



# Total phenolic content, total flavonoid content and *in vitro* antioxidant activities measured by the FRAP, ABTS, DPPH and ORAC assays of Sri Lankan black and green tea (*Camellia sinensis*) infusions

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## ABSTRACT

Tea is one of the most ancient and popular therapeutic beverages consumed by people all over the world. It is made from the leaves and buds of the plant "*Camellia sinensis*". Tea is cultivated in more than thirty countries around the world as a plantation crop. In the present study, thirty tea samples of 20 black and 10 green tea manufactured in Sri Lanka were screened for antioxidant activities. Black tea samples were obtained from the three main geographical regions low (Dust (I), BOPF, BOPI, OPI, Pekoe), mid (Dust (I), Dust, BOPF Local, BOPI, BOP, OPI, Pekoe) and up (Dust, BOPF, BOP, FBOP, FBOPI, OPA, OPI, Pekoe) country of Sri Lanka. Green tea samples are manufactured by two different processing methods; panning (Ceylon GT Chunmee (I), Ceylon GT Chunmee (II), Ceylon GT Fanning's, Ceylon GT Gun Powder (GP) (Extra Spl), Hyson, OPA green tea Ceylon GT Special Hyson) and steaming (Ceylon Sencha OPA, Ceylon Tencha, Cut Twist Curl (CTC) green tea) were used in the investigation. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities using ABTS, FRAP, DPPH and, ORAC assays were determined for each tea infusion. According to the results, phenolic content was higher in green tea than that of black tea and flavonoid content was lower in green tea than in black tea. Results of all antioxidants assays showed higher values for green tea than black tea. Neither the geographic area from which the samples of black tea came nor the method of preparation of green tea showed any significant differences in the activities. The results of the study have shown that Sri Lankan tea has compounds with antioxidant activities. Therefore, Sri Lankan tea can be used as a therapeutic beverage and a possibility to use the tea for producing antioxidant drugs through extraction and isolation processes.

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**KEYWORDS:** Black tea, green tea, upcountry, mid-country, low country, antioxidant activity

## INTRODUCTION

The tea plant, *Camellia sinensis* (L.) is indigenous to China and parts of India. Tea is grown worldwide, including in China, India, Sri Lanka, Kenya and Indonesia (Dharmadasa *et al.*, 2018). Tea leaves provide different types of beverages, which are the most popular non-alcoholic, soft, healthy drinks across the world (Schmidt *et al.*, 2005; Kamunya *et al.*, 2010; Falla *et al.*, 2021) and therefore rank as an important world food product (De-Heer, 2011). Tea is generally consumed for its attractive aroma

and taste as well as the unique place it holds in the culture of many societies (De-Heer, 2011).

Black tea, green tea, Oolong and white tea are all harvested from the same species but are processed differently to attain different levels of oxidation. Black tea is fully fermented, green and white tea is essentially unfermented and Oolong tea is partially fermented (Bancirova, 2010; Mahmood *et al.*, 2010; Namita *et al.*, 2012; Rohadi *et al.*, 2019; Falla *et al.*, 2021; Truong & Jeong, 2021; Wong *et al.*, 2022). Of the total teas produced

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worldwide are 78%, 20%, 2% are black, green and, Oolong tea respectively (Chan *et al.*, 2007; Omar *et al.*, 2016; Balaban *et al.*, 2022). Black tea is preferred by the consumers in India, Sri Lanka, Pakistan, the US, Russia, and the UK, green tea is preferred in Japan and China while Oolong tea is preferred by the people in China (Singh & Anita, 2012; Lange, 2022; Wong *et al.*, 2022).

Tea is cultivated in different districts in Sri Lanka, with variations in productivity due to both regional and seasonal differences. The varying landscapes of the tea-growing regions facilitate the production of the high, medium, and low-grown black teas; all of which have distinct differences in flavor (Kelegama, 2010). Sri Lanka has produced a lot of different grades of black teas (e.g., whole-leaf grades- OPI, OPA, OP, broken leaf grades- FB, BOP, BOPI, fanning's- PF, BOPF, and Dust grades - Dust, Dust (I)) and many grades of green teas (e.g., pan-fried- Gun Powder, Hyson, OPA, Sp. Hyson and steamed- Ceylon Sen. OPA, Ceylon Tencha, CTC). Sri Lanka exports approximately 95% of its production (Prematunga, 2009) and has been able to maintain 2<sup>nd</sup> largest exporter of tea worldwide with approximately 1.33 billion U.S. Dollars' worth of tea in 2020 (Ridder, 2022).

*C. sinensis* contains more than 200 chemicals which consist of 36% polyphenolic compounds, 20% carbohydrates, 10% proteins, 6% lignin, 0.05% ash, 4% amino acids, 2% lipids, 1.0% organic acids, 0.05% chlorophyll as well as carotenoids and volatile substances less than 0.1% of dry substance mass (Bancirova, 2010; Ponmurugan *et al.*, 2019; Zhang *et al.*, 2019; Chen *et al.*, 2022). The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin, and polyphenols. Among all tea types, green teas contain the highest amount of monomeric polyphenols, the most frequent are catechins. The most important catechins are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (Luczaj & Skrzydlewska, 2005; Ponmurugan *et al.*, 2019). Due to the oxidation of phenolic compounds during the fermentation process, black tea contains multimeric polyphenols (Luczaj & Skrzydlewska, 2005; Ponmurugan *et al.*, 2019) thearubigins and theaflavins (Lee *et al.*, 2002; Ponmurugan *et al.*, 2019). Black tea comprises 2–6% of theaflavins and more than 20% of thearubigins, whereas green tea has 30–42% of catechins (Lin *et al.*, 2003; Chan *et al.*, 2007; Lee *et al.*, 2019). There are some factors influencing the chemical content of tea such as the tea category, geographical location and growing conditions (soil, climate, agricultural practices, fertilizers), the type of tea (e.g., blended, decaffeinated, instant) and the preparation of the infusion (e.g., the amount of the product used, duration of steeping, the temperature of the water) (Hakim *et al.*, 2000; Wu & Wei, 2002; Ratnasooriya *et al.*, 2016; Ntezimana *et al.*, 2021).

Tea is simply considered a tasteful drink for most of the world. Moreover, the scientific community has recently rediscovered the therapeutic potential of this beverage (McKay & Blumberg, 2002; Luczaj & Skrzydlewska, 2005; Ntezimana *et al.*, 2021). Human studies suggested that tea may contribute to the reduction in the risk of cardiovascular disease and some forms of cancer, as well as the promotion of oral health

(Namita *et al.*, 2012; Yang & Hong, 2013; Lange, 2022). Harmful effects of free radicals and oxidative stress can be reduced by regular consumption of foods and beverages which exhibit antioxidant activity (Yashin *et al.*, 2011). Tea polyphenols are natural powerful antioxidants (Taniyazawa *et al.*, 1984; Farhoosh *et al.*, 2007; Truong & Jeong, 2021) and are considered to be responsible for the anticarcinogenic and antimutagenic properties of tea (Shahidi *et al.*, 1992; Tijburg *et al.*, 1997; Wiseman *et al.*, 1997; Truong & Jeong, 2021). However, the antioxidant activity of green tea is higher than that of black tea according to some previous investigations (Yokozawa *et al.*, 1998; Leung *et al.*, 2001; Lee *et al.*, 2002; Atoui *et al.*, 2005; Truong & Jeong, 2021). However, Leung *et al.* (2001) argued that theaflavins in black tea and catechins in green tea are equally effective antioxidants.

Although Sri Lanka is still regarded as a producer of superior-quality tea it is gradually losing its footing in the global tea industry. Intense competition from rivals has adversely affected the nation's competitive position. Meanwhile, the country's continued focus on producing orthodox tea as opposed to value-added tea could further erode its market share in the global market because of the inability to fulfil the global consumers rapidly changing preferences. Under these circumstances, the production of value-added tea may be a good solution to compete in the world market. The antioxidant capacity of tea can be used as value-added quality of tea as consumer preference is gaining interest in the health properties of food products. Only a few pieces of have been carried out on the antioxidant properties (Abeywickrama *et al.*, 2011; Aluthgamaa *et al.*, 2020) of Sri Lankan teas. Also, due to no previous evidence on comparative analysis of black and green tea manufactured in Sri Lanka one of an aim of the current research was to compare Sri Lankan black and green tea for antioxidant activities. The effect of geographical areas on total phenolic content, the total flavonoid content and the antioxidant activity of black tea was also estimated.

## MATERIALS AND METHODS

### Tea Samples

Twenty black tea samples of (Dust, Dust (I), Broken Orange Pekoe Fanning's (BOPF), Broken Orange Pekoe (BOP), Broken Orange Pekoe I (BOPI), Flowery Broken Orange Pekoe (FBOP), Flowery Broken Orange Pekoe I (FBOPI), Orange Pekoe A (OPA), Orange Pekoe I (OPI) and Pekoe) belonging to different geographical areas; up-St. Coombs estate, Thalawakelle; above 1,200 m, mid- St Joachim estate, Ratnapura; 600- 1200 m and low, Deniyaya tea estate; between sea level and 600 m obtained from Tea Research Institute, Thalawakelle, Sri Lanka. Ten green tea samples (Ceylon GT Chunmee (I), Ceylon GT Chunmee (II), Ceylon GT Fanning's, Ceylon GT Gun Powder (GP) (Extra Spl), Hyson, OPA green tea Ceylon GT Special Hyson, Ceylon Sencha OPA, Ceylon Tencha, Cut Twist Curl (CTC) green tea) were obtained from Melfort Green Teas (Pvt) Ltd, Peliyagoda, Sri Lanka.

## Preparation of Tea Infusion

Tea infusions were prepared by adding 100 ml of boiled water to 2 g of dry tea sample and cooled to room temperature. The dry matter content of tea infusions was determined using the oven method, dried at  $110 \pm 5^\circ\text{C}$  temperatures for constant mass (Faichney & White, 1983). Triplicates of infusion were prepared for each sample. Then, 2 ml of filtered tea infusion was centrifuged at 11000 rpm for 5 mins. The supernatants were stored at  $-40^\circ\text{C}$  until their use in the assays. Tea infusions were diluted for each assay separately by diluting the tea samples with relevant solutions (TPC- distilled water, TFC- methanol, FRAP- acetate buffer, ABTS- ABTS buffer, ORAC- ORAC buffer and DPPH- methanol). For TPC, TFC, FRAP, ABTS<sup>+</sup> and the ORAC assays only one concentration was prepared while for the DPPH assay five concentrations were prepared to determine the IC50 values.

## Determination of Total Polyphenolic Content (TPC) and Total Flavonoid Content (TFC)

The Folin-Ciocalteu method described by Blainski *et al.* (2013) was used to estimate the total polyphenolic content in the tea infusions. Total flavonoid content was determined according to the aluminium chloride method previously described by Wong *et al.* (2006) and Mundhe *et al.* (2011).

## Determination of Antioxidant Activities

Ferric Reducing Antioxidant Power was determined using the previously described FRAP method Maizura *et al.* (2011) with some modifications. In our study, the final volume of the reaction mixture used was 200  $\mu\text{l}$  instead of 1000  $\mu\text{l}$  in the previous study. For the ABTS<sup>+</sup> assay, the procedure followed the method of Thaiponga, (2006), with some modifications. In our

study, the final volume of the reaction mixture used was 200  $\mu\text{l}$  instead of 1000  $\mu\text{l}$  in the previous study. The ORAC assay was based on the procedure described by Kodama *et al.* (2010). A fluorescence microplate reader was used for this assay. ORAC assay is a kinetic assay. Decay curves (fluorescence intensity vs. time) were recorded and the area between the two decay curves (with or without antioxidants) was calculated. The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) the radical-scavenging method according to Liang and Kitts (2014). The results of all antioxidant assays were expressed as mg Trolox Equivalents (mg TE)/g of tea sample on a wet basis.

## Statistical Analysis

Determination of antioxidant activity was done using three replicates and the results were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using the SPSS software and Minitab version 16 software. The differences between means of each category of black tea, black and green tea were analyzed separately by ANOVA test and then Duncan's multiple range tests ( $p < 0.05$ ) to identify the best samples of each category.

## RESULTS

Total phenolic content, total flavonoid content and *in vitro* antioxidant activities measured by the FRAP, ABTS, ORAC and DPPH assays of black tea and green tea are interpreted in Tables 1 and 2 respectively.

## Total Polyphenolic Content (TPC)

From the used black tea samples the highest, the lowest total phenolic content (TPC) was found in mid-country BOPF (246.34 mg GAE/g tea sample) and mid-country Pekoe (40.54 mg GAE/g tea sample) tea

**Table 1: TPC, TFC and *in vitro* antioxidant activities of black tea infusions**

Geographical area	Leaf grade	Sample	TPC (mg GAE/g sample)	TFC (mg QE/g sample)	FRAP (mg TE/g sample)	ABTS+ (mg TE/g sample)	ORAC (mg TE/g sample)	DPPH (mg TE/g sample)	
Low country (LB)	Dust	Dust (I)	139.08 $\pm$ 1.92 <sup>b</sup>	2.60 $\pm$ 0.11 <sup>f</sup>	149.17 $\pm$ 1.63 <sup>b</sup>	162.07 $\pm$ 3.64 <sup>a</sup>	68.99 $\pm$ 3.55 <sup>f</sup>	<b>163.93 <math>\pm</math> 3.99<sup>a</sup></b>	
	Fanning's	BOPF	98.39 $\pm$ 5.22 <sup>de</sup>	1.62 $\pm$ 0.03 <sup>hi</sup>	<b>203.96 <math>\pm</math> 17.27<sup>a</sup></b>	112.60 $\pm$ 17.60 <sup>d</sup>	35.91 $\pm$ 4.39 <sup>hi</sup>	71.17 $\pm$ 1.56 <sup>e</sup>	
	Broken leaf	BOPI	69.23 $\pm$ 0.36 <sup>hi</sup>	1.65 $\pm$ 0.01 <sup>h</sup>	153.00 $\pm$ 21.71 <sup>b</sup>	122.34 $\pm$ 7.43 <sup>cd</sup>	59.12 $\pm$ 2.45 <sup>gh</sup>	<b>20.18 <math>\pm</math> 0.12<sup>j</sup></b>	
	Whole leaf	OPI	57.97 $\pm$ 0.84 <sup>j</sup>	3.03 $\pm$ 0.06 <sup>de</sup>	55.57 $\pm$ 4.58 <sup>d</sup>	88.31 $\pm$ 5.69 <sup>e</sup>	<b>22.46 <math>\pm</math> 1.02<sup>i</sup></b>	38.77 $\pm$ 0.53 <sup>g</sup>	
Mid country (MB)	Pekoe	Pekoe	58.33 $\pm$ 3.07 <sup>j</sup>	1.33 $\pm$ 0.03 <sup>l</sup>	142.83 $\pm$ 3.27 <sup>b</sup>	<b>168.69 <math>\pm</math> 3.52<sup>a</sup></b>	61.90 $\pm$ 3.71 <sup>gh</sup>	90.47 $\pm$ 1.63 <sup>d</sup>	
		Dust	Dust (I)	77.87 $\pm$ 2.43 <sup>g</sup>	2.59 $\pm$ 0.03 <sup>f</sup>	60.21 $\pm$ 1.16 <sup>d</sup>	87.66 $\pm$ 4.41 <sup>e</sup>	66.75 $\pm$ 1.95 <sup>f</sup>	38.34 $\pm$ 1.35 <sup>g</sup>
	Fanning's	Dust	Dust	75.86 $\pm$ 1.49 <sup>g</sup>	2.03 $\pm$ 0.02 <sup>g</sup>	92.71 $\pm$ 19.73 <sup>c</sup>	141.81 $\pm$ 4.17 <sup>b</sup>	40.19 $\pm$ 3.71 <sup>hi</sup>	39.48 $\pm$ 1.02 <sup>g</sup>
		BOPF	BOPF	<b>246.34 <math>\pm</math> 4.65<sup>a</sup></b>	3.44 $\pm$ 0.12 <sup>c</sup>	99.45 $\pm$ 6.04 <sup>c</sup>	109.27 $\pm$ 6.95 <sup>d</sup>	40.29 $\pm$ 4.33 <sup>hi</sup>	99.02 $\pm$ 6.17 <sup>c</sup>
	Broken leaf	BOPI	BOPI	98.58 $\pm$ 0.60 <sup>de</sup>	1.56 $\pm$ 0.03 <sup>hi</sup>	32.92 $\pm$ 3.10 <sup>f</sup>	128.04 $\pm$ 7.36 <sup>c</sup>	46.87 $\pm$ 5.46 <sup>ghi</sup>	38.46 $\pm$ 0.32 <sup>g</sup>
		BOP	BOP	78.28 $\pm$ 0.51 <sup>g</sup>	2.85 $\pm$ 0.04 <sup>ef</sup>	56.25 $\pm$ 5.84 <sup>d</sup>	79.09 $\pm$ 2.75 <sup>ef</sup>	60.97 $\pm$ 3.73 <sup>gh</sup>	39.49 $\pm$ 0.24 <sup>g</sup>
Up country (UB)	Whole leaf	OPI	121.42 $\pm$ 3.42 <sup>c</sup>	1.44 $\pm$ 0.02 <sup>hi</sup>	65.91 $\pm$ 6.38 <sup>d</sup>	59.81 $\pm$ 8.29 <sup>gh</sup>	37.95 $\pm$ 0.24 <sup>hi</sup>	65.40 $\pm$ 1.99 <sup>f</sup>	
		Pekoe	Pekoe	<b>40.54 <math>\pm</math> 1.14<sup>k</sup></b>	0.65 $\pm$ 0.15 <sup>jk</sup>	36.72 $\pm$ 1.04 <sup>ef</sup>	87.14 $\pm$ 4.41 <sup>e</sup>	52.75 $\pm$ 3.30 <sup>gh</sup>	27.49 $\pm$ 0.61 <sup>hi</sup>
	Dust	Dust	Dust	93.22 $\pm$ 1.14 <sup>f</sup>	3.08 $\pm$ 0.03 <sup>de</sup>	56.72 $\pm$ 1.84 <sup>d</sup>	68.84 $\pm$ 2.26 <sup>g</sup>	24.65 $\pm$ 4.25 <sup>i</sup>	31.62 $\pm$ 1.00 <sup>h</sup>
		BOPF	BOPF	73.68 $\pm$ 0.29 <sup>gh</sup>	3.25 $\pm$ 0.02 <sup>cd</sup>	61.35 $\pm$ 1.72 <sup>d</sup>	109.49 $\pm$ 3.58 <sup>d</sup>	37.52 $\pm$ 1.46 <sup>hi</sup>	137.70 $\pm$ 2.38 <sup>b</sup>
Broken leaf	BOP	BOP	95.86 $\pm$ 0.59 <sup>ef</sup>	<b>5.08 <math>\pm</math> 0.47<sup>a</sup></b>	30.58 $\pm$ 4.21 <sup>f</sup>	52.46 $\pm$ 2.87 <sup>h</sup>	445.88 $\pm$ 14.85 <sup>b</sup>	27.90 $\pm$ 0.65 <sup>hi</sup>	
		FBOP	FBOP	93.97 $\pm$ 2.00 <sup>ef</sup>	<b>4.96 <math>\pm</math> 0.04<sup>a</sup></b>	21.15 $\pm$ 5.38 <sup>f</sup>	<b>51.04 <math>\pm</math> 3.58<sup>h</sup></b>	324.55 $\pm$ 29.22 <sup>e</sup>	23.84 $\pm$ 0.51 <sup>ij</sup>
	FBOP	FBOP	102.07 $\pm$ 3.38 <sup>d</sup>	0.47 $\pm$ 0.04 <sup>k</sup>	53.00 $\pm$ 3.54 <sup>de</sup>	61.56 $\pm$ 2.45 <sup>gh</sup>	407.58 $\pm$ 18.62 <sup>c</sup>	31.22 $\pm$ 3.87 <sup>h</sup>	
		OPA	OPA	65.06 $\pm$ 1.79 <sup>j</sup>	4.14 $\pm$ 0.01 <sup>b</sup>	33.25 $\pm$ 8.20 <sup>f</sup>	61.68 $\pm$ 2.02 <sup>gh</sup>	464.04 $\pm$ 15.66 <sup>ab</sup>	22.76 $\pm$ 0.31 <sup>j</sup>
Whole leaf	OPI	OPI	102.70 $\pm$ 1.55 <sup>d</sup>	5.15 $\pm$ 0.29 <sup>a</sup>	<b>19.32 <math>\pm</math> 2.94<sup>f</sup></b>	84.66 $\pm$ 7.06 <sup>e</sup>	<b>486.33 <math>\pm</math> 23.90<sup>a</sup></b>	42.28 $\pm$ 1.07 <sup>g</sup>	
	Pekoe	Pekoe	124.14 $\pm$ 0.84 <sup>c</sup>	0.93 $\pm$ 0.18 <sup>l</sup>	55.92 $\pm$ 2.04 <sup>d</sup>	53.18 $\pm$ 1.43 <sup>h</sup>	357.11 $\pm$ 13.01 <sup>d</sup>	30.80 $\pm$ 0.60 <sup>h</sup>	

Values are mean ( $n=3$ )  $\pm$  standard deviation. Values with the same superscript letter within each column are not significant different ( $P < 0.05$ ). Bold letters are for tea samples with the highest and the lowest values in each column.

**Table 2: TPC, TFC and *in vitro* antioxidant activities of green tea infusions**

Processed method	Sample	TPC mg GAE/g sample	TFC (mg QE/g sample)	FRAP (mg TE/g sample)	ABTS+ (mg TE/g sample)	ORAC (mg TE/g sample)	DPPH (mg TE/g sample)
Pan fired (PG)	Ceylon GT Chunmee (I)	<b>73.91 ± 1.36<sup>a</sup></b>	0.69 ± 0.01 <sup>d</sup>	79.38 ± 6.06 <sup>d</sup>	186.35 ± 2.25 <sup>d</sup>	51.37 ± 13.16 <sup>cd</sup>	38.82 ± 0.87 <sup>e</sup>
	Ceylon GT Chunmee (II)	84.02 ± 3.25 <sup>fg</sup>	0.57 ± 0.02 <sup>e</sup>	<b>46.88 ± 6.75<sup>f</sup></b>	<b>282.15 ± 5.29<sup>a</sup></b>	<b>40.39 ± 10.77<sup>de</sup></b>	41.97 ± 0.25 <sup>e</sup>
	Ceylon GT Fanning's	134.54 ± 11.75 <sup>c</sup>	0.83 ± 0.01 <sup>c</sup>	<b>309.89 ± 3.38<sup>a</sup></b>	208.44 ± 4.59 <sup>c</sup>	59.40 ± 16.41 <sup>bcd</sup>	<b>188.23 ± 4.08<sup>a</sup></b>
	Ceylon GT Gun Powder	119.31 ± 2.27 <sup>d</sup>	0.98 ± 0.03 <sup>b</sup>	138.92 ± 4.05 <sup>b</sup>	176.89 ± 13.46 <sup>d</sup>	58.71 ± 7.30 <sup>bcd</sup>	109.09 ± 9.93 <sup>b</sup>
	Hyson	87.93 ± 0.61 <sup>ef</sup>	<b>0.31 ± 0.01<sup>g</sup></b>	82.33 ± 10.53 <sup>d</sup>	206.44 ± 3.30 <sup>c</sup>	27.77 ± 6.28 <sup>e</sup>	66.76 ± 0.73 <sup>d</sup>
	OPA green tea	95.86 ± 2.10 <sup>e</sup>	<b>2.68 ± 0.04<sup>a</sup></b>	120.99 ± 5.87 <sup>c</sup>	264.00 ± 6.89 <sup>ab</sup>	64.67 ± 2.57 <sup>bc</sup>	42.62 ± 0.07 <sup>e</sup>
Steam-Processed (SG)	Ceylon GT Special Hyson	74.67 ± 1.09 <sup>g</sup>	0.43 ± 0.01 <sup>f</sup>	63.44 ± 1.75 <sup>e</sup>	247.23 ± 11.37 <sup>b</sup>	55.59 ± 6.55 <sup>bcd</sup>	100.95 ± 1.41 <sup>c</sup>
	Ceylon Sencha OPA	137.89 ± 3.41 <sup>c</sup>	0.41 ± 0.00 <sup>f</sup>	85.08 ± 9.72 <sup>d</sup>	169.39 ± 7.41 <sup>de</sup>	76.20 ± 13.35 <sup>ab</sup>	40.23 ± 0.42 <sup>e</sup>
-Processed (SG)	Ceylon Tencha	<b>193.60 ± 4.35<sup>a</sup></b>	0.53 ± 0.01 <sup>e</sup>	140.26 ± 10.11 <sup>b</sup>	<b>153.18 ± 1.44<sup>e</sup></b>	72.34 ± 2.17 <sup>abc</sup>	65.43 ± 0.46 <sup>d</sup>
	Cut Twist Curl (CTC)	151.38 ± 5.95 <sup>b</sup>	0.54 ± 0.01 <sup>e</sup>	111.09 ± 8.32 <sup>c</sup>	266.25 ± 16.53 <sup>a</sup>	<b>88.81 ± 4.00<sup>a</sup></b>	<b>28.73 ± 0.29<sup>f</sup></b>

Values are mean (n=3) ± standard deviation. Values with the same superscript letter within each column are not significant different (p>0.05). Bold letters are for tea samples with the highest and the lowest values in each column.

samples respectively. The TPC of the mid-country BOPF tea sample was superior to all other samples. According to the results tested all green tea samples had high polyphenol content (> 64.74 mg GAE/g sample). The highest and the lowest TPC were observed in the Ceylon Tencha (189.64 mg GAE/g sample) and Ceylon GT Chunmee (I) 64.74 (mg GAE/g sample).

### Total Flavonoid Content

The total flavonoid content of tested all black tea samples was comparatively low values (< 6 mg QE/g sample). The highest and the lowest TFC were observed in upcountry OPI (5.15 mg QE/g sample) and FBOPI (0.47 mg QE/g sample) samples. Almost all the green tea samples had very low flavonoid content relative to the black tea. The highest and the lowest TFC were found in OPA (2.68 mg QE/g sample) and Hyson (0.31 mg QE/g sample) samples respectively. TFC of the OPA green tea sample was significantly higher than all other green tea samples.

### *In vitro* Antioxidant Assays

#### Ferric reducing antioxidant power (FRAP) assay

Antioxidant activities measured by the FRAP assay of black tea samples were distributed in a large range. Highest and the lowest values were the 203.96 mg TE/g sample (low country BOPF) and 19.32 mg TE/g sample (up country OPI). The highest and the lowest antioxidant activities of green tea measured by the FRAP assay are 309.89 mg TE/g sample (Ceylon GT Fanning's) and 46.88 mg TE/g sample (Ceylon GT Chunmee (II)) respectively. The FRAP value obtained by the Ceylon GT Fanning was significantly higher than all other samples. The high antioxidant activity measured by the FRAP assay was found in green tea samples followed by black tea.

#### ABTS + Radical Scavenging Assay

Tested Sri Lankan black tea samples have good antioxidant activities according to the results. The highest and lowest activity was shown by the low country Pekoe (168.69 mg TE/g tea sample) and the upcountry FBOP (51.04 mg TE/g tea sample) samples. The highest and the lowest antioxidant activity of green tea was measured by the ABTS assay given by the Ceylon GT Chunmee (II) (282.15 mg TE/g tea sample) and Ceylon

Tencha samples (153.18 mg TE/g tea sample). Tested all the green tea samples had a high content of antioxidants, according to the ABTS assay. Antioxidant activity measured by the ABTS assay of green tea was high, relative to the black tea.

#### ORAC Assay

According to the results up country OPI, OPA, BOP, FBOPI, Pekoe and FBOP samples had extremely higher ORAC values than all other tested black tea samples. However, the lowest ORAC value was found in the low country OPI sample (22.46 mg TE/g tea sample). Antioxidant activities of green tea samples measured by the ORAC assay were distributed within the low range. The highest and the lowest values were shown by the CTC (88.81 mg TE/g tea sample) and Hyson (27.77 mg TE/g tea sample) samples respectively. Antioxidant activities of black tea measured by the ORAC assay were the highest, followed by green tea. However, samples with high antioxidant activity of black tea belonged to the upcountry. All other black tea samples had lower antioxidant activity than upcountry black tea. Green tea samples had ORAC values between about 25-100 mg TE/g tea sample.

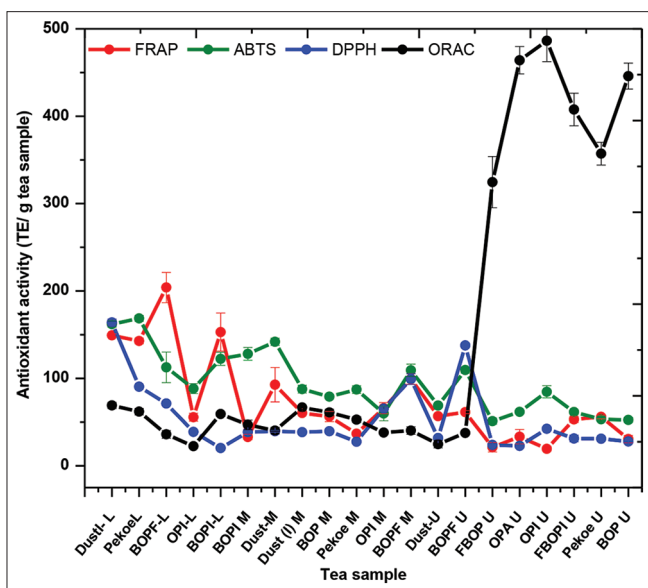
#### DPPH Assay

The highest and the lowest values for the antioxidant activity of black tea were obtained by the low country Dust(I) (163.93 mg TE/g sample) and low country BOPI (20.18 mg TE/g sample) samples respectively. Antioxidant activities of low country Dust (I), up country BOPF, mid-country BOPF low country Pekoe, low country BOPF and mid-country OPI were distributed in a broad range while all other tested black tea samples were in a narrow range. The highest and the lowest antioxidant activities of green tea were found in the Ceylon GT Fanning's (188.23 mg TE/g sample) and CTC green tea (28.73 mg TE/g sample) samples. There was a relatively broad distribution of data of green tea samples. Antioxidant activity measured by the DPPH assay had not much difference among different tea categories.

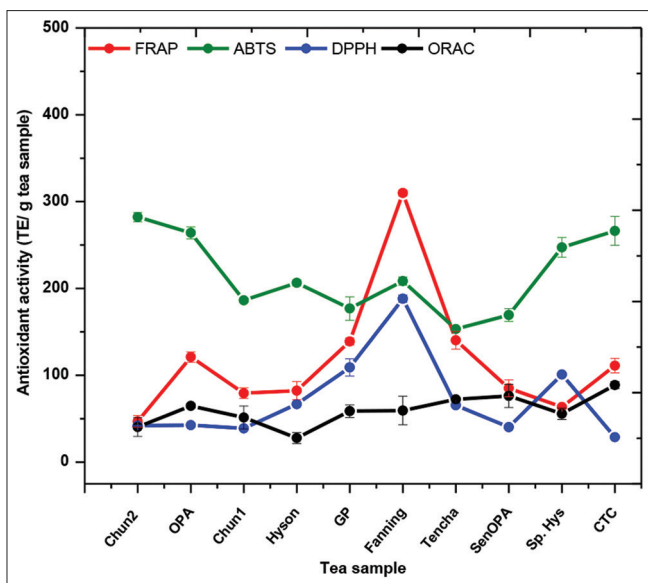
#### Comparative Analysis of the Results of FRAP, ABTS, ORAC and DPPH Assays

The results obtained from different antioxidant assays were compared for different tea categories separately. According to

Figure 1, out of twenty black tea samples, nine samples had given high antioxidant activity for ABTS assay. Upcountry FBOP, OPA, FBOPI, Pekoe and BOP grades had given high values for the ORAC assay and antioxidant activities measured by other assays were not given higher activities compared to the ORAC assay. Eight samples had given the lowest value for the ORAC assay. Antioxidant activity measured by the ORAC assay of five green tea samples had lower values than the values obtained by other assays. Tested eight green tea samples had the highest values for ABTS assay. Seven tea samples out of ten had the second-highest antioxidant activity for FRAP assay. The results of the DPPH and ORAC were relatively lower than other assays (Figure 2).



**Figure 1:** Comparison of the antioxidant activities of black tea samples measured by the FRAP, ABTS, DPPH and ORAC assays



**Figure 2:** Comparison of the antioxidant activities of green tea samples measured by the FRAP, ABTS, DPPH and ORAC assays

### Correlations between TPC, TFC Results with FRAP, ABTS, ORAC and DPPH Assays Results

The TPC and TFC assays and antioxidant assays of green tea had a high correlation which was significant at the 0.05 level and 0.01 levels. The TPC with FRAP and TFC with FRAP and ABTS of green tea were significantly correlated at 0.05 levels with 0.457, 0.459 and 0.524 correlation coefficients. The TPC with ABTS and DPPH and TFC with DPPH of green tea were significantly correlated at a 0.01 significance level. The ORAC assay was not given good correlations with both TPC and TFC assays. Although black tea used for this study had higher TPC than green tea, black tea was not shown good correlations between TPC and TFC with antioxidant activities (Table 3).

### Effect of different Geographical Areas on Total Phenolics Content, Total Flavonoid Content and *In vitro* Antioxidant Activity of Black Tea

- a. TPC  
TPC of same grade tea obtained from different geographical areas had wide variations of TPC. BOPF and OPI grades had the highest TPC for mid-country samples. Pekoe, BOP and Dust grades had higher TPC for up-country samples than for mid-country samples. Pekoe and Dust (I) grades, low country samples had higher TPC than mid-country.
- b. TFC  
TFC of OPI, Pekoe, BOP and Dust grade tea had higher TFC for up-country samples than mid-country samples. BOPI and Dust grades lower and mid-country samples had relatively similar TFC among different areas.
- c. FRAP  
BOPF, Pekoe, BOPI and Dust (I) grades, low country samples had the highest antioxidant activities measured by the FRAP assay. BOPF, OPI, BOP and Dust grade, mid-country samples had higher antioxidant activities than up-country samples. Up-country samples of BOPF, OPI, BOP and Dust grades had the lowest antioxidant activity.
- d. ABTS  
Pekoe, BOP and Dust grade tea had the lowest antioxidant activity for up-country samples. BOPF, OPI, Pekoe and Dust (I) grades had the highest antioxidant activity for low country samples.
- e. DPPH assays  
OPI, BOP and Dust grades had higher antioxidant activity measured by the DPPH assay for mid-country samples than

**Table 3:** Pearson’s correlation coefficient among the total phenolic content, the total flavonoid content and the antioxidant capacities of Sri Lankan black and green tea.

Tea categories	TPC x FRAP	TPC x ABTS	TPC x DPPH	TPCx ORAC
Black tea	0.145	0.012	0.386	0.003
Green tea	0.457*	0.581**	0.778**	0.012
Black tea	-0.390	-0.288	-0.022	0.433
Green tea	0.459*	0.524*	0.712**	-0.116

\*Correlation is significant at the 0.05 level (2-tailed).  
\*\*Correlation is significant at the 0.01 level (2-tailed).

up-country samples. BOPF, OPI and BOPI samples had high antioxidant activity for mid-country samples than low country samples.

f. ORAC assay

According to the ORAC assay, OPI, Pekoe and BOP grades had higher antioxidant activity than mid and low-country samples. Low country samples were the second, according to the ORAC values of low country Pekoe, BOPI and Dust (I) grades.

## DISCUSSIONS

Tea is the most popular beverage and it contains large amounts of polyphenols (Gramza-Michalowska & Regula, 2007; Falla *et al.*, 2021). Polyphenols in fresh tea leaves, manufactured black and green tea is considered compounds with high antioxidant activity. The fresh tea leaves contain four major catechins as colourless water-soluble compounds; epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (Karori *et al.*, 2007; Truong & Jeong, 2021). A limited numbers of studies have shown the antioxidant properties of black and green tea in Sri Lanka. Although the number of black tea grades manufactured in different geographical regions of the country, an adequate comparison of antioxidant activity among these has not been carried out (Abeywickrama *et al.*, 2005; Abeywickrama *et al.*, 2011; Aluthgamaa *et al.*, 2020). Therefore, in this study, twenty black tea samples were obtained from main three geographical regions of Sri Lanka and ten green tea samples were used for the determination of total phenolic content, total flavonoid content and antioxidant activities. A main phenolic compound present in green tea is catechin while theaflavins and thearubigins are the main components of black tea (Luczaj & Skrzydlewska, 2005; Ponmurugan *et al.*, 2019). These phenolic compounds are very important constituents of plants. Their radical scavenging ability is due to hydroxyl groups (Miller *et al.*, 1996, Yanab *et al.*, 2020).

According to a previous investigation, the TPC in black tea samples from different countries ranged between 80.5 to 134.9 mg GAE/g dry tea sample and the greatest activity were found in Ceylon black tea (Yashin *et al.*, 2011). However, the used tea grade of the Ceylon black tea was not mentioned. According to the present study range of the TPC was from 40.54 (mid-country Pekoe) to 246.34 mg GAE/g tea sample (mid-country BOPF). Green tea TPC were in the range from 65.8 to 106.2 mg GAE/g dry tea sample according to Yashin *et al.* (2011). Green teas manufactured in Sri Lanka had TPC in the range from 73.91 (Chunmee II) to 193.60 (Ceylon Tencha) mg GAE/g dry tea sample. Therefore, compared with the results of Yashin *et al.* (2011) Sri Lankan black and green tea TPC content were higher than the tea from other sources. Furthermore, Sri Lankan tea grades had high TPC and it was in agreement with the Yashin *et al.* (2011) findings. Flavonoids are biologically active polyphenolic compounds and the prominent flavonoids in the tea are the flavan-3-ols catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, their fermentation products, derived tannins-theaflavins and

thearubigins (Obanda *et al.*, 2004; Bancirova, 2010; Carloni *et al.*, 2013; Yanab *et al.*, 2020). High flavonoid content was observed in this study in black tea than green tea.

Most of the previous studies have found that green tea had higher antioxidant activity than black tea (von Gadow *et al.*, 1997; Richelle *et al.*, 2001; Roginsky *et al.*, 2003; Yashin *et al.*, 2011; Carloni *et al.*, 2013). However, some studies show that black teas are better than green teas (Hoff & Singleton, 1977; Khokhar & Magnusdottir, 2002; Carloni *et al.*, 2013), although some others report the absence of any significant differences (Lin *et al.*, 1996; Hodgson *et al.*, 1999; Carloni *et al.*, 2013). The numerous variables that affect tea components according to cultivar type, growth conditions (season, climate, soil), horticultural practices (mechanical- or hand-plucking, age of leaves) and the different technologies of the tea production (CTC and orthodox manufacturing of black tea, panning and steaming of green tea) may account for these conflicting investigations (Kan, 1980; Wong *et al.*, 2022).

According to the results of the antioxidant assays of the present study most green tea samples had high antioxidant activities than black tea according to the FRAP, ABTS and DPPH assays. This finding is in agreement with the previous findings (von Gadow *et al.*, 1997; Richelle *et al.*, 2001; Roginsky *et al.*, 2003; Gramza-Michalowska & Regula, 2007; Bancirova, 2010; Yashin *et al.*, 2011; Carloni *et al.*, 2013; Izzreen & Fadzelly, 2013; Rahman *et al.*, 2021). However, some samples had high antioxidant activity for black tea samples than green tea samples, same as in the case of some previous studies (Hoff & Singleton, 1977; Khokhar & Magnusdottir, 2002; Carloni *et al.*, 2013). According to Leung *et al.* (2001), theaflavins in black tea is effective as catechins in green tea. However, according to Pearson's correlations, tea samples were given significant correlations for only green tea samples. Green tea TPC and TFC results were well correlated with the FRAP, ABTS and DPPH assays result in this study.

FRAP measures the ability of compounds to act as electron donor (Chan *et al.*, 2007). Antioxidant activity measured by the FRAP assay had the highest results for the green tea followed by black tea samples. Therefore, green tea may contain a high number of compounds which can donor electrons. ABTS assay is a decolorization assay and it is applicable for quantifying both hydrophilic and lipophilic antioxidants (Alam *et al.*, 2013). However, according to the present study, used all the tea types had good antioxidant activity measured by the ABTS assay. However, green tea had the best activity followed by black tea and it was in agreement with the findings of Miller *et al.* (1996), Chan *et al.* (2007) and Yashin *et al.* (2011).

The DPPH assay is considered to be valid, rapid and easy (not involve with many steps and reagents) and inexpensive in comparison to other methods. It is a free radical scavenging colorimetric method for antioxidant property evaluation (Kodama *et al.*, 2010; Alam *et al.*, 2013). According to the DPPH assay, green tea had the highest activity followed by black tea. ORAC method is a physiologically significant assay (Kodama *et al.*, 2010) which determines the peroxy radical scavenging

capacities. According to the results of the ORAC assay, some black tea samples had higher antioxidant activities than green tea. Reasons for this may be the mechanism of the ORAC assay detected compounds of black tea than the compounds in green tea. Tea samples of China had high antioxidant activity measured by ORAC for green tea than black and oolong tea, according to a previous study (Zhao *et al.*, 2014).

### Comparative Analysis of the Results of FRAP, ABTS, ORAC and DPPH Assays

The unit of measurement used to quantify the antioxidant activity of tea samples was the same in FRAP, ABTS, ORAC and DPPH assays in the present research (mg TE/g tea sample). But, obtained antioxidant activity for the same sample using different assays was not given the same values. The main reason for this may be due to the different mechanisms involved in each assay. According to the antioxidant activity of fourteen black tea samples out of tested twenty samples measured by DPPH assay had the lowest values compared to other assays. Almost always results of the FRAP assay were in between other assays. According to the results of the study, different *in vitro* antioxidant assays had different results for each sample. As an example, Fanning's green tea had antioxidant activities measured by FRAP, ABTS, DPPH and ORAC assays as  $309.89 \pm 3.38$ ,  $208.44 \pm 4.59$ ,  $188.23 \pm 4.08$  and  $59.40 \pm 16.41$  mg TE/g tea sample respectively. Previous investigations also find that the different assays were given different antioxidant activities for the same sample (Kodama *et al.*, 2010; Zhao *et al.*, 2014; Bartoszek *et al.*, 2018).

The ORAC assay is a hydrogen donating assay while ABTS, FRAP and DPPH assays are electron transfer assays. Also, each different assays use different radicals such as peroxy radical, DPPH stable free radical, ABTS+ stable radical and Fe<sup>3+</sup>-TPTZ ion complex in ORAC, DPPH, ABTS and FRAP assay respectively. Due to these different antioxidant compounds measured by each assay may be different. Tea is reported to contain a lot of different antioxidant compounds (Yashin *et al.*, 2011). These different compounds may quantify using different assay methods. Therefore, the results of the different assays may differ from one another. Therefore, the antioxidant activity of a compound should not be concluded based on a single antioxidant assay. Due to the differences among each assay, it is difficult to compare fully one antioxidant test method to another (Alam *et al.*, 2013).

### Determination of Black Tea Grades' Effect on Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity

Overall, grade wise the order of activity of black tea for TPC assay was low country- Dust (I) > BOPF > BOPI > Pekoe > OPI; mid country- BOPF > OPI > BOPI > BOP > Dust (I) > Dust > Pekoe; up country- Pekoe > OPI > FBOPI > BOP > FBOP > Dust > BOPF > OPA. Grade wise the order of activity of black tea for TFC assay was low country- OPI > Dust (I) > BOPI > BOPF > Pekoe; mid country- BOPF > BOP >

Dust (I) > Dust > BOPI > OPI > Pekoe; up country- BOP > OPI > FBOP > OPA > BOPF > Dust > Pekoe > FBOPI. Grade wise the order of activity of black tea for FRAP assay was low country- BOPF > BOPI > Dust (I) > Pekoe > OPI; mid country- BOPF > Dust > OPI > Dust (I) > BOP > Pekoe > BOPI; up country- BOPF > Dust > Pekoe > FBOPI > OPA > BOP > FBOP > OPI. For the ABTS assay it was low country- Pekoe > Dust (I) > BOPI > BOPF > OPI > ; mid country - Dust > BOPI > BOPF > Dust (I) > Pekoe > BOP > OPI; up country- BOPF > OPI > Dust > OPA > FBOPI > Pekoe > BOP > FBOP. For the ORAC assay grade wise the order of activity of black tea was, low country- Dust (I) > Pekoe > BOPI > BOPF > OPI; mid country- Dust (I) > BOP > Pekoe > BOPI > BOPF > Dust > OPI; up country- OPI > OPA > BOP > FBOPI > Pekoe > FBOP > BOPF > Dust. For the DPPH assay it was low country- Dust (I) > Pekoe > BOPF > OPI > BOPI; mid country - BOPF > OPI > BOP > Dust > BOPI > Dust (I) > Pekoe; up country- BOPF > OPI > Dust > FBOP > Pekoe > BOP > FBOP > OPA. According to the above ranking, BOPF and Dust (I) tea grades were shown better activities than other grades in few assays. This may be due to the very small particle size of the Dust (I) grade may extract large amount of antioxidant compounds to the hot water. BOPF grade is a broken fannings grade, therefore it had relatively low particle size than the whole leaf grades and broken Pekoe grades. Although it had large particle size for OPI grade it also had higher activities may be due to the high-quality tea grade which produced mainly using immature tea leaves. Because previous investigation has found that immature tea leaves had highest antioxidant activity comparison with the mature leaves (Chan *et al.*, 2007; Izzreen & Fadzelly, 2013).

However, different assays had given different values so-called best tea grades due to the different mechanisms involved. For examples; TPC- mid-country Dust (I), BOPF and upcountry Pekoe, TFC- low country Dust (I) and OPI, mid-country BOPI, BOP and Dust (I), FRAP- Low country BOPF, BOPI, Dust (I) and Pekoe etc.

### Investigation of the Relationship of the Geographical Area of Black Tea on Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Tea.

Black tea samples were obtained for this study from up, mid and low countries of Sri Lanka to find out whether the different geographical areas affect on antioxidant activity of tea. Other than growing conditions, grades of manufactured tea are also related with the qualities of higher-grade black tea. Therefore, to compare the geographical areas of black tea same grades should have been used. At the time of this research, only available grades from different grades of black tea were used. Therefore, only three grades were available from three geographical areas of Sri Lanka (BOPF, OPI and Pekoe) and four grades were available from only two geographical areas (Dust (I), Dust, BOPI and BOP). Three tea grades were available only in up country of Sri Lanka (OPA, FBOPI, FBOP). According to the different assays best tea samples had in different geographical areas in different assays. Agro-climatic elevation wise rank order of potency for

BOPF grade was mid-country > up country > low country for TFC and ORAC assays, mid-country > low country > up country for TPC assay, low country > mid-country > up country for FRAP assay, low country > up country ≥ mid-country for ABTS assay and up country > mid-country > low country for ORAC assay. For OPI grade it was mid-country > up country > low country for TPC and ORAC assay. For the TFC assay it was up country > low country > mid-country, for FRAP assay mid-country > low country > up country, for ABTS assay low country > up country > mid-country and ORAC assay up country > mid-country > low country. For Pekoe grade it was, up country > low country > mid-country for TPC and ORAC assays. For TFC, FRAP and DPPH assays it was low country > up country > mid-country while for ABTS assay it was low country > mid-country > up country. Agro-climatic elevation wise rank order of potency for Dust (I) grade was low country > mid-country for all six bioassays while ranking concerning agro-climatic elevations for Dust grade was up country > mid-country for TPC and TFC assays and mid-country > up country for FRAP, ABTS, ORAC and DPPH assays. For BOPI grade it was mid-country > low country for TPC, ABTS and DPPH assays while for TFC, FRAP and ORAC assays it was, low country > mid-country. Agro-climatic elevation-wise rank order of potency for BOP grade was up country > mid-country for TPC, TFC and ORAC assays while mid-country > up country for FRAP, ABTS and DPPH assays.

When considering the results, there was not the same pattern for all the grades obtained from different geographical areas. As an example; according to the FRAP assay BOPF, Pekoe, BOPI and Dust (I) grades had the highest antioxidant activity in low country samples. OPI, BOP and Dust grades had the highest antioxidant activity in mid-country samples. According to the DPPH assay, BOPF grade had the highest activity in up country sample. It was not possible to determine whether the effects of the geographical area on antioxidant activity of black tea due to different tea grades and different mechanisms of the antioxidant assays.

### Determination of Correlations between TPC, TFC Results with FRAP, ABTS, ORAC and DPPH Assays Results

The primary natural antioxidants include flavonoids, phenols, oxiaromatic acids, vitamins C and E, carotenoids and other compounds (Namita et al., 2012; Lourenço et al., 2019). Results of antioxidant assays, TPC and TFC assays, it is possible to investigate whether there is any relationship between antioxidant activity and the total phenolic content and total flavonoid content. Positive high correlations for FRAP, ABTS, DPPH and ORAC assays results are accounts for the phenols or flavonoids are the reason for high antioxidant activity in tea samples. According to Pearson's correlations, tea samples were given significant correlations for only green tea samples. Green tea TPC and TFC results were well correlated with the FRAP, ABTS and DPPH assays results. According to the previous studies also TPC and TFC were well correlated with the results of the antioxidant assay (Ramirez-Aristizabal et al.,

2015). According to a previous study, high correlations had been observed in antioxidant capacities as well as total phenolic and flavonoid content of *C. sinensis*, when using 50% ethanolic extracts (Izzreen & Fadzelly, 2013).

## CONCLUSION

According to the findings of this study, Sri Lankan tea is rich in phenol and flavonoid compounds. Moderately high antioxidant activities were also observed for tea samples measured by the ABTS, FRAP, DPPH and ORAC assays. There were a lot of variations among different antioxidant assay results measured by different antioxidant assay methods. There were some relationships between total phenolic content, total flavonoid content and antioxidant activity with black tea grade and geographical area of the sample. However, due to other variations affecting these activities at the same time, it was not possible to derive a clear relationship. Of the antioxidant activities of black and green tea, green tea was the best followed by black tea. There were high correlations between TPC, TFC results with FRAP, ABTS and DPPH assay results for the only green tea sample.

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