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Evaluation of *in vitro* antioxidant activities of selected commercial tea brands

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Abstract

The generation of reactive oxygen species requires the availability of antioxidants to ensure a balance between oxidants and prooxidants. Teas are commonly consumed due to their beneficial antioxidant effects. This study evaluated the *in vitro* antioxidant effects of aqueous and ethanol extracts of five selected commercial tea brands using ascorbic acid as the reference standard. The results showed that total antioxidant capacity (TAC) of aqueous extract of brand 1 was significantly lower ($p<0.05$) compared to brand 2 with half maximal inhibitory concentration (IC_{50}) values of 96.82 and 25.60 mg/mL respectively while the ethanol extract of all tea brands (IC_{50} values between 53.94 to 120.40 mg/mL) were significantly lower ($p<0.05$) to vitamin C with IC_{50} of 22.72 mg/mL. In the ferric ion reducing antioxidant property (FRAP), four of the tea brands expressed none significant FRAP activities with IC_{50} values between 12.84 and 14.97 mg/mL compared with vitamin C with IC_{50} values of 17.16 mg/mL, while the ethanol extract of the brand expressed none significant FRAP activities at higher concentrations. Hydrogen peroxide scavenging activities of aqueous and ethanol extracts of all tea brands were significantly lower ($p<0.05$) with IC_{50} values between 20.23 - 44.56 mg/mL and 30.53 - 36.01 mg/mL compared with vitamin C with IC_{50} values of 13.72 and 15.69 mg/mL respectively. Similarly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging property of aqueous extracts of all brands were significantly lower ($p<0.05$) compared with vitamin C with IC_{50} values of 12.92 mg/mL. Furthermore, the nitric oxide scavenging property of aqueous extracts of three brands (IC_{50} = 18.65, 24.69 and 24.80 mg/mL) were significantly higher ($p<0.05$) than vitamin C (IC_{50} = 37.75 mg/mL), while ethanol extract of three brands (IC_{50} = 12.81, 12.84 and 13.89 mg/mL) were significantly higher ($p<0.05$) than vitamin C (IC_{50} = 32.80 mg/mL). The results of the study indicated that the selected commercial tea brands possess *in vitro* antioxidant properties with potential benefits of the removal of free radicals and thus the prevention of oxidative stress.

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1.0 Introduction

Antioxidants are capable of stabilizing or deactivating reactive oxygen species (ROS) such as hydroxyl radical, ferryl ion, superoxide radical anion, peroxyl radical and hydrogen peroxide, which are induced by oxidative stress, before they later attack cells and biological targets. Antioxidants are therefore believed to be crucial for maintaining optimal cellular and systemic health and well-being (Rahman, 2007; Dufresne and Mukamal, 2008).

The consumption of tea is an old tradition (Wu and Wei, 2002). The plant *Camellia sinensis* L. is a well-known cultivated evergreen tea, native to China, and with subsequent spread to India and Japan, then to Europe and Russia, and to the work in the late 17th century (Sharangi, 2009). Several types of tea exist; they are available as black, green and other flavored teas. Research has focused on the effects of tea and tea polyphenols for lowering the risk of cardiovascular diseases and cancers (Dufresne and Mukamal, 2008). Their ability to reduce body fat, systolic blood pressure and lower Low Density Lipoprotein (LDL) cholesterol (Nagle *et al.,* 2000). Among age-associated pathologies and neurodegenerative diseases, green tea has been shown to confer significant protection against Parkinson's disease and Alzheimer's disease (Rezai-Zadeh *et al.,* 2005; Hu *et al.,* 2007). Consumption of green teas is beneficial as anti-inflammatory, anti-proliferative, enhanced weight loss, and anti-atherosclerotic activities, and thus recommended for their inclusion in dietetic supplements, nutraceuticals, and functional foods (Wu and Wei, 2002). This study evaluated the *in vitro* antioxidant activities of selected commercial tea brands.

2.0 Materials and Methods

2.1 Chemicals

Potassium ferricyanide, ammonium molybdate, naphthylethylenediamine, ascorbic acid, sodium nitroprusside, sodium azide, sodium hydroxide, tetraoxosulphate (VI) acid, sulphanilamide, sulphosalicylic acid, trichloroacetic acid, ethanol, KH_2PO_4, $MgCl_2$, KOH, and $ZnSO_4$ were purchased from Merck Pharmaceutical Company, Darmstadt Germany. Sodium nitrite, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and thiobarbituric acid were purchased from Sigma Chemical Company, St. Louis, Mo, USA. All chemicals used were of analytical grade.

2.2 Sample Collection and Preparation

Five varieties of commercial tea brands namely: Akbar, Beyond Comments, Top Tea, Vivo and Yellow Label were purchased from Akbar Brothers LTD, Sri-Lanka, Scirrocco Int Ltd, Promasidor, Nigeria, Brisbane, Australia and Unilever Plc, Nigeria respectively. The tea brand samples (40 grams each) after removing from their respective tea bags were weighed and dissolved in water (heated to 100^{o}C and allow to cool) and ethanol (no heating applied) for 24 hours and then filtered using a Whatman filter paper and concentrated at 45^{o}C using a rotary evaporator to obtain the aqueous and ethanol samples respectively.

2.3 Methods

The tea samples *in vitro* antioxidant activities were evaluated using the total antioxidant capacity (TAC) according to the method of phomolybdenum assay as described by Prieto *et al*. (1999). The ferric ion reducing antioxidant property (FRAP) was determined according to the method reported by Girgih *et al*. (2013) while Ruch *et al*. (1989) method for hydrogen peroxide (H_2O_2) scavenging activities was used to estimate the H_2O_2 scavenging activities of the tea brands. The *in vitro* scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by McCune and Johns (2002), while nitric oxide (NO) scavenging capacity was measured according to the method of Fiorentino *et al*. (2008).

2.4 Statistical Analysis

The data obtained were analyzed using one-way analysis of variance and the significant difference were separated using Duncan Multiple Range Test (SPSS version 20, SPSS Inc., Chicago. IL, USA) and presented as mean ± Standard Error of Mean (SEM). Significant levels were considered at $p < 0.05$. Graphs were generated using GraphPad Prism 6 software (GraphPad Software, California, USA).

3.0 Results

The total antioxidant capacity, ferric iron reducing antioxidant capacity, DPPH scavenging activities, hydrogen peroxide (H_2O_2) and nitric oxide (NO) in aqueous and ethanol extract and as well the half maximal inhibitory concentration (IC_{50}) of five tea brands are presented in Figures 1 - 10 and Tables 1 – 5. The results showed that, the TAC of aqueous extract of brand 2 - 4 were significantly higher ($p<0.05$) than vitamin C with IC_{50} values of 25.60, 25.21, 37.81 and 45.83 mg/mL respectively (Figure 1, Table 1) while the ethanol extract of all tea brands (IC_{50} values between 53.94 to 120.40 mg/mL) were significantly lower ($p<0.05$) to vitamin C with IC_{50} of 22.72 mg/mL (Figure 2, Table 1). In the FRAP antioxidant activities, brands 1, 2, 4 and 5 expressed none significant FRAP activities with IC_{50} values between 12.84 and 14.97 mg/mL compared with vitamin C with IC_{50} values of 17.16 mg/mL, brand 3 expressed IC_{50} value >100 mg/mL (Figure 3, Table 2), while the ethanol extract of brand 3 expressed none significant FRAP activities at higher concentrations with IC_{50} values of 21.92 mg/mL compared with vitamin C with IC_{50} values of 16.33 mg/mL respectively (Figure 4, Table 2). Hydrogen peroxide scavenging activities of aqueous and ethanol extracts of all tea brands were significantly lower ($p<0.05$) with IC_{50} values between 20.23 - 44.56 mg/mL and 30.53 - 36.01 mg/mL compared with vitamin C with IC_{50} values of 13.72 and 15.69 mg/mL respectively (Figure 5 - 6, Table 3). Similarly, DPPH scavenging property of aqueous extracts of all brands were significantly lower ($p<0.05$) compared with vitamin C (Figure 7, Table 4) except the ethanol extracts of brand 1 which expressed none significant difference ($p>0.05$) with IC_{50} values of 17.04 mg/mL

compared with vitamin C with IC_{50} values of 14.17 mg/mL (Figure 8, Table 4). Furthermore, the nitric oxide scavenging property of aqueous extracts of brand 2 (18.65 mg/mL), brand 3 (24.80 mg/mL) and brand 4 (24.69 mg/mL) were significantly higher ($p<0.05$) than vitamin C (37.75 mg/mL), while ethanol extract of brand 3 (12.81 mg/mL), brand 4 (12.84 mg/mL) and brand 5 (13.89 mg/mL) were significantly higher ($p<0.05$) than vitamin C (32.80 mg/mL) (Figure 9 – 10, Table 5).

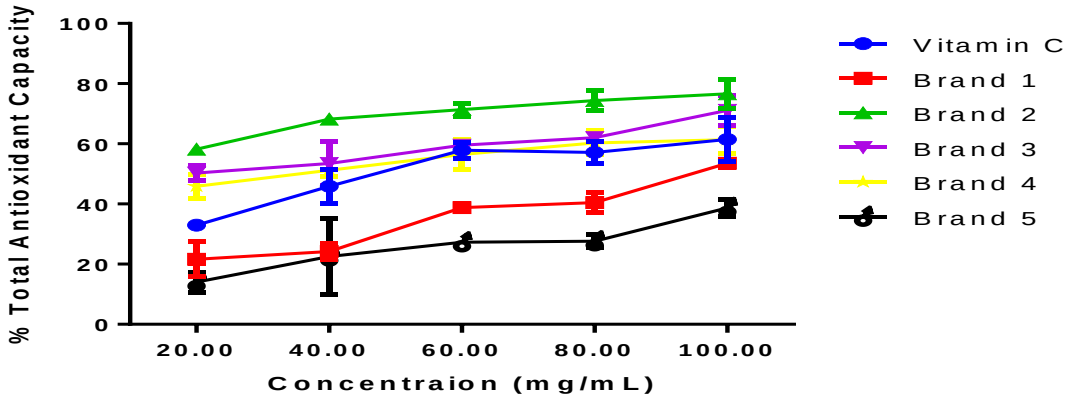

Figure 1: Total antioxidant capacity of aqueous extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

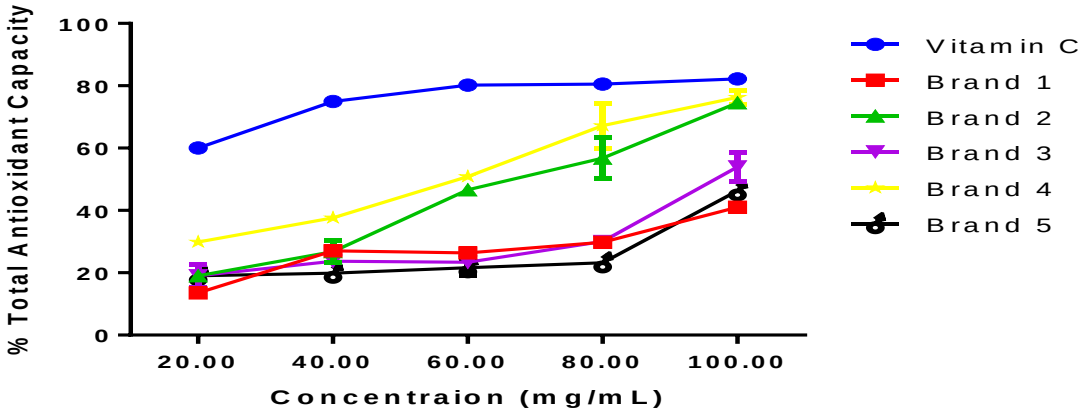

Figure 2: Total antioxidant capacity of ethanol extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

Table 1: IC_{50} values of total antioxidant capacity of aqueous and ethanol extracts of selected tea brands

	TAC IC_{50} (mg/mL)	
Sample	**Aqueous**	**Ethanol**
Vitamin C	45.83	22.72
Brand 1	96.82	>100.00
Brand 2	25.60	67.81
Brand 3	25.21	103.36
Brand 4	37.81	53.94
Brand 5	>100.00	120.40

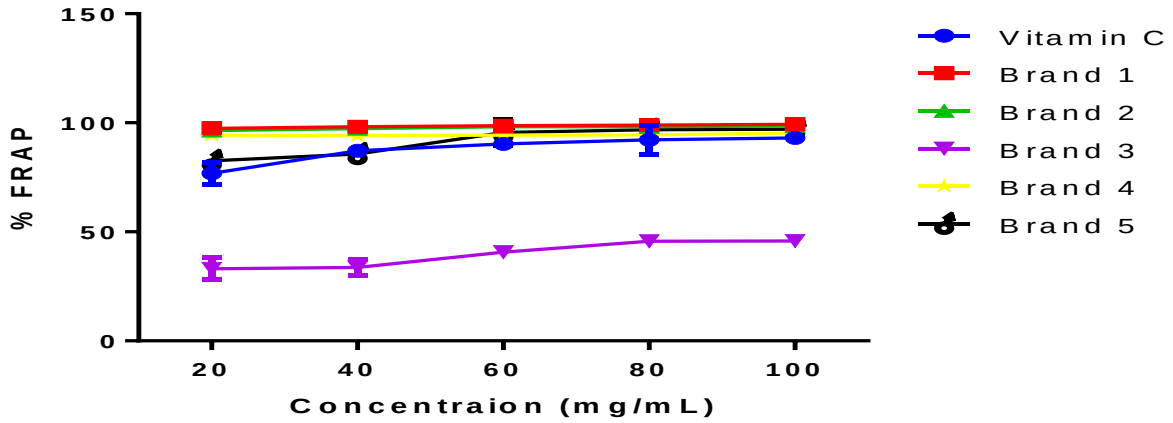

Figure 3: Ferric ion reducing antioxidant property of aqueous extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

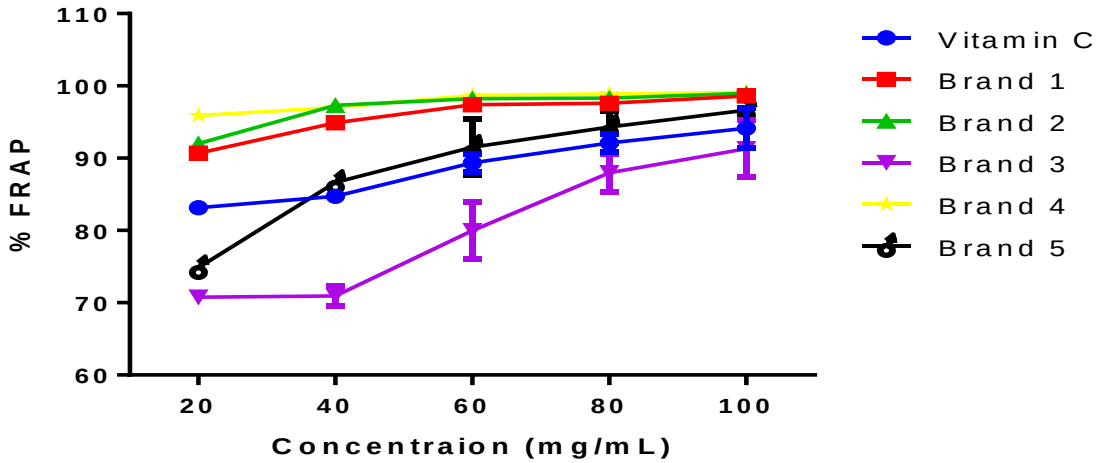

Figure 4: Ferric ion reducing antioxidant property of ethanol extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

Table 2: IC_{50} values of ferric ion reducing antioxidant property of aqueous and ethanol extracts of selected tea brands

	FRAP IC_{50} (mg/mL)	
Sample	**Aqueous**	**Ethanol**
Vitamin C	17.16	16.33
Brand 1	12.84	13.45
Brand 2	13.89	13.25
Brand 3	>100.00	21.92
Brand 4	13.36	12.98
Brand 5	14.97	17.43

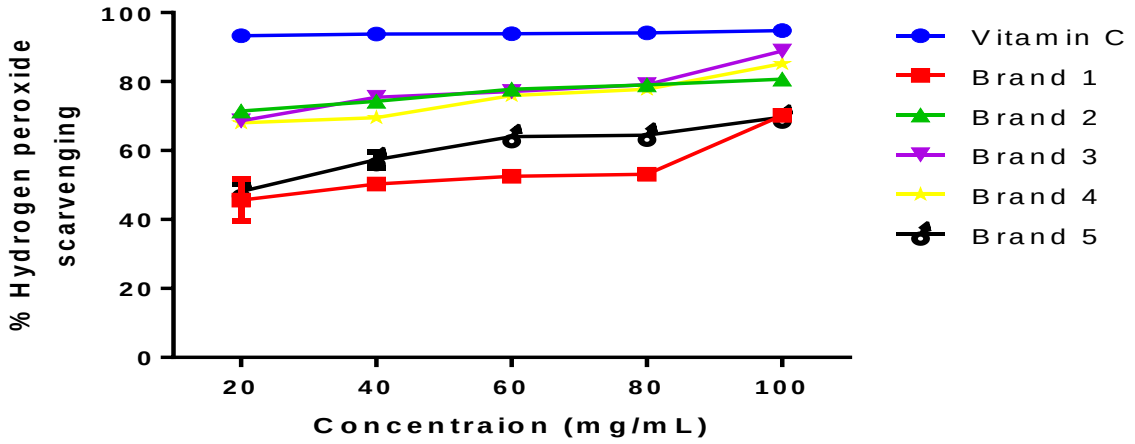

Figure 5: Hydrogen peroxide scavenging property of aqueous extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

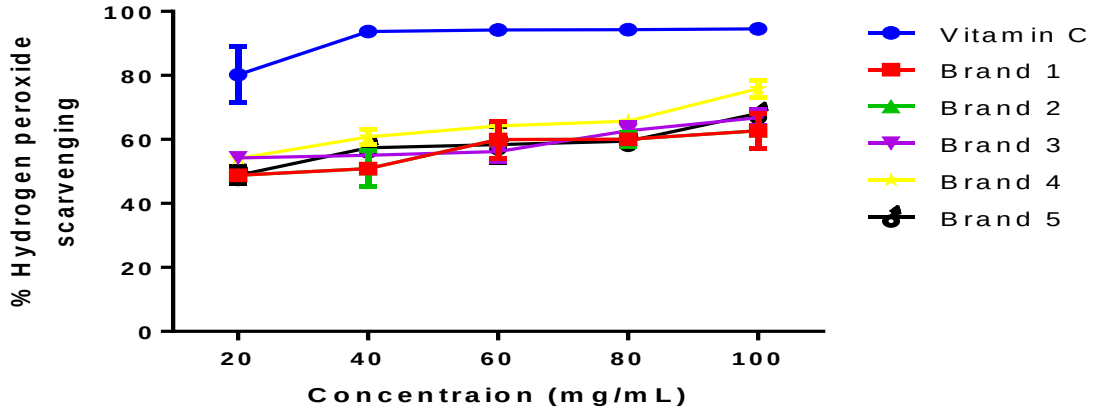

Figure 6: Hydrogen peroxide scavenging property of ethanol extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

Table 3: IC_{50} values of hydrogen peroxide scavenging property of aqueous and ethanol extracts of selected tea brands

	H_2O_2 IC_{50} (mg/mL)	
Sample	**Aqueous**	**Ethanol**
Vitamin C	13.72	15.69
Brand 1	44.56	36.01
Brand 2	20.23	35.76
Brand 3	21.72	34.40
Brand 4	22.61	30.53
Brand 5	32.33	34.48

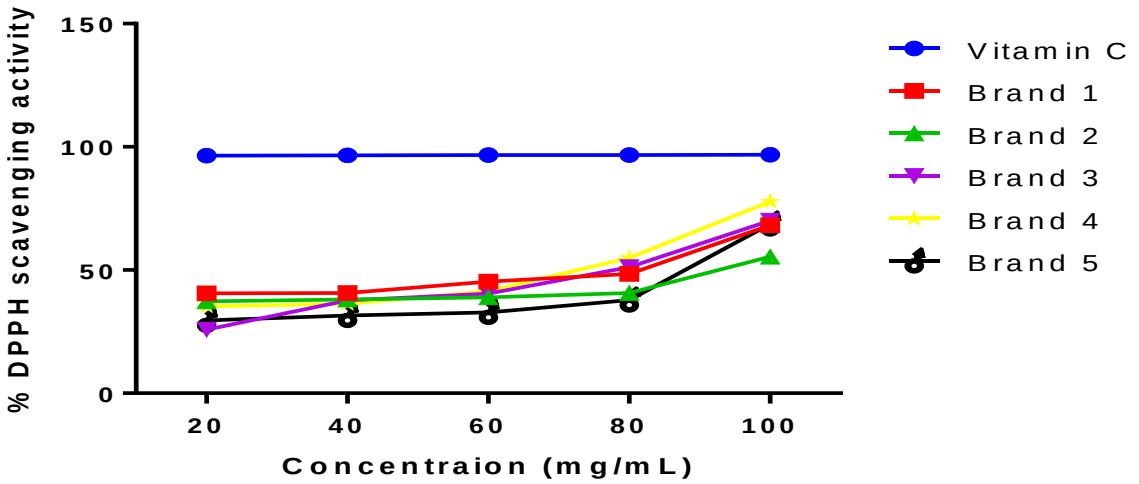

Figure 7: DPPH scavenging property of aqueous extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

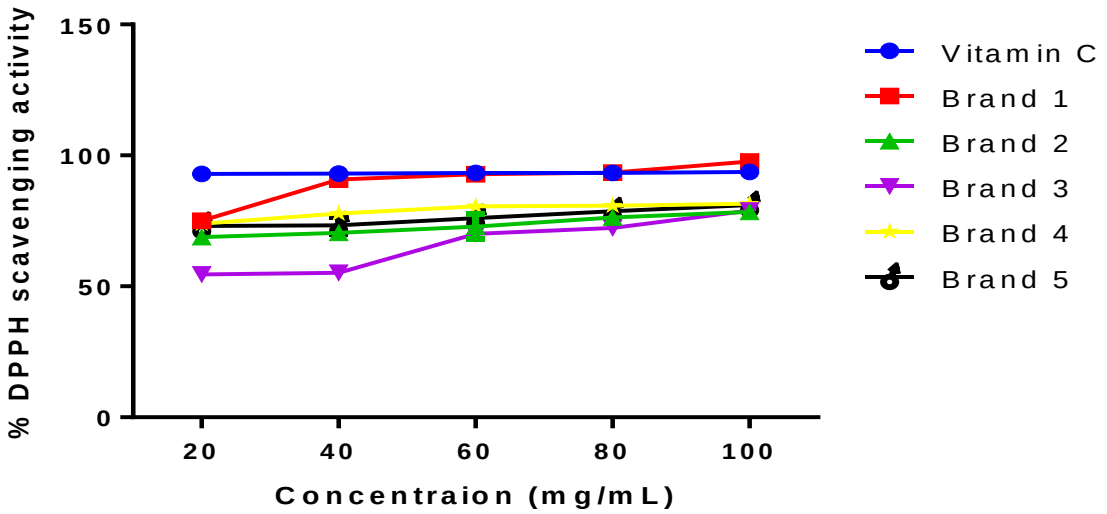

Figure 8: DPPH scavenging property of ethanol extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

Table 4: IC_{50} values of DPPH scavenging property of aqueous and ethanol extracts of selected tea brands

	DPPH IC_{50} (mg/mL)	
Sample	**Aqueous**	**Ethanol**
Vitamin C	12.92	14.17
Brand 1	60.67	17.04
Brand 2	101.53	21.92
Brand 3	68.26	30.28
Brand 4	63.30	18.93
Brand 5	82.43	20.48

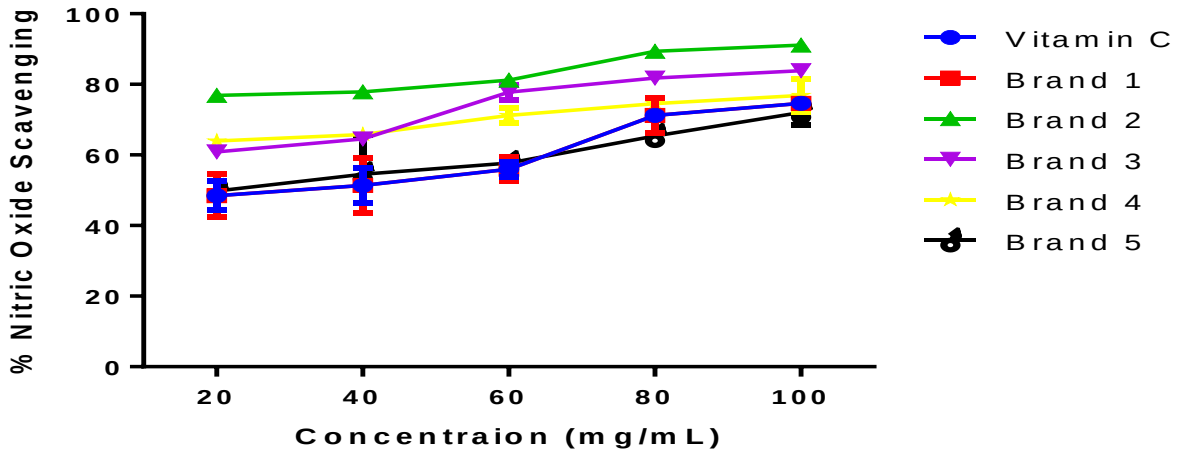

Figure 9: Nitric oxide scavenging property of aqueous extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

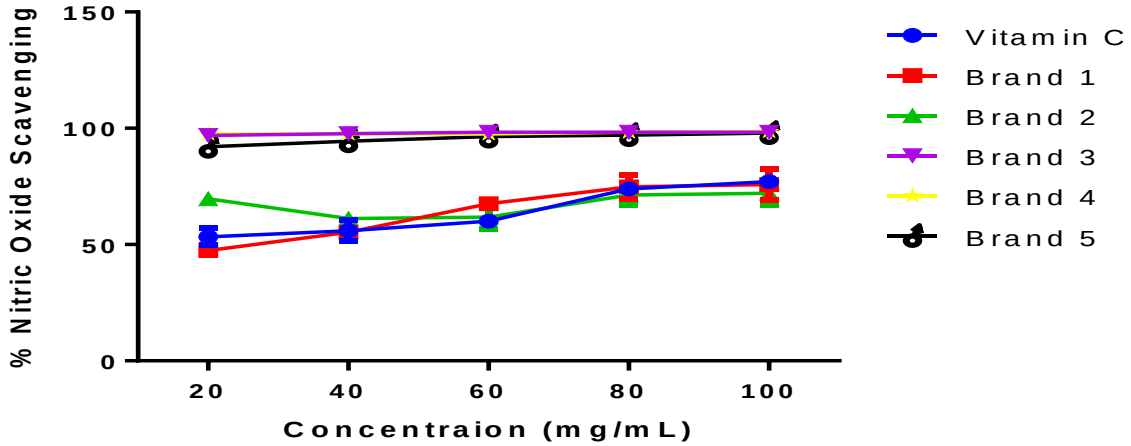

Figure 10: Nitric oxide scavenging property of ethanol extracts of selected tea brands. Values are mean ± Standard Error of Mean (SEM) of triplicate determination.

Table 5: IC_{50} values of nitric oxide scavenging property of aqueous and ethanol extracts of selected tea brands

	NO IC_{50} (mg/mL)	
Sample	**Aqueous**	**Ethanol**
Vitamin C	37.75	32.80
Brand 1	38.30	32.10
Brand 2	18.65	25.60
Brand 3	24.80	12.81
Brand 4	24.69	12.84
Brand 5	36.89	13.89

4.0 Discussion

Teas are among the most popular beverages consumed globally. They are available as black (fully fermented), green (unfermented), or oolong (semi-fermented) tea (Chan *et al*., 2011). The methods for processing these teas are different. In green tea, the leaves for its preparation are harvested fresh and steamed immediately to prevent fermentation and provide a dry and stable green tea (Chacko *et al.,* 2010). These techniques destroy the enzyme required for colour pigment degradation in the leaves and preserve natural components of the teas, such as polyphenols (Chacko *et al.,* 2010).

The benefits of tea consumption are not only limited to the recreation and relaxation they provide. The benefits of teas also include their health benefits; including antiangiogenic, antiarthritic, antibacterial, anticancer, anti-inflammatory, antioxidative, antiviral, prevention of cardiovascular diseases, their cholesterol-lowering effects and neuroprotection (Vanessa and Gary, 2004; Roomi *et al.,* 2007; Unno *et al.,* 2007; Chacko *et al.,* 2010).

Medicinal teas are made up of different components just as herbal medicines. These components act synergistically to ensure a desired beneficial effect (Raederstorff *et al.,* 2003). Tea are rich in proteins, carbohydrates, minerals and trace elements, lipids, vitamins, carotenoids, flavonoids and volatile compounds (Graham, 1992; Belitz and Grosch, 1997; Ifemeje *et al*., 2020). However, most of the tea brands used in this study do not contain sugars, fat, fiber or sodium, with considerable amount of energy and low proteins in their nutritional information. More so, flavonoids are among the phytoconstituents ready available from the consumption of these tea brands. Studies have demonstrated that tea catechins, a type of flavonoids (Tijjani *et al.,* 2018) provides protection against degenerative diseases (Vanessa and Gary, 2004). Catechins from green tea possess antitumorigenic properties (Roomi *et al.,* 2007) and could act as immune modulators in immunodysfunction from carcinogen treatment or transplanted tumors (Vanessa and Gary, 2004).

In the present study, the aqueous tea brands expressed higher *in vitro* radical scavenging properties compared with the ethanol extracts of the tea brands. However, their activities were significantly lower compared with vitamin C in hydrogen peroxide and DPPH scavenging activities. The results indicate that the tea brands express beneficial antioxidant activities, which are similar to reports of Babu *et al*. (2006), on therapeutic benefits of green tea extracts on oxidative stress. Other beneficial properties of tea (green) extract in areas of drug metabolizing enzymes, intestinal metal absorption, in diseases such as diabetic mellitus, and in health of both humans and animals have been reported (Chacko *et al.,* 2010).

The tea brands are thus recommended due to their antioxidant benefits. Excessive/overconsumption of some tea is discouraged in some health conditions including cardiovascular problems, pregnancy or breastfeeding (Bruneton, 2001). Concomitant consumption of green tea requires monitoring during the use of some drugs (Bruneton, 2001). The harmful effects are attributed to some factors including the presence of some phytochemicals such as caffeine and tea polyphenols, which affects iron bioavailability (Chacko *et al.,* 2010).

5.0 Conclusion

The results of the study indicated that the selected commercial tea brands possess *in vitro* antioxidant properties with potential benefits of the removal of free radicals and thus the prevention of oxidative stress.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

All data are presented in the report.

Competing interests

All authors declare no competing interests.

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Reference

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