled water on 2 g of dried tea leaves and left for 5 minutes. The second method (Boiled tea) was prepared by addition 2 g of tea leaves to 100 ml of boiled water in glass beaker on a hot plate at 100°C for 5 min. The extracts were collected and filtered using filter paper to be used for subsequent analysis.

Determination of total phenolic compounds:

The total phenolic compounds (TPC) content was determined by the Folin-Ciocalteu method. Spectrophotometric measurements were performed using can Agilent 8453 UV-Visible Spectroscopy System (Germany). 1.0 ml of the diluted sample extract (in triplicate) was added separately to tube containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The TPC was expressed as mg gallic acid equivalents / 100 g of tea leaves (mg GAE/100g) ISO 14502-1 (2005).

Determination of flavonoids content:

The total flavonoids content (TFC) was determined by a method described by Makris *et al.* (2007). An aliquot of tea infusion 1 ml (in triplicate) was added to a volumetric flask containing 0.3 ml a solution of $NaNo_2$ (0.3 ml, 0.5 g/L). After 5 min, a 0.3 ml of $AlCl_3$ solution (1 g/l) was added and 6 min later, 2 ml of NaOH (40 g/l) was added to the mixture. The total volume was made up to 10 ml with distilled water, the solution was mixed and the absorbance was measured at 510 nm against methanol blank. Quercetin was used as the standard for the construction of a calibration curve and the concentrations were expressed as mg quercetin equivalent / 100 g of tea leaves (mg QE/100g).

Assay of antioxidant activity of extracts:

Scavenging effect of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was measured by the method of (Chou *et al.,* 2009). Where, 0.1 ml of 1 mM solution of DPPH was incubated with sample extracts with concentration of 50µg/ml. After 30min incubation periods at room temperature, absorbance was measured at 517 nm. DPPH radical scavenging activity was expressed as inhibition percentage and was calculated as:

$$\% \text{ inhibition} = (\text{Abs control} - \text{Abs test})/\text{Abs control}) \times 100$$

Then, curves were constructed by plotting percentage of inhibition against concentration in µg/mL. The equation of this curve allowed to calculate the IC50 corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%.

Statistical analysis:

Obtained results were analyzed by the analysis of variance (ANOVA) procedures and Fisher's least significant difference (L.S.D) with significance defined at $P < 0.05$ and correlation between phenolic compounds, flavonoids and antioxidant activity was investigated. All statistical analysis were carried out using the software program STATISTICA 8 (Stat Soft, Inc, USA). The results were expressed as means ± standard deviation, (Anonymous, 1989).

Results and Discussion

The total phenolic compounds of black and green tea samples:

The total phenolic compounds of black and green tea extracts (5 for each) of two methods of preparations (Koshary or boiled) were estimated and its amounts were tabulated in Table (1). Significant differences in the total phenolic contents were observed between the different brands of black and green tea through the different methods of preparation.

According the extraction method, the total phenolic contents of boiled tea ranged from 236.88 (for black tea) to 1076.30 (for green tea) mg Gallic acid equivalent (mg GAE)/100g. However infusion method (Koshary) resulted in amounts of total phenolic contents ranged from 515.63 (for green tea) to 997.9 (for black tea) mg GAE/100g. For Koshary black tea, labeled No.3 had the highest total phenolic content (997.97 mg GAE/100g) which is local economic brand, but labeled No.1 which is a famous local brand showed the lowest total phenolic content (574.49 mg GAE/100g) among the

tested samples. For boiled black tea, labeled No.4 had the highest total phenolic content (875.13 mg GAE/100g) which is local economic brand, but labeled No.1 which is a famous local brand showed the lowest total phenolic content (236.88 mg GAE/100g). On the other hand, label No.7 showed the highest total phenolic content (775.57 and 1076.31mg GAE/100g) which is global brand, for Koshary and boiled green tea respectively.

Table 1: The phenolic compounds of black and green tea extracts (mg GAE/100g).

Tea Type	Brand	Koshary tea	Boiled tea
Black tea	1	574.16±0.47^{e}	236.88± 0.40^{e}
	2	834.28±0.39^{c}	776.33± 0.53^{c}
	3	997.97±0.50^{a}	640.59± 0.52^{d}
	4	627.68±0.46^{d}	875.13±0.48^{a}
	5	863.37±0.52^{b}	826.29±0.42^{b}
	L.S.D.	3.86	5.46
	Mean	779.5	671.04
Green tea	6	683.29±0.42^{b}	770.06 ± 0.42^{c}
	7	775.57±0.37^{a}	1076.30 ± 0.46^{a}
	8	515.63±0.36^{e}	671.58 ± 0.36^{d}
	9	563.39±0.56^{c}	806.99 ± 0.48^{b}
	10	537.31±0.40^{d}	559.11± 0.45^{e}
	L.S.D.	5.682	5.59
	Mean	615.04	776.81

But for Koshary green tea labeled No.8 local economic brand showed the lowest total phenolic content (515.63 mg GAE/100g). While for boiled green tea labeled No.10 which is Arabian famous brand showed the lowest total phenolic content (559.11mg GAE/100g). Generally, the mean values of the phenolic contents for Koshary black tea (779.5 mg GAE/100g) were higher than those of boiling black tea (671.04 mg GAE/100g). On the contrariwise, the mean values of the phenolic contents for the boiled green tea (776.81 mg GAE/100g) were higher than those of Koshary green tea (615.04 mg GAE/100g). These results suggested that, infusion method (Koshary tea) for black tea and boiling method for green tea were the best methods for maintaining the high levels of phenolic compounds and keeping the benefits of tea accordingly. The higher levels of polyphenols in boiled green tea (unfermented tea) than boiled black tea may be due to conversion of the tea polyphenols into thearubigins and theaflavin during the fermentation process of black tea (Jain, 1999). Finding of other studies also revealed that, total polyphenol contents of green tea are higher than those of black tea (Anesini *et al*., 2008; Shrestha *et al*., 2010; Nor Ohairul Izzreen and Mohd Fadzelly, 2013 and Hashish, 2016). Moreover, the variations in polyphenol levels in black tea and green tea could be due to variations in the climate, agronomic practices, commercial brands of tea, tea plantation area, etc. (Suteerapataranon and Pudta , 2008).

The total flavonoid content of black and green tea extracts:

The total flavonoid content of black and green tea was determined using aluminum chloride colorimetric method as shown in Table (2). Significant differences among the total flavonoid contents were obtained for the different brands of black and green tea through the two preparation methods (Koshary and boiled).

For Koshary black tea, labeled No. 3 a local economic brand had the highest total flavonoid content (74.53 mg QE/100g), but labeled No.1 famous local brand showed the lowest total flavonoid content (20.86 mg QE/100g) among the tested samples. For boiled black tea labeled No.2 a global brand showed the highest total flavonoid content (35.67 mg QE/100g), but labeled No.4 a local economic brand showed the lowest total flavonoid content (16.15 mg QE/100g). Label No.7 a global brand showed the highest total flavonoid content (35.67 and 53.29.30 mg QE/100g) for Koshary and boiled green tea respectively. Label No. (9 and 8) local economic brands showed the lowest total flavonoid content (16.62 and 5.74 mg QE/100g), in Koshary and boiled green tea respectively. Generally, the mean values of the flavonoid content for Koshary black and green tea (39.85 and 24.06 mg QE/100g) were higher than those of boiling black and green tea (23.94 and 18.83 mg QE/100g), which reveals

that, infusion method (Koshary tea) for black tea and green tea was effective for keeping the high levels of flavonoid contents.

This may be due to some flavonoid compounds in black and green tea might not be heat resistant and degraded at high temperatures of boiling preparation. Catechins are a class of phenolic and flavonoid compounds and chemically unstable (Higdon and Frei 2003). In solution, catechins readily undergo oxidation, involving the loss of hydrogen atoms, generation of a semiquinone radical intermediate and formation of oxidized quinone products (Yuan *et al.,* 2009 and Honzel *et al.,* 2008).

Table 2: The total flavonoid content of black and green tea extracts (mg QE /100gm).

Tea Type	Brand	Koshary tea	Boiled tea
Black tea	1	20.86± 0.49^{e}	17.70 ± 0.41^{d}
	2	41.78 ± 0.53^{b}	35.67 ± 0.43^{a}
	3	74.53 ± 0.40^{a}	22.51 ±0.47^{c}
	4	24.53 ± 0.41^{d}	16.15 ±0.34^{e}
	5	37.55± 0.44^{c}	27.69 ± 0.37^{b}
	L.S.D.	0.86	0.75
	Mean	39.85	23.94
Green tea	6	17.69 ± 0.33^{d}	14.56 ± 0.37^{b}
	7	35.67 ± 0.35^{a}	53.29 ± 0.48^{a}
	8	22.51 ± 0.54^{c}	5.74 ± 0.22^{e}
	9	16.62± 0.45^{e}	6.82 ± 0.41^{d}
	10	27.83 ± 0.38^{b}	13.73 ± 0.29^{c}
	L.S.D.	0.76	0.66
	Mean	24.06	18.83

Antioxidant activity "DPPH" of black and green tea extracts:

Significant differences in the antioxidant activity levels were observed among the different brands of black and green tea for the two preparation methods (Koshary and boiled) (Table 3). The results have shown that the antioxidant activity levels were significantly higher in green tea for both preparation methods as compared to those of black tea ($p<0.05$). Similar results were reported in earlier studies (Chan *et al.,* 2010; Dutta *et al.,* 2013 and Hashish, 2016).

Table 3: The DPPH activity of black and green tea extracts.

Tea Type	Brand	Koshary tea	Boiled tea
Black tea	1	38.84 ± 0.47^{b}	16.68±0.44^{c}
	2	32.00 ± 0.39^{e}	21.76±0.40^{a}
	3	37.76 ± 0.50^{c}	11.67±0.28^{d}
	4	43.01± 0.48^{a}	19.37±0.42^{b}
	5	35.63 ± 0.52^{d}	22.50±0.47^{a}
	L.S.D	0.98	0.74
	Mean	37.45	18.40
Green tea	6	52.69 ± 0.59^{a}	35.69 ± 0.45^{d}
	7	11.92 ± 0.51^{c}	44.64 ± 0.38^{a}
	8	46.85 ± 0.31^{b}	10.89 ± 0.40^{b}
	9	52.82 ± 0.61^{a}	37.87 ± 0.35^{c}
	10	52.55 ± 0.40^{a}	42.82 ± 0.28^{b}
	L.S.D	1.08	0.68
	Mean	43.37	34.38

Furthermore, the results have shown that the antioxidant activity mean levels in tea extract prepared by Koshary method (37.45 and 43.37 mg/100g for black and green tea respectively) were significantly higher than those of boiled method (18.40 and 34.38 mg/100g for black and green tea respectively) for both the black and green tea. In Koshary black tea, the antioxidant activity levels ranged from 32.00 to 43.01mg/100g, while in boiled black tea it ranged from 11.67 to 22.50 mg/100g. On the other hand, In Koshary green tea, the levels ranged from 11.92 to 52.82 mg/100g. However in Boiled green tea it ranged from 10.89 to 44.64 mg/100g.

The higher antioxidant activity is due to potent antioxidant activities of catechins in green tea which are due to their three adjacent hydroxyl (OH) groups on the β-ring as in epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epigallocatechin (EGC) and gallocatechin (GC) which are more effective in scavenging free radicals than the two adjacent OH groups as in catechin gallate (CG) and epicatechin (EC). The content of EGCG and EGC in green tea is much higher than in black tea (Almajano *et al.,* 2008). In case of black tea the antioxidant properties have been attributed to its chemical components of the arubigins, phenolic acids, catechins, and the aflavins. The aflavins which impart color, brightness, and astringency to black tea extract also possess potent antioxidant properties (Shivaki *et al.,* 1994 and Miller *et al.,* 1996).

The correlation coefficients between phenolic compounds, flavonoids and antioxidant activity (DPPH) of tea drink "extract".

The data in table (4) showed the correlation between three estimates (phenolic compounds, flavonoids and antioxidant activity "DPPH") in the two types of tea (green and black) prepared by two methods (Koshary and boiled) as mentioned by Anonymous (1989). The results showed that there was moderate correlation between the antioxidant activity "DPPH" and the total phenols and flavonoids contents in boiled treatments (r= 0.470 and 0.483 for black tea and r= 0.407 and 0.532 for green tea) in the two types of tea for all the tested brands, , while there was negative correlation between the antioxidant activity and the total phenols and flavonoids contents in Koshary black tea (r= -0.528 and -0.310) and the total phenols in Koshary green tea (r= -0.757). Beside that the results revealed that there was strong positive correlation between phenols and flavonoids in Koshary black tea (r=0.920) and in boiled green tea (r=0.938), while it was weak correlation in boiled black tea (r= 0.362) and Koshary green tea (r= 0.244).

Table 4: The correlation coefficients between phenols, flavonoids and antioxidant activity (DPPH) for both preparation methods in black and green tea extract.

Black	**Preparation methods**		**Phenols**	**Flavonoids**	**DPPH**
	Koshary	**Phenols**	1.000	0.920	-0.528
		Flavonoids	0.920	1.000	-0.310
		DPPH	-0.528	-0.310	1.000
	Boiled	**Phenols**	1.000	0.362	0.470
		Flavonoids	0.362	1.000	0.483
		DPPH	0.470	0.483	1.000
Green	**Preparation methods**		**Phenols**	**Flavonoids**	**DPPH**
	Koshary	**Phenols**	1.000	0.244	-0.757
		Flavonoids	0.244	1.000	0.313
		DPPH	-0.757	0.313	1.000
	Boiled	**Phenols**	1.000	0.938	0.407
		Flavonoids	0.938	1.000	0.532
		DPPH	0.407	0.532	1.000

The obtained results are similar to previous studies. Weak correlation was observed between the antioxidant activity determined with DPPH radical scavenging abilities and total phenolic contents "TPC" for fruit residue extracts where r2=0.36 (Babbar *et al.,* 2011). While Ronowicz *et al.,* (2013) reported that there was a significant negative correlation (R=–0.7668, P≤0.01) between antioxidant activity (using DPPH method) and total phenolic content of ginkgo preparations. On the other hand, a strong positive correlation (R=0.9273, N=162, P<0.001) was found between total phenolic content and antioxidant capacity of ginkgo extracts (Koczka *et al.,* 2016). The results are also compatible with Pereira *et al.* (2014) who reported that there are individual differences between phenolic contents and antioxidant activity. For black teas, a group was observed with lowest content of total phenols and flavonoids and lower antioxidant activity. Another group is formed by white and green teas with highest levels of total phenols and flavonoids and higher antioxidant activity.

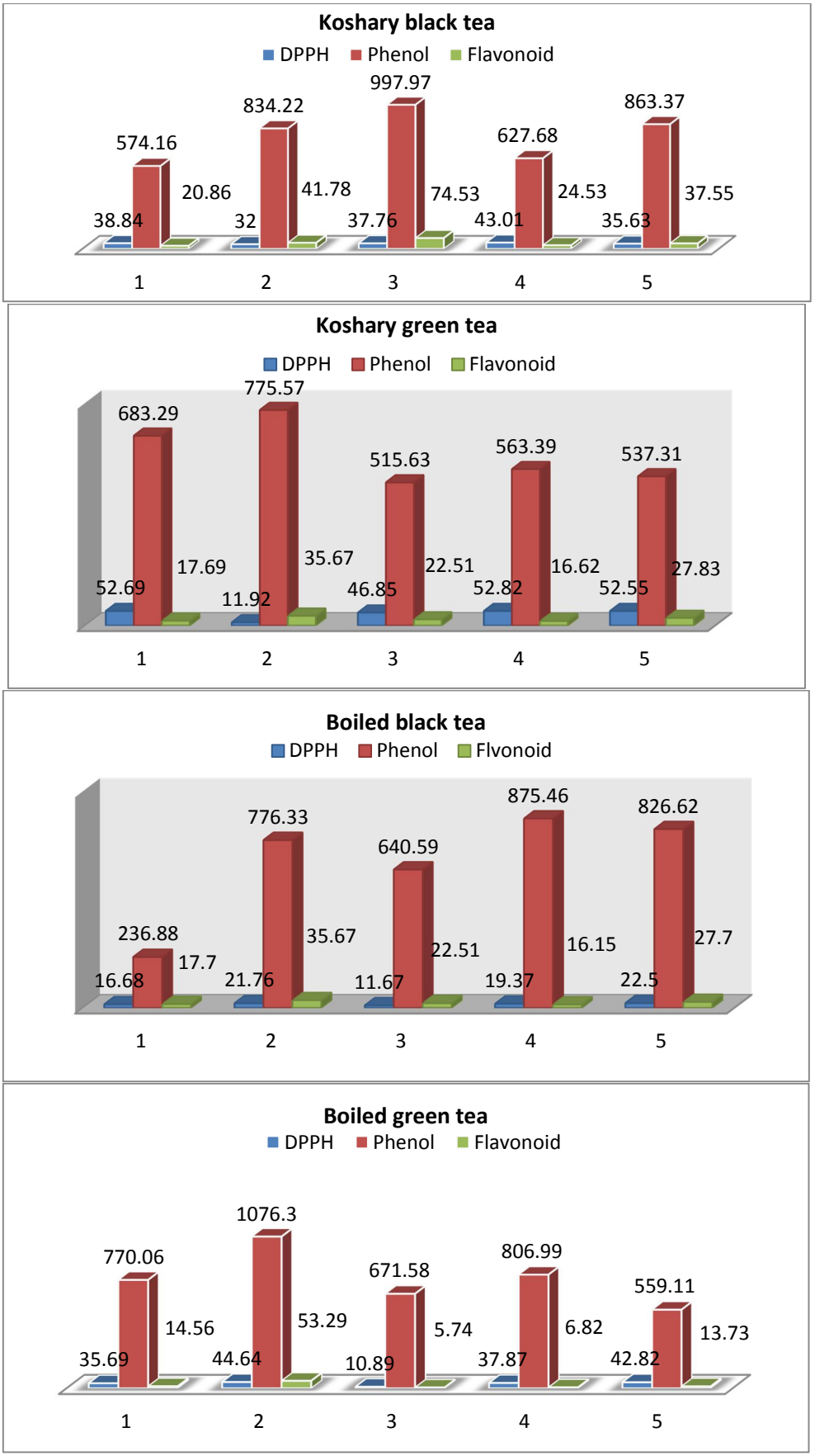

Fig. 1: The correlation coefficients between phenols, flavonoids and antioxidant activity (DPPH) for both preparation methods in black and green tea extract.

Moreover, results showed that the antioxidant activity is most pronounced in boiled treatment for both of green and black tea, agreeing with the amount of total phenols and flavonoids. Green tea has antioxidant activity mainly attributed to catechins, while black tea is dependent on theaflavins and thearubigins (Yashin *et al.,* 2011). This fact could explain individual differences between phenolic contents and antioxidant activity observed in this study. In the same context, Heijnen *et al.* (2000) reported that, catechins content of black tea (belongs to fermented tea), was reduced to 20% of that in green tea.

Conclusion

This study showed the presence of antioxidant compounds (phenolic acids and flavonoids) and demonstrated level of antioxidant activity in different types of black and green tea (*Camellia sinensis* L.) and that not exclusive to the global and famous brands but also found in the economic commercial brands. It can be concluded that high total phenols and flavonoid content and antioxidant activity were observed in black and green tea prepared by infusion (Koshary). There was significant correlation between the total flavonoid content (higher than the phenolic) and the antioxidant activity of the tea extracts. Moreover, Koshary tea is the best method to maintain the high levels of polyphenols and antioxidants activity.

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