Pak. j. sci. ind. res. Ser. A: phys. sci. 2014 **57** (2) 74-85

Evaluation of Free Radical Scavenging Activity of Tea Infusion of Commercial Tea Products Available in UAE

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(received September 25, 2012; revised November 19, 2012; accepted December 5, 2012)

Abstract. In the present study, twenty four commercial tea samples were assayed to determine their free radical scavenging activity and polyphenolic contents based on the brewing/infusing period. Tea samples were infused/brewed in 200 mL boiled water at 120 °C for 1, 2 and 5 min, respectively. The radical scavenging activities of tea infusion/brewing were measured using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay method. The results were ranged from 67.81-90.51% for black tea bags, 90.37-94.51% for green tea bags, 24.66-92.25% for black tea powder, 16.08-93.06% for green tea powder and 32.90-45.54% for Camomile herbal infusion. The results showed that 1 or 2 min black tea bags infusion exhibited highest radical scavenging activity than 5 min infusion. Antioxidant activities of tea powders were variable with the amount of tea powder. It was observed that antioxidant activity increased with increasing boiling time for smaller amount of sample. In contrary, shorter boiling time was better for larger amount of sample. The polyphenol contents of tea infusion were determined and the results were expressed as milligram quercetin equivalent/200 mL of tea infusion. The polyphenol content was increased with increased brewing period. In contrary, brewing for longer time rendered extract less antiradical activity. This study suggests that infusing tea bag for 1 or 2 min is sufficient for getting infusion with maximum radical scavenging activity and in case of tea powder, shorter boiling time is better for larger amount of powder or small amount of powder should be boiled for minimum 5 min for rendering extract with maximum radical scavenging activity.

Keywords: tea bag, tea powder, antioxidants, polyphenols, 1,1-diphenyl-2-picrylhydrazyl radical

Introduction

Tea is an infusion of the leaves of the *Camellia sinensis* (Theaceae) plant. It is one of the most popular beverages in the world and currently revealed that it can promote health and helps to prevent a number of diseases (Arab *et al.*, 2009; Peters *et al.*, 2001). Tea is rich in flavonoids and other polyphenols known as catechins. The type of flavonoids found in different types of tea will depend on the level of processing the tea leaves. Depending on the manufacturing process, teas are classified into three major types, green tea non-fermented, oolong tea semi-fermented and black tea-fermented (Gupta *et al.*, 2008; Zuo *et al.*, 2002). During the oxidation process, enzymatic activity allows for the catechins to be polymerised and thus alter their structure. Typically, green tea leaves undergo minimal oxidation hence, retaining the majority of catechins. Black tea receives significant oxidation under controlled temperatures and humidity and results in the polymerisation of catechins into theaflavins and thearubigins. Theaflavins possess benzotropolone rings with dihydroxy or trihydroxy substitution systems which give the characteristics colour and taste of the black tea (Menet *et al.*, 2004). These catechins and their polymer can protect against heart disease and cancer (Higdon and Frei, 2003; Lambert and Yang, 2003; Vita, 2003; Yang and Landau, 2000; Lin *et al.*, 1999; Buschman, 1998). Black tea extracts containing thearubigins, also effectively protect against the paralytic actions of botulinum neurotoxins (Satoh *et al.*, 2001). Recently, reported green tea polyphenols exhibited beneficial effect on pathological states related to oxidative stress of the kidney (Yokozawa *et al.*, 2012) and in oral health (Narotzki *et al.*, 2012). The beneficial effects of tea are many, be ascribed to tea's antioxidant activity for their polyphenol contents. A number of studies reported that tea contained a number of polysaccharides which also exhibited good antioxidant activity (Wang *et al.*, 2012; Xiao *et al.*, 2011). However, the structural criteria of flavonoids for the potent free-radical scavengers are the presence of *ortho*-hydroxylation on the B-ring, a C_2-C_3 double bond in C-ring and the presence of 3-hydroxyl groups (Nessa *et al.*, 2004; Bors *et al.*, 1997; 1990; Rice-Evans *et al.*, 1996). Levels of flavonoids in a tea brew will depend on many factors that include

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type of tea used or present in the tea bag as well as how long the tea is left to infuse in the water etc., (Peterson *et al.*, 2004). Though, extensive work already done on the antioxidant potential of tea products (Nkubana and He, 2008; Su *et al.*, 2007; Gramza *et al.*, 2005; Cao *et al.*, 1996), but very little information is available to study the free radical scavenging activity of tea infusion based on the brewing period. Therefore, the main aim of this research was to qualify the effectiveness of black, green and herbal tea infusion as the free radical scavengers based on the brewing period.

Materials and Methods

Samples. A set of 24 processed commercial tea products that includes: 8 black tea bag samples, 9 black tea powder samples, 5 green tea bag samples, 1 green tea powder sample and 1 herbal tea sample were purchased from the supermarket of Dubai, UAE during the September-October, 2011. The samples had been manufactured in different commercial factories using standard manufacturing conditions. The descriptions of samples are shown in the Table 1.

Table 1. Description of tea products

Product	Manufacturer	Origin/country
Black tea bags		
Ahmed Tea London (English Tea No.1)	Packed in Sirlanka Ahmed Tea Limited	England (Sirlanka, India)
Red Label (Brooke Bond)	Packed in UAE by Unilever Gulf FZE	India
Lipton (Yellow Label Tea)	Packed in UAE by Unilever Gulf FZE	-
Lulu (Blender's special)	Packed in UAE by Unilever Gulf FZE	India , Sirlanka, Africa
Alokozay (Premium Tea)	Packed in UAE by Alokozay Tea International	Sirlanka
Tetley London	Packed in India for Tetley GB Ltd	England
(Drawstring Pure Black Tea)	TATA Tea Enterprise	
Kanan devan (TATA Tea)	Packed & Exported by TATA Tea Limited	India
Premium (TATA Tea)	Packed & Exported by TATA Tea Limited	India
Black tea powder		
Ahmed Tea London (English Tea No.1)	Packed in Sirlanka Ahmed Tea Limited	India
Red Label (Brooke Bond)	Packed in UAE by Unilever Gulf FZE	-
Lipton (Yellow Label Tea)	Packed in UAE by Unilever Gulf FZE	India , Sirlanka, Africa
Lulu (Blender's special)	Packed in UAE by Unilever Gulf FZE	Srilanka
Alokozay (Premium Tea)	Packed in UAE by Alokozay	Tea International Ltd., India
Kanan Devan (TATA Tea)	Packed & Exported by TATA Tea Limited	India
Premium (TATA Tea)	Packed & Exported by TATA Tea Limited	India
Leone (Finest Indian Tea)	Crown Oriental Food Ltd Wembley Mildx HAO U.K	India
Society Tea (From the house of Hasmukhxai & Co)	Packed by Amerty privet Ltd	-
Green tea bags		
Twinings of London (Green Tea & mint)	Packed R.Twining & Company Limited	-
Lipton (Clear Green)	Packed in UAE by Unilever Gulf FZE	England
Tetley (Drawsting Pure Green Tea)	Packed in India for Tetley GB Ltd	Sirlanka
Alokozay (Premium Tea) Green Tea	Packed in UAE by Alokozay	Sirlanka -
Tea International Ltd.,		
Dilma (Special Green Tea with natural Jasmine Petals)	Packed in Sirlanka Dilmah Australia Pty Ltd	UAE
Green tea powder		
Packed R.Twining & Company Limited	Twining of London (Green Tea & mint)	-
Herbal tea product		
Camomile Herbal Infusion (Safa)	Hassani Tea & Herbs Factory,	UAE

Chemicals. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, quercetin, anhydrous sodium carbonate, Folin-Ciocalteu reagent, L-ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl, and methanol (spectroscopic grade) were purchased from Sigma Chemical Co. (USA).

Sample preparation. Tea infusions were prepared by infusing and steeping without stirring. For tea infusion, different tea bags were infused in 200 mL boiled distilled water at 120 °C for 1, 2 and 5 min, respectively. The tea brew were prepared for tea powder, where different amounts of tea powder were boiled in 200 mL at 120 °C in a thermostatic hot plate for 1 and 5 min, respectively. The tea infusion and brew was filtered through Whatman No. 1 filter paper, and brought at room temperature (25 °C). The aqueous tea filtrates were then immediately used for the determination of solid contents (yield), total polyphenolic contents and antioxidant activities.

Measurement of antioxidant activity. ***DPPH free radical scavenging method.*** The antioxidant activity of different aqueous tea extracts were evaluated using a stable radical, 1,1-diphenyl-2-picrylhydrazyl in a methanol solution according to the method of Nessa *et al.* (2004). Briefly, 100 µL tea infusion solution was placed in a cuvette and 3.9 mL of 0.1 mM methanolic solution of DPPH radical was added. The solution was incubated for 30 min at 25 °C in dark and the absorbance was measured at λ517 nm with a Shimadzu-1700 uv-vis spectrophotometer. A blank sample (without tea infusion) containing methanolic DPPH radical was measured with the sample. All determinations were performed in three replicates. The percent inhibition of DPPH radical by the samples was calculated according to the formula, as follows:

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \times 100$$

where:

$A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min.

A dose response curve was plotted for few selected tea products for studying hydrogen donating abilities of tea extracts. In this study, absorbance measurement commenced immediately. The decrease in absorbance at λ517 nm was determined continuously with data capturing at 2 min intervals until absorbance stabilised (± 30 min). L-ascorbic acid was used as positive control.

Determination of total polyphenol content. Total polyphenolic contents were determined by the Folin-Ciocalteau method described by Scalbert *et al.* (1989). Folin Ciocalteu reagent was diluted (1:10) with distilled water. Briefly, the 100 µL tea infusion/brewing solution (three replicates) were mixed with 2 mL diluted Folin Ciocalteu reagent and then 2 mL aqueous Na_2CO_3 (7.5%). The mixture was allowed to stand for 1.5 h and the absorbance was measured at λ765 nm with a Shimadzu-1700 uv-vis spectrophotometer. The standard curve was prepared from 1-300 g/mL solutions of quercetin in methanol: water (80:20, v/v). The regression equation was y = 0.004x + 0.0166 with regression coefficient (r) 0.999. Total polyphenol values were expressed as milligram of quercetin equivalents per 200 mL of tea infusion.

Results and Discussion

Antioxidant activity of tea product infusion. Antioxidant activities of tea leaves infusion were determined according to the DPPH radical scavenging method. The DPPH radical has been widely used to test the free radical scavenging ability of various natural products (Sanchez-Moreno, 2002; Chen *et al.*, 1999; Yamaguchi *et al.*, 1998). According to this method, a compound with high antioxidant activity effectively binds with the radical hence, prevent its propagation and the resultant chain reaction. The results were expressed as mean ± standard deviation (SD). The data of 1, 2 and 5 min infusion of tea bags were compared for each product and were subjected to a one-way analysis of variance (ANOVA). Tukey's test ($P<0.05$) was performed to determine the significance of the difference between means.

Black tea bag products. Eight black tea bags and one herbal sample were infused for 1, 2 and 5 min, respectively. For 1 min infusion, Tetley and Kanan Devan showed the highest free radical scavenging activity as decreased in the order of: Tetley ≥ Kanan Devan > Lulu > Alkozay > Red Label > Lipton > Ahmed > Camomile. In case of 2 min infusion, Lipton, Red Label and Kanan Devan exhibited highest free radical scavenging activity as decreased in the order of, Kanan Devan ≥ Lipton ≥ Red Label ≥ Lulu > Tetley > Ahmed > Alkozay > Tata > Camomile. For 5 min infusion, Lipton, Red Label, Kanan Devan and Ahmed showed equal and highest free radical scavenging activity than other black tea bag products as decreased in the following order: Lipton ≥ Red Label ≥ Ahmed Kanan Devan ≥ Alkozoay ≥

Tetley > Lulu > Tata > Camomile. Overall, Lipton, Red Label, Kanan Devan and Tetley showed the highest free radical scavenging activity and Camomile was the least free radical scavengers (Table 2). In comparison between 1, 2 and 5 min tea infusion for each product, overall 2 min tea infusion gave higher antioxidant activity than 1 min. Tea infusion for 5 min gave lowest antioxidant activity except Ahmed tea bag, which gave higher antioxidant activity with increasing infusion time (5 min > 2 min > 1 min) (Fig. 1). Amongst the studied black tea bag products Tata tea bag showed comparatively lower antioxidant activity than other tea products and showed highest antioxidant activity at 2 min infusion. But the mean differences were not significantly different ($P<0.05$) for Lulu (1, 2 min), Lipton (2, 5 min), Red Label (2, 5 min), Tetly (1, 2 min), Kanan (1, 2, 5 min), Ahmed (2, 5 min) and Alkozay (1, 5 min). Camomile, a herbal infusion showed lowest antioxidant activity and was significantly, lower than the other tea bag samples (Fig. 1). The antioxidant activity of tea infusion depends on many factors, one of these factor is the amount of leaves present in a tea bag (Peterson *et al.*, 2004). All the studied black tea bags contained tea leaves in the ranges of 2.03 to 2.68 g except Camomile herbal tea which contained about 0.95 g leaves in a bag. It seems the lowest antioxidant activity of Camomile was due to the lower amount of leaves present in the tea bag. From the results it was concluded that brewing the black tea bags for 1 to 2 min was sufficient to obtain higher antioxidant activity.

Green tea bag products. Five tea bag samples were infused for 1 and 5 min for determination of their free

Table 2. Free radical scavenging activity and total polyphenol concentration of tea infusion of different black tea bag samples. Results are mean ± SD ($n = 3$)

Products	Av. wt. of tea leaves/bag (g)	Brewing period (min)	Solid contents (mg/mL)	Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol /200 mL products tea infusion (mg ± SD)
Lulu	2.04	1	2.01	88.64 ± 0.50	35.13 ± 1.95
		2	2.98	88.50 ± 0.77	73.14 ± 1.35
		5	3.98	86.49 ± 0.33	124.25 ± 2.55
Lipton	2.07	1	2.21	82.18 ± 0.66	30.51 ± 1.47
		2	3.13	90 ± 0.55	49.82 ± 1.65
		5	4.29	88.9 ± 0.43	156.2 ± 2.41
Tata	2.03	1	1.76	78.44 ± 0.11	59.84 ± 1.28
		2	2.44	82.90 ± 0.88	82.14 ± 2.72
		5	4.01	80.17 ± 0.67	122.25 ± 1.25
Red Label	2.07	1	2.4	84.77 ± 0.50	24.64 ± 1.99
		2	3.78	89.94 ± 0.66	66.49 ± 0.91
		5	4.35	88.64 ± 0.88	103.34 ± 1.25
Tetley	2.68	1	2.36	89.79 ± 0.99	75.22 ± 0.95
		2	3.69	88.79 ± 0.32	106.75 ± 1.14
		5	4.43	87.06 ± 0.58	209.47 ± 0.75
Kanan Devan	2.07	1	1.72	89.94 ± 0.34	46.37 ± 1.16
		2	2.39	90.51 ± 0.88	78.4 ± 1.23
		5	3.87	88.21 ± 0.95	121.25 ± 1.35
Ahmed	2.06	1	1.74	67.81 ± 0.54	21.43 ± 1.15
		2	2.01	87.5 ± 0.56	77.68 ± 1.12
		5	3.66	88.6 ± 0.37	133.52 ± 1.50
Alkozay	2.06	1	1.50	86.06 ± 0.66	29.69 ± 1.77
		2	2.41	85.63 ± 0.44	74.5 ± 1.11
		5	3.33	87.35 ± 0.79	139.68 ± 0.94

radical scavenging capacity (Table 3). All products exhibited in the ranges of 90.37-94.51% free radical scavenging activity as long as it brewed. In comparison between 1 min infusion of green tea bag samples, Tetley tea bag showed highest antioxidant activity than other studied samples as decreased in the order of: Tetley > Dilma ≥ Lipton > Alkozay > Twinings. For 5 min infusion, the antioxidant activity decreased in the order of: Lipton > Dilma > Alkozay > Tetley > Twinings (Fig. 1). But the mean differences between 1, 2 and 5 min infusion were not statistically significant (P > 0.05). It seemed 1 min infusion was enough to get maximum antioxidant activity and infusion for longer time did not significantly increase or decrease the antioxidant activity. The detailed analytical value for all studied tea bag herbal products are tabulated in Table 2-3.

Black and green tea powder products. In this study, 9 black tea powders and one green tea powder samples were analysed. Four different amounts (0.1 g, 0.5 g, 1.0 g and 2.0 g) of tea leaves powder for each product were boiled for 1 min and 5 min separately (Fig. 2). For 1 min infusion of all samples, the free radical scavenging activity significantly (P > 0.05), increased

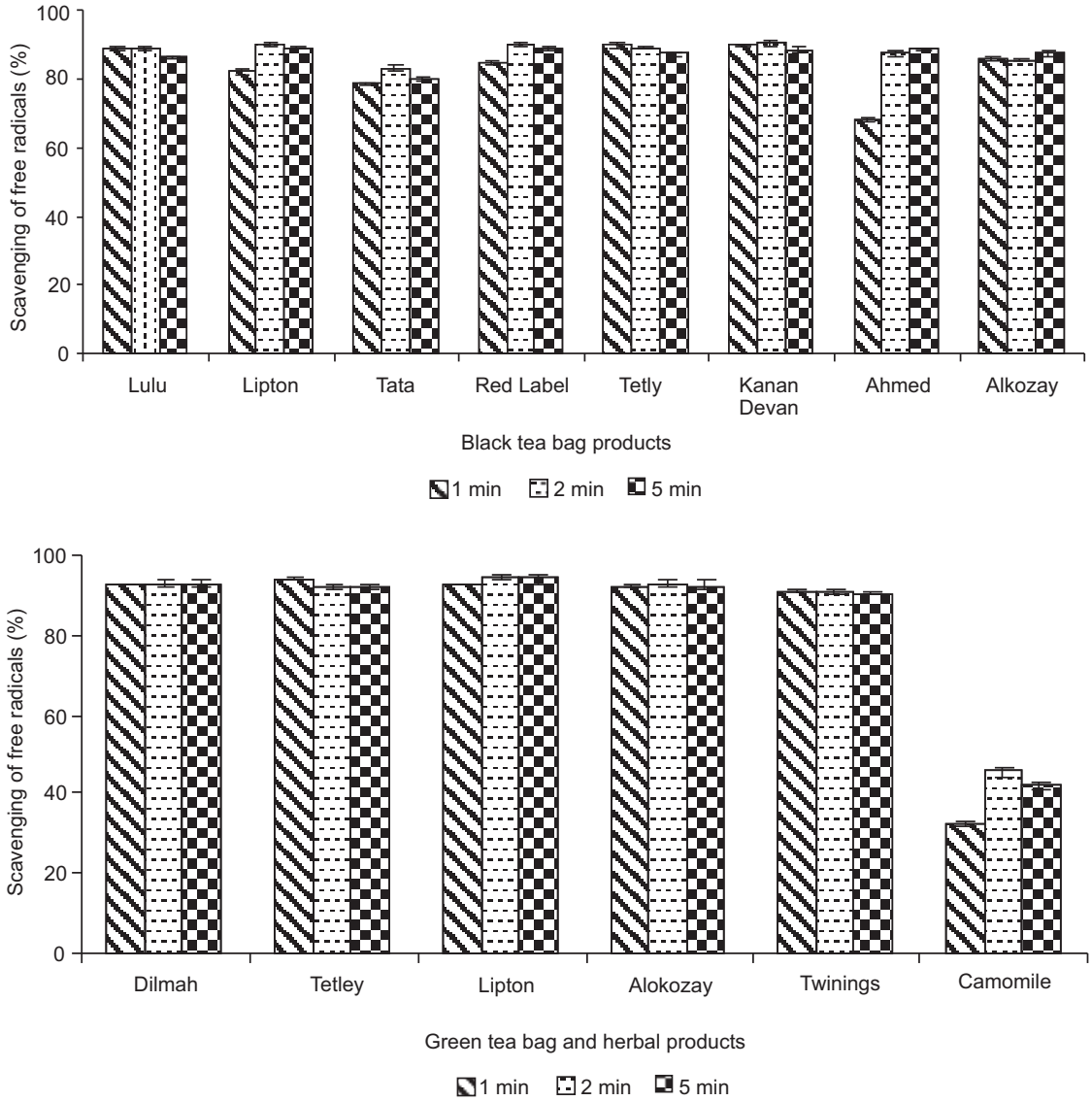


Fig. 1. Free radical scavenging activity of different manufacturer's tea bags (black, green and herbal) samples measured using the DPPH assay. Results are mean ± SD (n = 3).

with increasing the amount of samples. For 5 min infusion, free radical scavenging activity only increased for 0.1 and 0.5 g samples but in contrary, decreased for 1.0 and 2.0 g samples with increasing boiling period from 1 min to 5 min (Table 4-5). It seems shorter brewing period was better for larger amount of powder, in contrary, longer brewing period was necessary for brewing smaller amount of tea powder for getting higher antioxidant activity.

For a few selected tea samples the rate of reaction with the free radicals was also evaluated. In dose response studies it was found that tea infusion scavenged the free radicals very fast as it can be seen in Fig. 3-4 and reached a steady state within 5 min. Camomile, a herbal sample very slowly scavenged free radicals as compared to Lipton tea samples (Fig. 3B). In comparison of green tea with black tea bag samples, black tea scavenged free radicals comparatively slowly than green tea bag

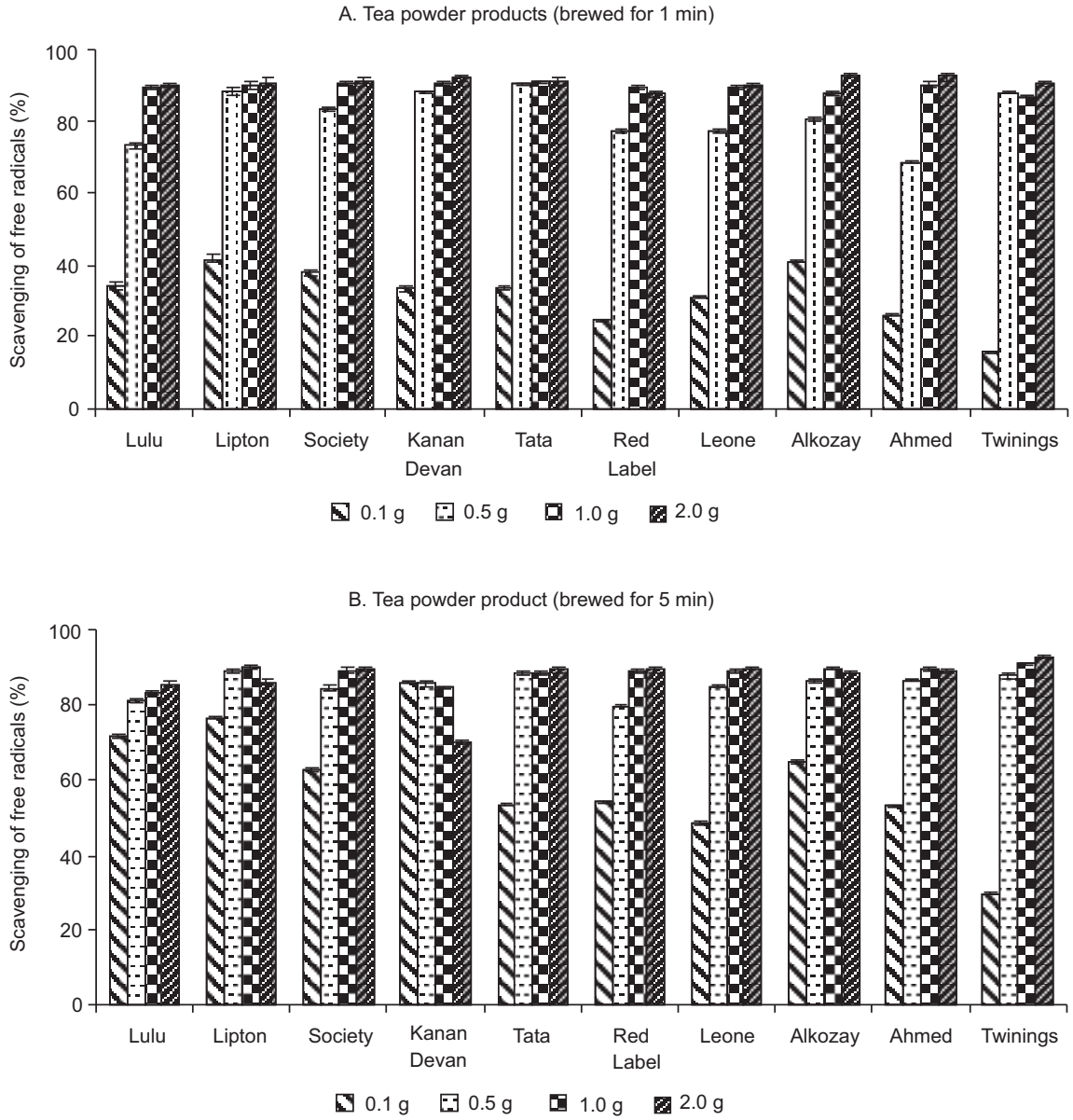


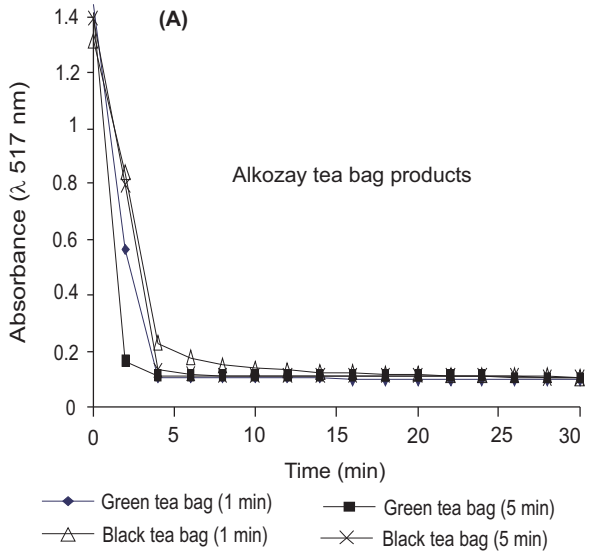
Fig. 2. Free radical scavenging activities of tea infusion prepared from different amount of tea powder leaves *vs*. the scavenging of free radicals, measured using the DPPH assay. Results are mean ± SD (n = 3). Samples were brewed for 1 min (A) and 5 min (B).

Table 3. Free radical scavenging activity and total polyphenol concentration of tea infusion of different green tea bag/herbal samples. Results are mean SD (n = 3)

Products	Av. wt. of tea leaves/bag (g)	Brewing contents (min)	Solid period (mg/mL)	Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol /200 mL products tea infusion (mg ± SD)
Dilma	2.11	1	1.82	93.11 ± 0.32	41.49 ± 1.05
		2	2.99	93.32 ± 0.73	86.45 ± 0.9 5
		5	3.53	93.32 ± 0.92	137.55 ± 1.11
Tetley	2.16	1	1.43	94.07 ± 0.34	63.17 ± 0.96
		2	3.11	92.46 ± 0.73	95.23 ± 1.26
		5	3.92	92.46 ± 0.63	165.50 ± 1.84
Lipton	1.54	1	1.52	93.11 ± 0.25	64.65 ± 0.60
		2	3.04	94.51 ± 0.62	72.67 ± 0.60
		5	3.68	94.51 ± 0.83	92.23 ± 1.63
Alkozay	1.95	1	1.90	92.68 ± 0.25	68.33 ± 2.45
		2	3.16	92.89 ± 0.62	89.34 ± 1.60
		5	4.01	92.89 ± 0.15	164.57 ± 0.73
Twinings	2.04	1	1.31	91.09 ± 0.45	140.85 ± 2.62
		2	2.97	90.94 ± 0.74	158.65 ± 2.62
		5	3.65	90.37 ± 0.31	171.85 ± 1.21
Camomile	0.95	1	0.99	32.90 ± 0.73	62.17 ± 1.89
		2	1.34	45.54 ± 0.92	61.46 ± 1.39
		5	2.44	42.09 ± 0.87	63.2 ± 1.27

infusion, but after 5 min there were no significant differences in their scavenging activities (Fig. 3A). In dose-response studies of tea power (2.0 g) and tea bag (2.04 g), infusion of tea bag scavenged free radicals comparatively little faster than the powder leaves infusion as it can be depicted from the Fig. 4. In dose-response studies, L-ascorbic acid was used as a reference standard and reacted with DPPH immediately and reached a steady state within a minute (Nessa *et al.*, 2004). Its scavenging capability was rapid and dependent on concentration/dose (Fig. 3B). In this study, all the studied tea infusions also reacted rapidly with DPPH radical and reached a steady state within 2 to 5 min. It seemed tea infusion also acted as fast free radical scavenger as ascorbic acid.

The total polyphenols content of tea bag and tea powder products. Free radical scavenging activity of tea extracts depends mainly on its polyphenolic content (Gupta *et al.*, 2008; Higdon and Frei, 2003; Yang and Landau, 2000). Therefore, the polyphenolic contents of all studied tea products were determined and the results were expressed as milligram quercetin equivalent/ 200 mL of tea infusion. The results are presented in Table 2-5. In case of black and green tea bags, the polyphenolic content increased with increasing brewing time from 1 min to 5 min. In comparison amongst 1 min infusion of black tea bag samples, lowest concentration was recorded in Ahmed tea bag (21.43 ± 1.15) and highest amount recorded in Tetly tea bag (75.22 - 0.95). The overall results were decreased in the order of: Tetly > Tata > Kanan Devan > Lulu > Lipton ≥ Alkozay > Red Label > Ahmed. In case of 2 min infusion, highest concentration was recorded in Tetly tea bag (106.75 ± 1.14) and the results decreased in the order of: Tetly > Tata > Ahmed ≥ Kanan Devan > Alkozay > Lulu > Red Label > Lipton. For 5 min infusion, the lowest amount recorded in Red Label (103.34 ± 1.25) and the results decreased in the order of: Tetly > Lipton > Alkozay > Ahmed > Lulu > Tata > Kanan Devan > Red Label. However, Tetley exhibited highest polyphenolic content amongst the studied tea bag samples, in contrary, the polyphenolic content of Camomile (a herbal infusion) was not increased with increasing infusion time and the values were in the ranges of 61.24 mg to 63.2 mg. The polyphenolic content of all studied black tea bag samples are tabulated in Table 2.



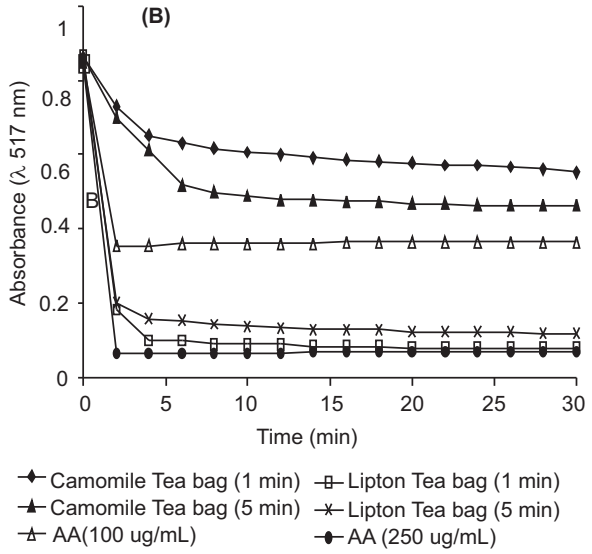
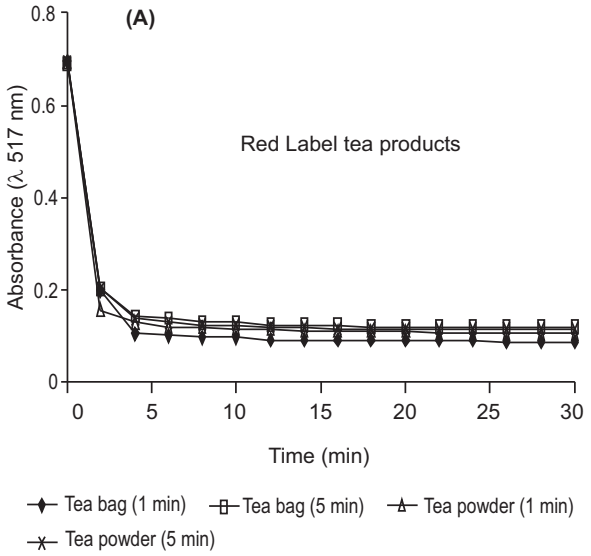


Fig. 3. Hydrogen donating abilities of Alkozay tea bag products (A) Camomile, Lipton tea bag products and Ascorbic acid (AA, 100 and 250 μg/mL) (B) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.



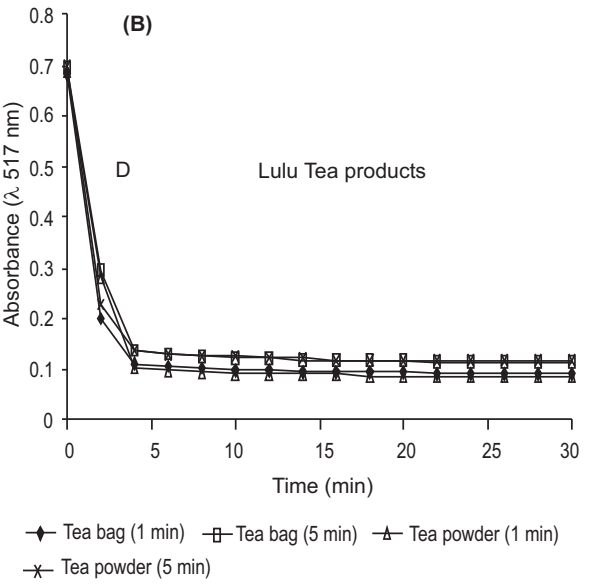


Fig. 4. Hydrogen donating abilities of Red Label tea products (A) and Lulu tea products (B) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

For green tea bag samples, 5 min infusion exhibited higher concentration of polyphenols than 1 min infusion. Dilma tea bag showed lowest polyphenol content (41.49 mg) for 1 min infusion and Twinings exhibited highest content about 140.85 mg. For 5 min infusion, again Twinings showed highest polyphenol content (171.85 mg) and lowest amount recorded in Tetley (63.17 mg). Though, polyphenol content of all tea bag samples increased with increasing infusion time but in contrary, DPPH radical scavenging activity was not increased with increasing polyphenol content. Though polyphenol content of Dilma was lower for 1 min infusion than 5 min, but both infusions scavenged free radicals effectively. Other studied green tea bag samples also exhibited similar results, as it can be depicted from Table 3. It seems that some other chemical compounds such as tannin extracted at longer boiling time, which might interfered the free radical scavenging

Table 4. Free radical scavenging activity and total polyphenol concentration of infusion of tea leaves powder samples (Lulu, Lipton, Society, Kanan Devan, Tata and Red Label). Results are mean SD (n = 3)

Tea product (powder)	Wt. of tea powder (g)	Brewing period			
		(1 min)		(5 min)	
		Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol mg/200 mL tea infusion	Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol mg/200 mL tea infusion
Lulu	0.1032	34.19 ± 0.97	2.94 ± 0.52	71.79 ± 0.33	7.94 ± 1.05
	0.5012	73.24 ± 0.87	26.26 ± 2.09	81.05 ± 0.51	34.57 ± 1.06
	1.023	89.47 ± 0.56	58.06 ± 1.59	83.33 ± 0.45	83.03 ± 1.50
	2.012	90.32 ± 0.57	86.2 ± 1.42	85.75 ± 0.73	155.83 ± 1.71
Lipton	0.1002	41.83 ± 0.89	0.29 ± 0.16	76.43 ± 0.38	4.06 ± 0.49
	0.5015	88.33 ± 1.22	23.34 ± 2.00	89.04 ± 0.57	39.62 ± 1.11
	1.011	90.06 ± 0.99	58.16 ± 1.03	89.90 ± 0.59	104.90 ± 1.41
	2.015	90.86 ± 0.89	175.36 ± 1.87	86.35 ± 0.91	116.99 ± 1.56
Society	0.1012	37.87 ± 0.78	5.15 ± 0.06	63.08 ± 0.51	8.68 ± 0.89
	0.5011	83.49 ± 0.56	30.47 ± 0.99	84.77 ± 0.69	48.43 ± 1.00
	1.014	90.61 ± 0.45	77.79 ± 0.63	89.06 ± 0.81	103.73 ± 1.62
	2.005	91.21 ± 0.55	107.03 ± 0.93	89.47 ± 0.57	166.99 ± 1.56
Kanan Devan	0.1000	33.24 ± 0.64	8.73 ± 0.76	86.36 ± 0.44	9.48 ± 0.83
	0.5024	88.31 ± 0.16	20.79 ± 1.67	85.97 ± 0.65	41.94 ± 1.27
	1.024	90.61 ± 0.65	46.51 ± 1.04	84.90 ± 0.38	104.51 ± 1.03
	2.055	91.71± 0.54	89.53 ± 1.12	70.21 ± 0.49	145.43 ± 1.02
Tata	0.1004	33.51 ± 0.39	11.55 ± 1.08	53.13 ± 0.33	8.92 ± 0.96
	0.5025	90.61 ± 0.19	29.36 ± 1.64	88.47 ± 0.41	41.24 ± 1.50
	1.014	91.18 ± 0.43	62.51 ± 2.02	88.33 ± 0.49	105.85 ± 1.13
	2.013	91.09 ± 0.81	185.36 ± 1.87	89.36 ± 0.71	93.72 ± 1.61
Red Label	0.1011	24.66 ± 0.22	2.38 ± 1.30	53.95 ± 0.36	14.5 ± 0.93
	0.5021	77.38 ± 0.43	19.29 ± 1.84	79.8 ± 0.48	43.81 ± 1.69
	1.004	89.61 ± 0.35	40.52 ± 1.78	89.04 ± 0.39	93.29 ± 0.87
	2.015	87.63 ± 0.73	170.75 ± 2.56	89.36 ± 0.71	101.58 ± 2.46

activity as it can be observed from the appearance of tea infusion. The appearance of 1 min infused tea was clear in colour and 5 min infused tea was cloudy and turbidity appeared upon standing as it contained more solids. The solid contents of tea infusion were higher in 5 min infused/brewed tea than 1 min and the values are tabulated in Table 2-3.

For black and green tea powder brew, when lower amount of tea leaves powder (0.1 g) boiled for 1 min produced infusion with lower polyphenol content, on the other hand when same quantity (0.1 g) boiled for 5 min produced infusion having higher polyphenol contents. Similarly, tea brew prepared with increasing the quantity of tea leaves powder from 1.0 g to 2.0 g, 2.0 g of tea leaves powder produced infusion with higher polyphenol contents than 1.0 g powder. From this study, it was clear that the polyphenol contents of tea leaves powder products increased with increasing infusion/ steeping time as well as with increasing the amount of tea leaves powder (Fig. 5). Table 4-5 represents the total polyphenol contents of all studied tea powder samples.

Correlation between DPPH radical scavenging activity of black tea leaves powder and their polyphenol content by linear regression analysis was also evaluated and correlation coefficients values were decreased for 1 min infusion in the order of: Lulu (0.7883) > Ahmed (0.7755) > Alkozay (0.7152) > Society (0.6543) > Leone (0.4599) > Kanan Devan (0.4164) > Twinings (0.3365) > Lipton (0.3365) > Red Label (0.3049) > Tata (0.2705); and for 5 min infusion in the order of: Red Label (0.8499) > Lulu (0.7201) > Alkozay (0.6697) > Kanan Devan

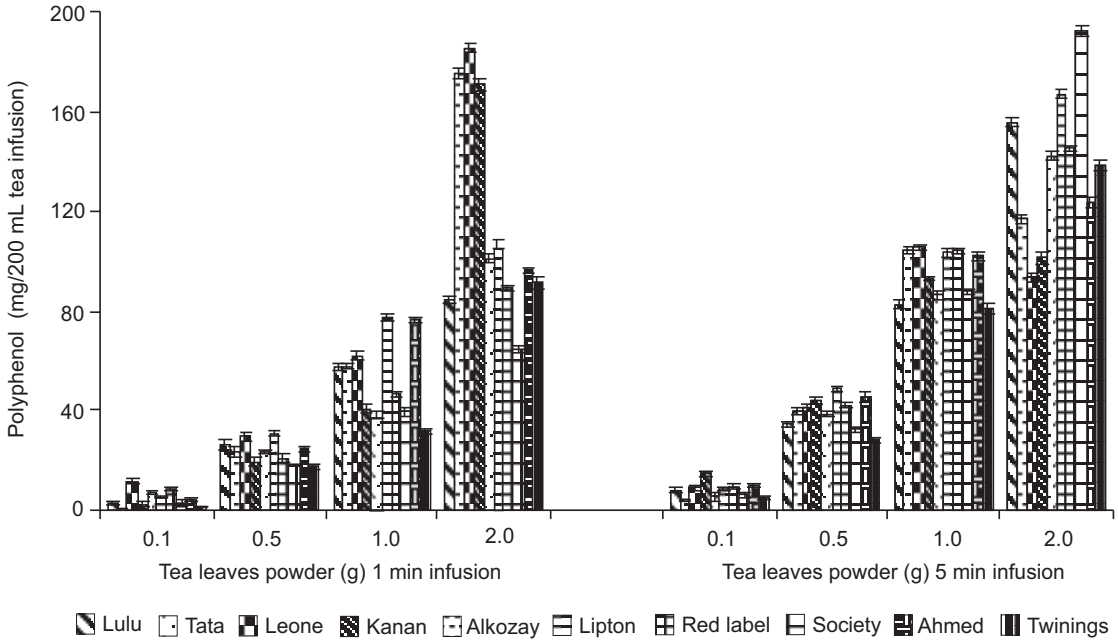


Fig. 5. Total polyphenol concentration of black tea and green tea leaves powder products boiled in 200 mL distilled water for 1 and 5 min. Results are mean ± SD (n = 3).

Table 5. Free radical scavenging activity and total polyphenol concentration of infusion of tea leaves powder samples (Leone, Alkozay, Ahmed and Twinings). Results are mean SD (n = 3)

Tea product (powder)	Wt. of tea powder (g)	Brewing period			
		(1 min)		(5 min)	
		Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol mg/200 mL tea infusion	Scavenging of DPPH$^{\bullet}$ (% ± SD)	Polyphenol mg/200 mL tea infusion
Leone	0.1001	30.65 ± 0.28	7.37 ± 0.06	48.50 ± 0.63	5.47 ± 0.89
	0.5001	77.52 ± 0.39	23.34 ± 0.99	85.34 ± 0.43	38.51 ± 1.85
	1.008	89.61 ± 0.51	38.11 ± 1.63	89.04 ± 0.55	86.73 ± 1.72
	2.005	90.40 ± 0.53	101.75 ± 1.93	89.59 ± 0.32	142.35 ± 1.88
Alkozay	0.1018	41.28 ± 0.38	4.01 ± 0.59	64.58 ± 0.63	9.94 ± 0..86
	0.5004	80.22 ± 0.45	24.42 ± 1.02	86.91 ± 0.54	45.35 ± 1.90
	1.014	87.91 ± 0.44	76.79 ± 1.26	89.75 ± 0.44	102.4 ± 1.80
	2.059	92.25 ± 0.61	96.53 ± 1.01	88.67 ± 0.43	123.68 ± 2.12
Ahmed	0.1011	26.15 ± 0.19	2.37 ± 1.30	52.99 ± 0.29	6.19 ± 1.07
	0.5010	68.27 ± 0.28	18.02 ± 0.51	86.91 ± 0.22	32.44 ± 1.10
	1.002	90.46 ± 0.99	39.30 ± 1.66	89.61 ± 0.45	88.04 ± 1.11
	2.051	92.25 ± 0.77	64.81 ± 1.53	89.0 ± 0.42	192.03 ± 2.12
Twinings (Green tea leaves powder)	0.1014	16.08 ± 0.27	1.45 ± 0.29	29.43 ± 0.39	4.89 ± 0.91
	0.5004	88.05 ± 0.31	15.85 ± 1.51	87.9 ± 0.49	28.14 ± 1.18
	1.011	86.91 ± 0.19	31.5 ± 1.07	91.03 ± 0.39	81.25 ± 1.89
	2.014	90.98 ± 0.20	91.7 ± 2.35	93.06 ± 0.33	138.36 ± 2.01

(0.6605) > Society (0.649) > Tata (0.6232) > Leone (0.578) > Twinings (0.4951) > Ahmed (0.3916). The analytical data clearly showed that their relationship were not linear (Table 6). However, in the present study, it was observed that, free radical scavenging activity of tea infusion determined by DPPH radical scavenging method were not increased with increasing of their polyohenol contents. Moreover, antioxidant activity decreased with increasing brewing period for larger amount of tea leaves powder inspite of their higher concentration of polyphenolic content.

Table 6. Correlation coefficients between free radical scavenging activity and polyphenol content of black tea leaves powder samples

Tea leaves powder products	Correlation coefficients (r)* brewing period	
	1 (min)	5 (min)
Leone	0.4599	0.578
Alkozay	0.7152	0.6697
Ahmed	0.7755	0.3916
Lulu	0.7883	0.7201
Lipton	0.3365	0.4698
Society	0.6543	0.6490
Kanan Devan	0.4164	0.6605
Tata	0.2705	0.6232
Red Label	0.3049	0.8499
Twinning	0.3601	0.4951

*In the linear regression analysis, polyphenolic content was regarded as X and % DPPH radical scavenging activity as Y.

Conclusion

Free radical scavenging activity depends on the amount of tea leaves used and the brewing period. From this study, it is clear that infusion of black or green tea bag for 1 or 2 min is enough to get higher antioxidant activity. Boiling of black tea powder products for longer time loses its antioxidant properties as well as decrease its health beneficial effect. Therefore, shorter boiling time is better for large amount of powder or small amount of powder should brew for minimum 5 min for getting higher antioxidant activity. However, several methods need to employ to study the antioxidant activity of tea infusion to find out the reason behind decreased free radical scavenging activity with increasing infusion time inspite of increasing polyphenol content.

Acknowledgement

The authors are grateful to Sana E. Taher, Shaima A. Jenebi, Asma Y. Rashid, Nehad M. Nafad and Hunain N. Salman, B. Pharm students of Dubai Pharmacy College for their valuable help in the preparation of tea infusion during carrying out this study.

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