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Polyphenolic contents of some instant tea brands and their anti-oxidant activities

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ABSTRACT

Tea which is one of the most widely consumed beverages after water, contains diverse polyphenols such as flavonoids and other catechins. These polyphenols confer anti-oxidant properties on tea with the capacity to slow down or prevent oxidation of other molecules. Ten instant tea brands were tested for purity and anti-oxidant properties. Qualitative tests for anti-oxidant activities were visualized by thin layer chromatography (TLC) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) spray. The extent of purity was determined by tests for extractable matter, ash values and moisture content whereas total polyphenolic content was determined by the colorimetric method using Folin-Ciocalteu reagent. Antioxidant activity was determined by DPPH free radical scavenging assay using rutin, vitamin C and gallic acid as standards. The test with TLC-DPPH spray showed that all ten brands had anti-oxidant components and the tea with the highest total phenolic content had a value of 360.62 ± 31.47mg GAE/g whereas the lowest was 150.76 ± 23.13mg GAE/g in contrast to 33.24 ± 15.89 and 6.47 ± 1.20mg RE/g for flavonoid content. The tea (black tea) with the most potent anti-oxidant activity had a potency value of 5.90 while the least potent (green tea) had a value of 4.20.The results are suggestive of good antioxidant properties of instant tea.

INTRODUCTION

Teas are reported to be the most widely consumed beverage after water. They are classified basically as green, black and oolong tea but they are all sourced from *Camellia sinensis*(L.) O. Kuntze, Family Theaceae. They, however differ in the way the leaves are processed. Oxidative processes by enzymes are minimized by steaming (Japan) or by panning (China) in green tea (Cloughley, 1983; Graham, 1984; Muktar, 2000). On the other hand, black tea leaves undergo crushing and fermentation. Oolong tea, found in some regions of China, is an intermediate variant between black and green tea (Graham, 1983). Polyphenolic compounds in the form of catechins have been reported to be present in green and black teas and studies on their anti-oxidant properties indicate that they contain up to 30% of the dry weight as phenolic content (Lin et al., 1998). The manufacture of instant tea is essentially carried out by exhaustive extraction of black tea with hot water (IARC WHO, 1991). The anti-oxidant capacity of phenolic compounds found in teas especially green tea has been associated with health benefits and this in turn has been regarded as an indicator of the quality. The study was conducted to determine the extent of purity, total phenolic, total flavonoid and anti-oxidant activities of ten instant tea brands commonly consumed within Jos metropolis, Nigeria.

MATERIALS AND METHODS

Collection of samples

The various brands of instant tea were purchased from the open market in Jos, Nigeria. Six (6) out of the ten (10) tea samples were black tea while the remaining were labelled as green tea.

Extraction of Polyphenols

The polyphenols from the extract were extracted according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 200 mg of each sample was weighed and transferred to an extraction tube containing 5 mL of 70% methanol at 70 ^{o}C. The instant tea was mixed and heated at 70 ^{o}C over water bath for 10 min.

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After cooling at room temperature, the extract was vortexed for 5 min and filtered into a 10 mL volumetric flask. The extraction procedure was repeated once and the extracts were combined together. The volume was then adjusted to 10 mL with cold 70 % methanol. One mL of the extract was diluted with water to 100 mL.

Thin layer chromatography

Rutin standard was prepared by dissolving 1 mg of rutin in 10 ml methanol. Tea samples (500 mg) were mixed with 5 ml of methanol and extracted by sonication for 10 min. After centrifugation, the supernatant was spotted on the TLC plate (20 x 20 cm, silica gel F_{254}). The plate was developed using the solvent system; ethyl acetate, acetic acid, formic acid and water in a ratio of 100:11:11:27 (www.camag-laboratory.com). DPPH reagent (0.05 %) was used to visualize the TLC plates.

Purity tests

The purity of the samples was determined by carrying out tests for extractable matter, ash value, and moisture content. These tests were carried out according to the WHO quality control methods for herbal materials (WHO, 2011).

Determination of extractable matter

This method determines the amount of active constituents extracted with solvents from a given amount of herbal material. It is employed for materials for which no suitable chemical or biological assay exists. 2.0 g of coarsely air-dried material was accurately weighed and placed in a glass stoppered conical flask. It was then macerated with 100 ml of water for 6 hrs, frequently shaken, and allowed to stand for 18 hrs. This was then carefully and rapidly filtered after which 25 ml of the filtrate was evaporated to dryness on a water bath. Further drying at 105˚C for 6 hrs, was followed by cooling in a desiccator for 30 min and weighing without delay. The content of extractable matter in mg/g of air-dried material was then calculated.

Determination of ash

For total ash, 2 g of the ground air-dried material was accurately weighed, in a crucible and ignited by gradually increasing the heat to 500˚C until it turned white indicating the absence of carbon. This was then cooled in a desiccator and weighed. For acid-insoluble ash, 25 ml of hydrochloric acid (70 %) was added to the crucible containing the total ash and boiled gently for 5 minutes. The watch glass used as a cover was rinsed with 5 ml of hot water and added to the crucible.

The insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 minutes, and then weighed without delay. The content of acid insoluble ash in mg /g of air-dried material was then calculated. For water-soluble ash, 25 ml of water was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature of 300 ˚C. The content of water soluble ash was then calculated in mg/g of air-dried material.

Determination of moisture content

Two (2) g of the tea sample was placed in an oven at 103˚C for six hours and cooled in a desiccator to prevent absorption of atmospheric moisture. Moisture content was calculated using the formula

$$\text{Moisture content (\%)} = \frac{(\text{Weight of air-dried sample} - \text{Weight of oven dried sample}) \times 100}{\text{Weight of dried sample}}$$

Determination of the total phenolic content

Total phenolic content of the extracts were evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent according to the method described in (Adedapo et al., 2008). Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing blue coloured complex. The phenolic concentration of instant teas was evaluated from a gallic acid calibration curve. 500µL aliquots of 10, 20, 30, 40, 50, and 60 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate.

The tubes were vortexed for 10 sec and allowed to stand for 2 hr at 25 ^{o}C. After incubation at 25 °C for 2 hr, absorbance was measured at 765 nm against reagent blank using the Shimadzu UV-Vis Spectrophotometer 1650 (Japan). Total phenolic content was expressed as mg gallic acid equivalent/gusing the following equation based on the calibration curve: $y = 0.0069x + 0.0673$, $R^2 = 0.9947$, where x was the absorbance and y was the gallic acid equivalent (mg/g) (Figure 2). A similar procedure was adopted for the extract as described above in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of tea.

Determination of total flavonoid content

The total flavonoid content of the instant tea extracts was measured by employing aluminium chloride colorimetric assay reported by Zhishen et al., (1999). An aliquot (1 mL) of extract (40mg) or rutin standard solution with the following concentrations (10, 20, 40, 60, 80 & 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask, 300 µL of 5% $NaNO_2$ and 300 µL of 10 % $AlCl_3$ were added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was brought to 10 mL by the addition of 2.4 mL H_2O. The solution was vortexed in order to mix the mixture thoroughly and the absorbance was measured at 510 nm against reagent blank using the UV-Vis Spectrophotometer 1650 Shimadzu, Japan. The total flavonoid contents of the tea extracts were expressed as mg rutin equivalents mg (RE)/g of tea extracts. All treatments were

carried out in triplicate. The results were calculated using the standard calibration curve of rutin in methanol (R^2 = 0.9957) and expressed as rutin equivalents (RE mg/g).

DPPH radical scavenging activity

The antioxidant activity (free radical scavenging activity) of the tea on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described in (Brand-Williams et al., 1995). The following concentrations of extract were prepared in methanol; 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/mL. 2 mL of each concentration was mixed with 4 mL of 50µM DPPH solution in methanol in triplicate. The mixture was vortexed for 10 sec to homogenise the mixture and test tubes were incubated for 30 min at room temperature in the dark and the absorbance was measured at 515 nm using UV-vis spectrophotometer (Shimadzu, 1620 Japan). Lower absorbance readings of the reaction mixture indicate higher free radical scavenging activity. Gallic acid, ascorbic acid and rutin were used as standards at the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391, & 0.195 µM. Blank solutions were prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solution in methanol. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation;

% inhibition = $100\ x\ (Abs\ control - Abs\ sample)/Abs\ control$

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

RESULTS AND DISCUSSION

The qualitative analysis using TLC separation and visualization by DPPH spray showed that all the tea samples contained compounds with antioxidant activity. This is attributable to the flavonoids present in the tea samples and was visualized as yellow spots against a purple background (Figure 1). The International Organization for Standardization (ISO, 1981) standard for black tea requires that the tea should be clean and reasonably free from extraneous matter (IARC WHO, 1991). The ash which remains after herbal materials are ignited can be determined by three different methods as total ash, acid-insoluble ash and water-soluble ash. The total ash method measures ash derived from the plant tissue itself and residue of extraneous matter such as sand and soil which adheres to the plant surface. Acid-insoluble residue measures the amount of silica present especially as sand and siliceous earth and water soluble ash gives the difference in weight after treatment with water (WHO, 2011). The values obtained from the determinations indicate the level of purity of the herbal preparation. The standard chemical requirements for extractable matter and ash values for black tea is 32 %, 4 – 8 % for total ash, 5 % for water soluble ash and 1% for acid insoluble ash (IARC WHO, 1991). The values of extractable matter for all the tea samples in this study were below the standard requirement of 32 % with values ranging between 16.25 – 27.5 % (Table 1).

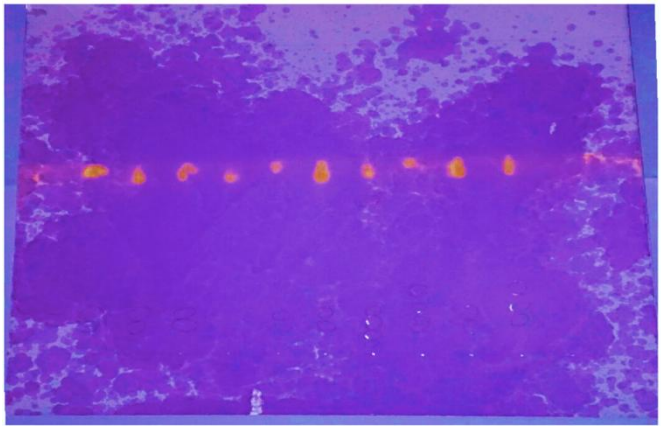

Fig. 1: screening for antioxidant activity of flavonoid containing compounds in instant tea (1-10).

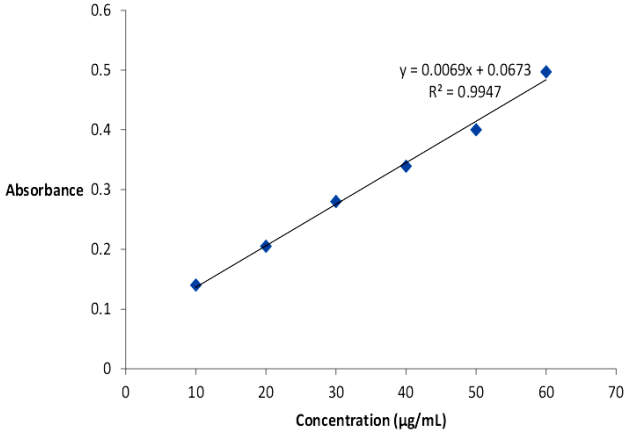

Fig. 2: calibration curve for standard gallic acid.

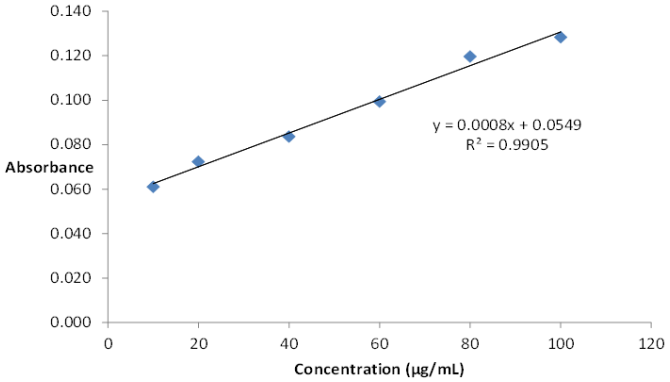

Fig. 3: calibration curve for standard rutin.

Only two of the black tea samples (2-B, London and 5-B, Sri Lanka origin) out of the ten tea brands met the requirement for total ash values of 4 – 8%. All the other tea samples had higher percentage values (Table 2). Tea brands, 2 and 5 (black tea) had water soluble ash values less than 5 % which was within

specifications while all the tea samples had acid-insoluble ash values above the recommended requirements (Tables 3 and 4).

Table 1: Extractable matter.

Tea sample	Weight of air-dried sample	Weight of extractable matter		Mean	% Weight of extractable matter
		1	2		
1-B	2	1.48	1.50	1.49±0.010	25.5
2-B	2	1.50	1.55	1.53±0.025	23.8
3-B	2	1.45	1.45	1.45±0.000	27.5
5-B	2	1.44	1.45	1.45±0.005	27.5
6-B	2	1.67	1.60	1.64±0.035	18.2
9-B	2	1.50	1.55	1.53±0.025	23.8
4-G	2	1.55	1.50	1.53±0.025	23.8
7-G	2	1.50	1.55	1.53±0.025	23.8
8-G	2	1.70	1.65	1.68±0.025	16.3
10-G	2	1.65	1.60	1.64±0.025	18.3

B- Black tea; G- Green tea

Table 2: Total ash values.

Tea samples	Weight of air-dried sample	Total ash values		Mean	%Total ash content
		1	2		
1-B	2	1.86	1.80	1.83±0.030	8.5
2-B	2	1.95	1.97	1.96±0.010	2.0
3-B	2	1.72	1.70	1.71±0.010	14.5
5-B	2	1.90	1.95	1.93±0.025	3.74
6-B	2	1.76	1.89	1.83±0.065	8.50
9-B	2	1.80	1.78	1.79±0.010	10.5
4-G	2	1.64	1.65	1.65±0.005	17.5
7-G	2	1.78	1.83	1.81±0.025	9.75
8-G	2	1.80	1.76	1.78±0.020	11.0
10-G	2	1.76	1.80	1.78±0.020	11.0

Table 3: Water-soluble ash.

Tea samples	weight of total ash	weight of water-soluble ash	%Water-soluble ash
1-B	1.86	1.80	3.2
2-B	1.95	1.81	7.2
3-B	1.72	1.66	3.4
5-B	1.90	1.82	4.2
6-B	1.76	1.69	3.9
9-B	1.80	1.64	8.9
4-G	1.64	1.51	7.9
7-G	1.78	1.59	10.8
8-G	1.80	1.55	13.8
10-G	1.76	1.50	14.8

Table 4: Acid-insoluble ash.

Tea samples	Weight of total ash	Weight of acid-insoluble ash	% Weight of acid-insoluble ash
1-B	1.80	1.75	2.8
2-B	1.97	1.92	2.5
3-B	1.70	1.65	2.9
5-B	1.95	1.89	3.1
6-B	1.89	1.81	4.2
9-B	1.78	1.71	3.9
4-G	1.65	1.51	8.5
7-G	1.83	1.70	7.1
8-G	1.76	1.71	2.8
10-G	1.80	1.73	3.9

The regulatory body in Nigeria, National Agency for Food and Drug Administration and Control (NAFDAC SOP, 2000) recommends a maximum value of 8% for moisture content however from the data, five of the tea samples (four black and one green) had less than 8% (Table 5). The values obtained for the samples compared to the chemical requirements show that some of the tea samples possibly contain extraneous matter. The total phenolic content ranged between 150.76 – 360.62 GAE mg/g while total flavonoid content was 10.02 – 33.24 RE mg/g. Total polyphenolic and flavonoid content was observed to be higher in black tea samples than green tea samples with values ranging from 288.73 ± 18.82 in tea 9 to360.62 ± 25.70 mg GAE/g in tea 2 while total flavonoid contents varied from 20.27 ± 1.80 in tea 9 to 33.24 ±2.36 mg RE/g (Table 6).

Table 5: Moisture content test.

Tea samples	Weight of air-dried sample	Weight of sample after oven drying		Mean	% Moisture content
		1	2		
1-B	2	1.8	1.9	1.85±0.05	7.5
2-B	2	1.8	1.9	1.90±0.05	5.0
3-B	2	1.8	1.8	1.80±0.00	10
5-B	2	1.8	1.8	1.80±0.00	10
6-B	2	1.8	1.9	1.85±0.05	7.5
9-B	2	1.9	1.9	1.90±0.00	5.0
4-G	2	1.7	1.9	1.80±0.00	10
7-G	2	1.8	1.9	1.85±0.05	7.5
8-G	2	1.8	1.8	1.80±0.00	10
10-G	2	1.8	1.8	1.80±0.00	10

Table 6: Total phenolic content (TPC) and total flavonoid content (TFC).

Brands of instant Tea	TPC (mg GAE/g tea)	TFC (mg RE/g tea)
1-B	323.52 ± 13.55	21.37 ± 1.53
2-B	360.62 ± 25.70	21.16 ± 1.70
3-B	360.23 ± 13.42	29.55 ± 2.84
5-B	319.85 ± 52.80	33.24 ± 2.36
6-B	358.30 ± 17.51	30.38 ± 0.30
9-B	288.73 ± 18.82	20.27 ± 1.80
4-G	195.60 ± 22.26	19.13 ± 0.04
7-G	150.77 ± 18.89	6.48 ± 0.98
8-G	205.26 ± 17.29	10.02 ± 1.09
10-G	195.21 ± 18.65	17.36 ± 3.33

TPC- total phenolic content, TFC- total flavonoid content, GAE- gallic acid equivalent, RE- rutin equivalent. The results are average of triplicate analysis (n = 3; data expressed as Mean ± SEM).

Table 7: DPPH radical scavenging activity (%) of ten tea samples.

	Brand of instant tea									
Conc. µg/mL	1	2	3	4	5	6	7	8	9	10
500	82.2	86.2	84.4	86.7	87.0	84.1	83.7	90.5	90.0	87.0
250	81.4	85.9	83.4	83.6	86.6	83.6	76.6	90.1	89.7	80.5
125	81.2	83.2	82.9	74.6	86.1	83.5	71.5	84.5	89.0	78.6
62.5	78.1	80.9	82.8	72.1	84.6	82.2	49.9	82.5	88.6	77.6
31.25	77.6	78.1	79.2	66.0	81.4	79.3	43.7	79.1	86.3	74.0
15.62	75.4	75.8	71.5	56.0	74.8	71.9	40.1	66.2	81.4	61.1
7.81	69.0	63.3	64.7	47.6	74.0	65.2	40.0	57.0	72.4	51.1
3.91	58.1	54.7	51.4	44.7	59.6	52.8	35.8	49.9	59.6	49.0
1.95	46.8	50.1	42.7	41.6	56.3	45.3	34.0	48.1	54.9	43.8
0.98	38.9	47.2	39.2	41.1	46.9	36.4	33.3	47.3	49.3	41.9
0.49	35.1	46.6	39.1	37.6	45.5	35.8	33.1	45.7	47.5	37.3
0.25	34.4	43.8	37.7	37.3	45.3	34.5	32.6	45.4	45.6	33.7
0.12	34.3	38.8	36.8	35.7	44.5	31.1	32.5	45.5	45.4	35.1

On the other hand, the green tea samples (4, 7, 8 and 10) had total polyphenolic content ranging from 150.77 ± 18.89 in tea 7 to 205.26 ± 17.29 mg GAE/g in tea 8 with total flavonoid content of 6.48 ± 0.98 in tea 7 to 19.13 ± 0.04 mg RE/g in tea 4 (Table 6). In comparing black tea samples to green tea samples, the highest polyphenolic content, 360.62 ± 25.70 mg GAE/g

occurred in tea 2 (black tea, London brand) while the least content was found in tea 7 (green tea, USA brand). Furthermore, the highest flavonoid content, 33.24 ± 2.36 mg RE/g was found in tea 5 (black tea, Sri Lanka brand) while the least flavonoid content, 6.48 ± 0.98 mg RE/g occurred in tea 7 (USA brand). DPPH radical scavenging activity of the tea samples were observed to be over 80 % at 500µg/mL whereas the reference antioxidant compounds gallic acid, rutin and ascorbic acid exhibited values between 75 – 92 % at 100 µg/mL (Tables 7 & 8). In Table 9, the antioxidant activity conducted with DPPH was also expressed as IC_{50}corresponding to a reduction of the absorbance of DPPH by 50 % and transformed into potency values (-log IC_{50}). A lower IC_{50} indicates a greater ability to neutralise free radicals (Pontis et al., 2014). The black tea samples (1, 2, 3, 5, 6, and 9) had IC_{50}values ranging from 1.26 to 3.98 while values for green tea samples (4,7, 8 and 10) ranged from 3.98 to 63.0. The results indicate that high polyphenolic and flavonoid contents in black tea samples (1, 2, 3, 5, 6, 9 of Sri Lanka, London and Nigeria brands) may account for stronger antioxidant activities compared to the green tea samples (Table 9).

Table 8: DPPH radical scavenging activity (%) of standards.

Conc. µg/mL	Gallic acid	Rutin	Ascorbic acid
100	92.12	89.27	75.20
50	91.93	82.20	81.00
25	92.49	60.41	83.60
12.5	92.02	52.43	85.50
6.25	71.58	46.55	86.10
3.12	53.97	45.63	86.60
1.56	48.39	43.47	86.70
0.78	43.67	44.12	73.40
0.39	40.56	44.50	50.70
0.19	39.57	45.04	42.00

Table 9: Potency of scavenging activity on free radical DPPH.

Brands of instant Tea	Country	IC_{50}(µg/mL)	Potency
1-B	Sri Lanka	2.34	5.63
2-B	London	2.45	5.61
3-B	Nigeria	3.98	5.40
5-B	Sri Lanka	1.35	5.87
6-B	Nigeria	3.16	5.50
9-B	Nigeria	1.26	5.90
4-G	France	10.00	5.00
7-G	USA	63.00	4.20
8-G	China	3.98	5.40
10-G	Nigeria	7.08	5.15
Rutin	Sigma, UK	75.30	4.12
Gallic acid	Sigma, UK	47.40	4.32
Ascorbic acid	BDH, UK	11.30	4.95

Potency = - Log (IC_{50})

Conversely, Anesini *et al.,* (2008) conducted a study on commercially available tea in Argentina where they reported that the polyphenolic content was higher in green tea bags than black tea bags. However, the results of this study are corroborated by previous reports of other investigators who observed that extracts enriched with polyphenols or flavonoids show higher antioxidant activity than those with lower phenolic contents (Wang et al., 2012). Also it has been reported that catechins and anthocyanins present in teas possess higher antioxidant activities than those of vitamin C and E as well as those of synthetic antioxidants such as butylated hydroxyl toluene , BHT (Kerio et al., 2013). In order to explore possible relationships between total polyphenolic contents as well as total flavonoid contents, the correlation values were determined by Excel program using potency values against TPC and TFC.

The linear correlation (r = 0.735) calculated for potency against TPC showed stronger positive correlation than for potency against TFC (r = 0.665) in all the tea samples. However, when the correlation was determined for black tea samples and compared to green tea samples, it was observed that there was stronger correlation of potency against TPC (r = 0.986) than against TFC (r = 0.508) for green tea samples while negative correlation was found for black tea samples which had higher antioxidant capacities (Table 10).

Table 10: Correlation of potency with TPC and TFC.

	TPC	TFC
Potency (10 tea samples)	0.735	0.665
Potency (6 black tea samples)	-0.870	-0.194
Potency (4 green tea samples)	0.986	0.508

CONCLUSION

The present study has established black tea samples of Nigerian and Sri Lankan origin have higher phenolic contents and the best antioxidant capacities compared to green tea samples based on IC_{50}values. Although, the total polyphenolic and total flavonoid content was higher in the black tea samples, no correlations with higher antioxidant properties were observed in this study instead there was strong correlation of potency against TPC in green tea samples. The potency of the tea samples suggests that instant tea has good antioxidant properties.

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