Comparative Analysis of the Phytochemical Content and Antioxidant Capacities of Five Pure Green Tea Brands

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ABSTRACT

Background: *There are different brands of tea in the market with diverse claims on their antioxidant potentials. It is necessary*
to verify these claims and help consumers make informed choices.

Objectives: *This study was undertaken to analyze and compare the phytochemical composition and antioxidant capacity of five brands of pure green teas (coded: GTQ, GTL, GTN, GTLp and GTT) sold in Nigeria.*

Methods: *Five brands of pure green tea were purchased and infused to obtain their extracts. The extracts were screened for phytochemical content using standard methods and their in-vitro antioxidant capacities were also determined using 1, 1- diphenyl-2- picrylhydrazyl radical (DPPH.), nitric oxide (NO.) and total antioxidant capacity assays.*

Results: *The result indicated that all the tea samples recorded the presence of phenolic compounds, GTQ and GTT recorded the highest and lowest total phenol content respectively. All the samples differed significantly ($p < 0.05$) in their flavonoid content. The tannins content of GTQ (8.76±0.07 mg/100g) and GTL (8.78±0.06 mg/100g) did not differ significantly ($p > 0.05$). The alkaloid content of GTQ (527.33±21.93) was significantly ($p < 0.05$) higher than the others, except GTN. GTQ recorded the highest steroids (0.42±0.02 mg/100g). The results showed the tea samples scavenged 1, 1- diphenyl-2-picrylhydrazyl radical (DPPH.) and nitric oxide (NO.). They also possessed strong total antioxidant capacity. However GTL was more potent among all the tea samples.*

Conclusion: *All the green tea samples processed strong phytochemical content and were potent in scavenging free radicals.*

Keywords: Green tea, Phytochemicals, Antioxidant capacity

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INTRODUCTION

Green tea (*Camellia sinensis*) is known to be one of the most frequently consumed beverages in the world, especially in Asian countries (1). *Camellia sinensis* plant is presently cultivated in more than thirty nations of the world (2), including Nigeria. Green tea is one of the different classes of teas made from the leaves of *Camellia sinensis* plant. It is considered to possess the most effective antioxidant activity among the other tea types (3). This is because it is processed without fermentation. The processing involves a method known as fixing which deactivates enzymes in leafs to prevent oxidation, fermentation and to maintain the tea's green colour. Green tea as a natural herb is gaining great popularity and there has been tremendous increase in its consumption

within the recent years owing to its high antioxidants concentration.

Low incidences of various pathological conditions including cardiovascular disease, diabetes, obesity and cancer may be associated with green tea consumption (4). These benefits are believed to be attributed to their phytochemical content of which majority are polyphenolic compounds (4). Plants are known to have medicinal values because of their phytochemicals composition which produce definite physiological actions in the human body. Alkaloids, tannins, flavonoids and phenolic acids are considered as most important among the other phytochemicals (5).

Antioxidant activity is one of the major and important mechanisms by which phytochemicals exert their protective action. This is because they are able to scavenge free radicals in a system (6). Free radicals are electrically charged molecules which contain unpaired electrons on their orbit. The presence of the unpaired electrons in free radicals makes then highly reactive and unstable and are capable of damaging essential biological molecules such as DNA, proteins, carbohydrates, and lipids in the nucleus and in the membranes of cells (7).They attack healthy cells of the body and cause them to lose their structure and function. The production of free radical occurs continuously in all cells as part of normal cellular function. However, excessive free radical production leads to oxidative damages which have been implicated in the development of many diseases (7).

In order to cope with the oxidative damages, the body cells have developed a complex antioxidant defense system. However, the antioxidant defense system may be outweighed by various pathological or environmental factors. This necessitates the need to supply other exogenous sources of antioxidants through diet. Green tea is believed to be a good source of the phenolic compounds which have antioxidant properties. There has been a proliferation of green teas in the market with different claims hence the objective of this present study is to compare the antioxidant capacity of different brands of pure green tea in the market and establish the brand with the highest antioxidant effects.

METHODS

Procurement of samples

Five different brands of pure green tea by different companies were purchased from Ogige market, Nsukka in Enugu state, Nigeria. The samples were coded: GTQ, GTL, GTN, GTLp and GTT. They were transported to Shalom Laboratories at No. 157, Onuiyi road, Nsukka, Enugu state the laboratory where they were investigated for their phytochemical content and antioxidant capacity using standard procedures.

Tea Infusion

Two grams of the tea in tea bags from each brand was weighed and dipped into 100mls of distilled water of 80°C for 5 minutes to obtain the extracts. The extracts were then subjected to phytochemical determination and antioxidant assays.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Determination of Total Phenolic content (TPC)

The TPC of the extract was determined by the Folin–Ciocalteu method (8). One gram of the sample was macerated with 20 ml of 80 % ethanol for 10 minutes and was centrifuged for 5 minutes. Two ml of supernatant was transferred into triplicate tubes, 3 ml of water was added, 0.5 ml of Folin–Ciocalteu reagent was mixed, allowed to stand for 5 minutes, 2 ml of 20 % sodium carbonate mixed was added and allowed to stand for 30 minutes. The absorbance was taken at 760 nm.

Determination of Flavonoids

This was done using the method of Harborne (9). One gram of the sample was macerate with 20 ml of ethyl acetate for 10 minutes and centrifuged for 5 minutes. Five ml of the supernatant was transferred into triplicate tube, shaken vigorously between 2 to 5 minutes. It was centrifuged for 5 minutes, and the upper layer was discarded. The absorbance of the lower layer was taken at 470 nm.

Determination of Tannins

This was done using the method of Harborne (9).

One gram of the sample was macerated with 20 ml of methanol for 10 minutes, centrifuged for 5 minutes and 2 ml of supernatant was transferred into triplicate tubes, 3 ml of methanol, 0.3 ml of 0.1 M Ferric chloride was added in 0.1 M HCL and mixed and 0.3 ml of 0.0008 M Potassium ferricyanic was also added, mixed and the absorbance was taken against blank at 720 nm after 5 minutes but below 30 minutes.

Determination of Alkaloids

This was done using the method of Harborne (9). One gram of the sample was macerated with 10 ml of ethanol and 10 ml of 20 % surphuric acid for 10 minutes, centrifuged for 5 minutes. Into triplicate tube, 0.5 ml of the supernatant was transferred, 2.5 ml of 60 % surphuric acid was added and also 2.5 ml of 0.5 % formaldehyde in 60 % surphoric acid, mixed and allowed to stand for 3 hours, the absorbance at 565 nm was taken against the blank.

Determination of Steroids

This was done using the method of Harborne (9). One gram of sample was macerate with 20 ml petroleum ether for 10 minutes and allowed to stand for 1 hour with intermittent shaking every 10 minutes, centrifuged for 5 minutes. Two ml of the supernatant was transferred into triplicate tubes, evaporated to dryness, 2 ml of alcoholic potassium hydroxide will be added, boiled for 30 minutes, cooled. Three ml of petroleum ether was added and shaken for 2 minutes, centrifuged for 5 minutes. Two ml of the upper layer was transferred into another test tube. Evaporated to dryness, cooled, 2 ml of ethanol will be added to dissolve the residue, 2 ml of steroid color reagent was also added, shaken vigorously and allowed to stand for 30 minutes. The absorbance was taken at 550 nm against the blank.

***In- vitro* Antioxidant Capacity Analysis**

1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging activity assay

The free radical scavenging activity of the extract was analyzed by the DPPH photometric assay as described by Mensor et al. (10) using a spectrophotometer. The extract (2 ml) at concentrations of 20, 40, 80, 160, 320 and 640

Nitric oxide scavenging ability assay

This was carried out by the method described by Jaiswal et al. (11). The nitric oxide scavenging activity of the extract was conducted based on Greiss assay method. Sodium nitroprusside (2.0 ml of 10 mM) and 5.0 ml of phosphate buffer was mixed with 0.5 ml of different concentrations (20, 40, 80, 160, 320 and 640 μg/ml) of the extract and incubated at room temperature for 150 minutes. After the incubation period, 2 ml of the incubated solution was added to 2 ml of Greiss reagent (1%sulphanilamide, 0.1%

$$\% \text{ inhibition} = 100 - \left(\frac{\text{ABS sample} - \text{ABS blank}}{\text{ABS control}} \times 100\right)$$

The EC_{50} (the effective concentration of the teas that is able to scavenge 50% of a radical) of the samples against DPPH and NO were calculated using equations generated after plotting the graphs of the different concentration against the inhibition percentages.

Total Antioxidant Capacity (TAC) assay

The total antioxidant capacity assay of the extract was carried out by the phosphomolybdate method according to a method described by Prieto et al. (12). A zero point three milliliter (0.3ml) aliquot of different concentrations (20, 40, 80, 160, 320 and 640 μg/ml) of the extract and ascorbic acid were mixed with 3ml of reagent solution (600mm sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate, 1:1:1). The test tubes were covered with aluminum foil and incubated in a water bath at 95°C for 90 minutes. Later, extract was cooled to room temperature, the absorbance of the mixture was determined at 765nm against a blank containing 1ml of the reagent solution. Ascorbic acid was used as standard. The Total Antioxidant Capacity (TAC) is expressed as equivalents of ascorbic acid. The TAC will be estimated using:

$$\% \text{ inhibition } \frac{A_{Control} - A_{Sample}}{A_{Control}} \times \frac{100}{1}$$

A_0 = Absorbance of control

A_S = Absorbance of extract or sample

TAC values of the samples were obtained by taking the means of the inhibition percentages caused by the different concentrations of the samples

$$TAC\ (\%) = \frac{A_0 - A_S}{A_0} \times \frac{100}{1}$$

Data Analysis

The data obtained were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 23 from windows. Descriptive statistics (mean and standard deviation) was used to describe the data. The mean values were compared using Analysis of Variance (ANOVA) and turkey test. Statistical significance was accepted at $p<0.05$.

RESULTS

Table 1 indicates the mean quantitative phytochemical composition of the tea samples. The result indicated that GTQ had the highest (4835.48±73.28 mg/100g) and GTT had the lowest (1590.32±164.52 mg/100g) TPC. However, the TPC of GTL and GTN were not significantly ($p > 0.05$) lower when compared to that of GTQ. All the samples differed significantly ($p < 0.05$) in their flavonoid content. The tannins content of GTQ and GTL did not differ significantly ($p > 0.05$) while a significant ($p < 0.05$) difference occurred among samples. GTN, GTL_P and GTT. The alkaloid content of GTQ was significantly ($p < 0.05$) higher than that of GTL, GTL_P and GTT but was not significantly ($p > 0.05$) higher than that of GTN. GTQ recorded the highest steroids composition (0.42±0.02 mg/100g) which was significantly ($p < 0.05$) higher than the steroid contents of the other samples.

Values in the same column bearing different superscript letters were significantly different ($p < 0.05$) while values in the same column bearing the same superscript letters were not significantly different ($p>0.05$).

Antioxidant activity of GTQ, GTL, GTN, GTLp, GTT against 1,1 Diphenyl-2-Picrylhydrazyl (DPPH) radical.

Figure 1 shows the EC_{50} values of the extracts against DPPH. The result recorded the EC_{50} of the five samples as follows: GTQ; 8.73 ± 0.31 μg/ml, GTL; 6.79± 0.18 μg/ml, GTN; 8.71± 0.83 μg/ml, GTL_P; 7.78 ± 0.77μg/ml, GTT; 6.80± 0.18 μg/ml, ASC; 6.10± 0.053μg/ml, It was also observed that GTL had the least EC_{50} (6.79± 0.18 μg/ml) while GTQ recorded the highest EC_{50} (8.73 ± 0.31 μg/ml).

Antioxidant activity of GTQ, GTL, GTN, GTLp, GTT against nitric oxide (NO) radical.

Figure 2 shows the EC_{50} of the five green tea samples against nitric oxide radical. The result showed that the samples obtained the following EC_{50} values: GTQ; 61.99±0.22, GTL; 59.95±1.85, GTN; 69.66±1.70, GTLp; 81.66±4.82, GTT; 322.85±13.82 and ASC; 21.09± 0.66μg/ml. GTT recorded the highest EC_{50} value (322.82μg/ml) which was significantly higher ($p<0.05$) than the other samples while GTL had the least EC_{50} value (59.95±1.85 μg/ml) when compared to the other green tea samples.

Total Antioxidant Capacity of GTQ, GTL, GTN, GTL_P and GTT

Figure 3 indicates the Total Antioxidant Capacity in Ascorbic acid Equivalent (AAE) of the green tea samples. The figure shows that the mean TAC values of the samples are GTQ; 103.03 AAE, GTL; 98.36 AAE, GTN; 134.63 AAE, GTL_P; 142.06 AAE, GTT; 105.63 AAE. It can be deduced from the figure that GTL_P had the highest TAC value (142.06 AAE) while GTL had the least TAC value (98.36 AAE).

Table 1: Quantitative phytochemical composition of GTQ, GTL, GTN, GTL_P, GTT (mg/100g)

Green teas	Total Phenols	Flavonoids	Tannins	Alkaloids	Steroids
GTQ	4835.48±73.28^{c}	39.00±0.25^{c}	8.76±0.07^{a,b}	527.33±21.93^{c}	0.42±0.02^{c}
GTL	4720.43±102.07^{c}	22.83±1.42^{b}	8.78±0.06^{a,b}	305.00±50.83^{b}	0 .29±0.06^{b}
GTN	4801.07±135.34^{c}	160.75±3.90^{e}	9.30±0.40^{c}	480.28±25.60^{c}	0.33±0.02 ^{b}
GTL_P	4429.03±145.26^{b}	12.63±1.44^{a}	8.84±0.34^{b}	303.06±40.35^{b}	0 .18±0.01^{a}
GTT	1590.32±164.52^{a}	72.38±1.07^{d}	8.43±0.10^{a}	200.56±2.65^{a}	0.17±0.03^{a}

Mean ±SD (n=3)

Values in the same column bearing different superscript letters were significantly different ($p < 0.05$) while values in the same column bearing the same superscript letters were not significantly different ($p>0.05$).

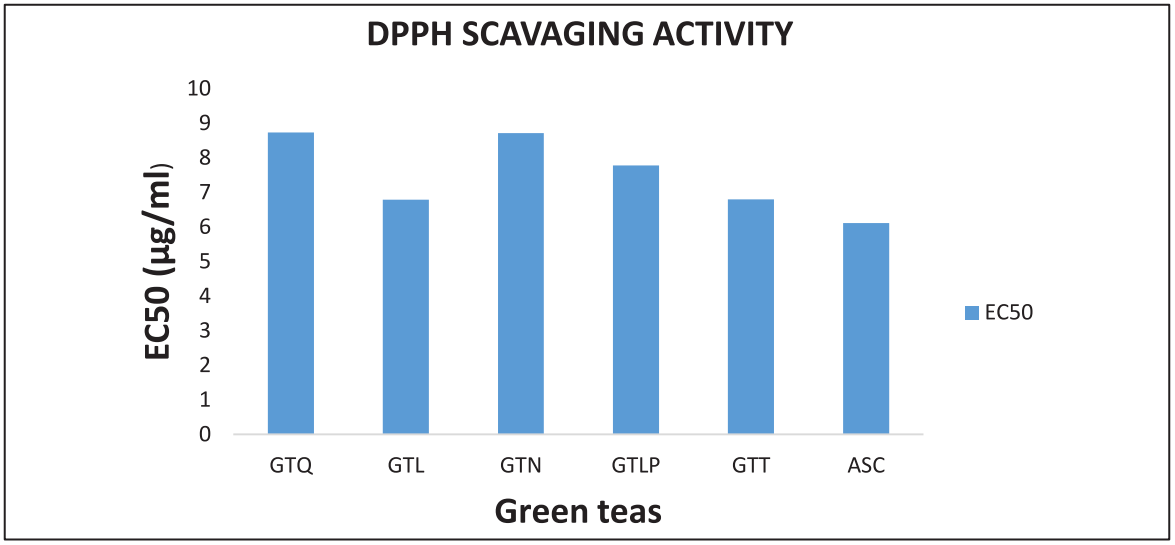

Figure 1: The antioxidant activity of Green teas against DPPH radical

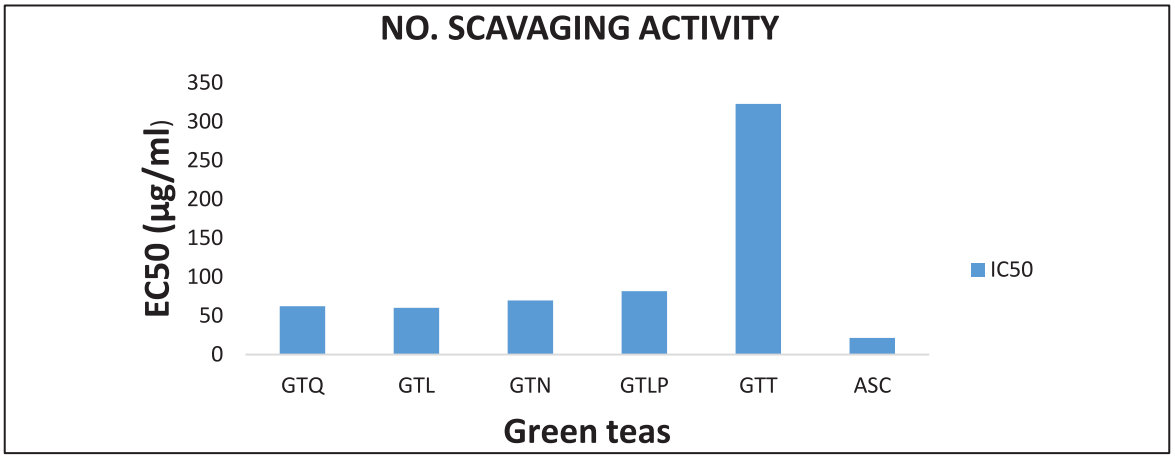

Figure 2: The antioxidant activity of Green teas against NO. radical.

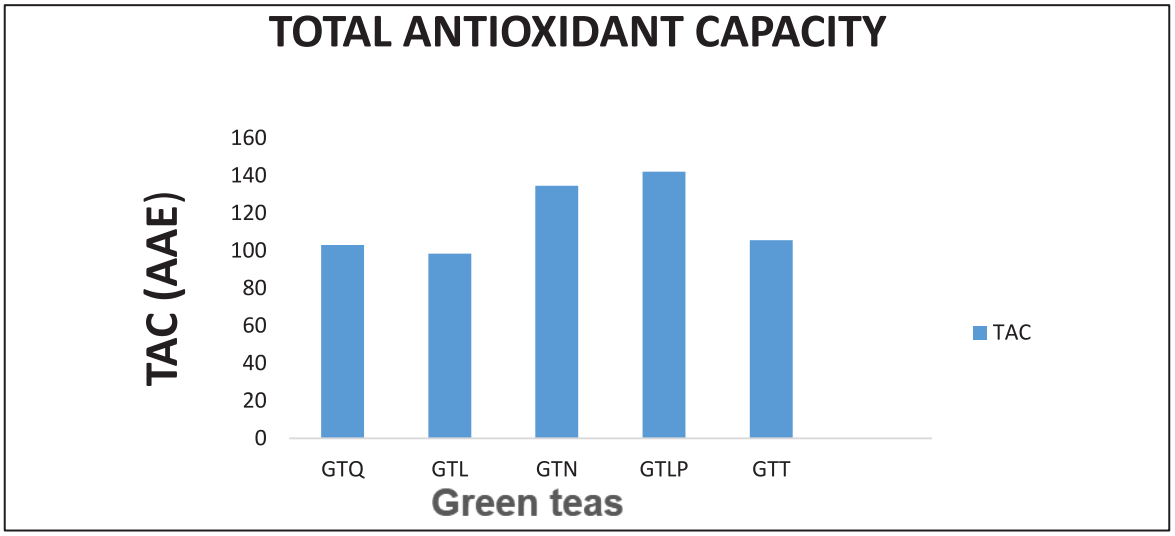

Figure 3: The Total Antioxidant Capacity of Green tea samples.

DISCUSSION

Phenols were the most abundant phytochemicals present in the green teas and ranged from 1590.32±164.52 mg/100g in GTT to 4835.48±73.28 mg/100g in GTQ. The result of the TPC found in this work was similar to the result of a work carried out by Olatidoye et al. (13). They recorded the TPC of green tea to be (4993.00 mg/100g). Phenols are widely spread compounds in plant foods, including tea. They are responsible for the organoleptic properties of plant foods as well as their antioxidant properties (14). Nutritionists, researchers and food manufacturers have become very interested in plant phenols and this is attributed to their antioxidant properties, abundance in the diet, and oxidative stress related diseases preventive effects (14).

All the brands of green tea possess steroids, tannins, flavonoids and alkaloids. This was in line with the findings of Ananthi and Giri (15) who also reported the presence of these phytochemicals in green tea. The results of this study show a high content of flavonoids in all the tea samples. The types and amounts of flavonoids present in tea depends on the variety of leaf, soil condition, processing, particle size of ground tea leaves and infusion preparation (16). Flavonoids are secondary metabolites widely spread in plants. They have been shown to possess free-radical scavenging activity and protection against reactive oxygen species (17). Studies have found that the consumption of foods rich in flavonoids protect against human diseases related to oxidative stress such as diabetes mellitus, artherosclerosis, ischemic heart disease and others. Their alkaloid content was high, ranging from 527.33±21.93 mg/100g in GTQ to 200±12.65 mg/100g in GTT. The presence of flavonoids and alkaloids in green tea suggests that they can be useful in inhibiting some pathogenic microorganisms. Pradhan and Dubey (18) reported that soluble extract of green tea showed an inhibitory effect on the growth of six bacterial species. This antimicrobial attribute of green tea could have served as a preservative while the product remained on the shelf. The high content of alkaloid in the green tea also samples suggests that they could be helpful in the prevention of some cardiovascular diseases (19).

The tannins content of GTQ and GTL (8.76±0.07 and 8.78±0.06) respectively did not differ significantly ($p > 0.05$), while the tannin content of GTN (9.30±0.40) was significantly ($p < 0.05$) higher than those of GTLp and GTT (8.84±0.34 and 8.43±0.10) respectively. Tannins are

astringent polyphenols found in plants that can bind and precipitate proteins. They also possess anti-oxidative and anticancer properties. Excessive tannins are thought to toxic because tannins act as act as metal ion chelators and can decrease the bioavailability of iron. This, most times leads to anaemia (20). However studies have shown that condensed tannins are responsible for depleted iron bioavailability and not tea tannin. The type of tannin present in tea is chemically different from the tannins found in other plants (16). There is absent of tannic acid in tea (16). According to Beverly et al. (21), there was no significant ($p > 0.05$) difference in the iron absorption of rats fed green tea and control diet for 30 days. Another study by Samman et al. (22) involving women as subjects found out that there was no significant difference in the iron absorption of women fed diet containing green tea and a control diet containing no green tea. Low levels of steroids were found in the green teas. GTQ recorded the highest steroids composition (0.42 ± 0.02). Steroids play crucial roles in hormonal balance by acting as precursors in the synthesis of sex hormones (23). The low concentration of steroids in the green teas found in this study suggests that the plant may not be effective in the management of hormonal imbalance. Consequently, women of child bearing age should be careful not to be involved in over consumption of green tea. The steroids result in this study also suggests that green tea could be used as a slimming therapy.

The radical scavenging activity of an extract is expressed as EC_{50} value. Generally, the lower the EC50 of a substance against a radical, the higher radical scavenging power of that substance while the higher the TAC value of a substance the higher the antioxidant power against free radicals.

The result of the 1,1 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the green tea samples, as shown in Figure 1, indicated that all the samples were very potent in scavenging the radical. However, GTL which recorded the least EC_{50} (6.79 ± 0.18 μg/ml) was more potent, followed by GTT (6.80 ± 0.018 μg/ml). The result of the EC_{50} gotten from this study were lower than those gotten from other samples of green tea used by Suljagic et al. (24). They obtained EC_{50} values ranging from 14 μg/ml to 27 μg/ml. (16) obtained the EC_{50} of different samples of green tea against DPPH ranging from 471 μg/ml to 894 μg/ml.

Nitric oxide (NO) is a reactive oxygen species which has been thought to be produced by responses generated by vascular endothelial cells. When NO comes in contact with superoxide (O-) in the epithelium, a reaction occurs leading to the formation of Peroxynitrite (ONOO-) which may lead to blood pressure build up and oxidative damage of DNA (25). The green tea samples were capable of inhibiting Nitric oxide as indicated by the low EC_{50} shown in figure 2 as compare with ascorbic acid. The EC_{50} obtained from this result also shows that GTL was more potent in inhibiting NO.

Total Antioxidant Capacity (TAC) assay measures the ability of an extract to destroy a free radical by transferring an electron to it. The assay is based on the reduction of molybdate VI to molybdate V by an extract with the formation of a green phosphate and molybdate V complex at an acidic pH (13). Figure 3 shows the TAC values of the five samples of green tea. It indicates that GTLp was more capable in challenging the free radical, followed by GTN. The five brands of green teas were ranked based on the results obtained from the three antioxidant assays used. The following order of potency was obtained: GTL>GTLp>GTN>GTT>GTQ.

CONCLUSION

The findings in this study confirms that all the five green tea green tea brands available in Ogige market in Nsukka, Enugu state are rich in essential phytochemicals such as flavonoids and tannins. They demonstrate antioxidant potency and scavenging potential against DPPH and NO radicals. However, there were variations in their phytochemical composition and antioxidant capacity. GTL was the most potent against free radicals after ranking using the three antioxidant

assays as shown in the appendix.. Green tea is thus recommended for the prevention of oxidative stress

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APPENDIX

RANKING OF THE TEA SAMPLES USING EC_{50} VALUES FROM THE THREE ANTIOXIDANT ASSAYS

DPPH. SCAVENGING ASSAY

GREEN TEA	EC	NO. OF POINTS
GTQ		1
GTL		5
GTN		2
GTLp		3
GTT		4

NOTE: The most potent tea gets the highest point.

NITRIC OXIDE SCAVENGING ASSAY

GREEN TEA	EC_{50}	NO. OF POINTS
GTQ	61.99	4
GTL	59.95	5
GTN	69.66	3
GTLp	81.66	2
GTT	322.85	1

NOTE: The most potent tea gets the highest point.

TOTAL ANTIOXIDANT CAPACITY (TAC) ASSAY

GREEN TEA	TAC VALUE	NO. OF POINTS
GTQ	103.03	2
GTL	98.36	1
GTN	134.63	4
GTLp	142.06	5
GTT	105.63	3

NOTE: The most potent tea gets the highest point.

SUMMARY OF POINTS OBTAINED BY THE TEA SAMPLES

GTQ 1+4+2 =7
GTL 5+5+1 =11
GTN 2+3+4 =9
GTLp 3+2+5 =10
GTT 4+1+3 =8

RANKING OF THE TEAS IN A DESCENDING ORDER OF POTENCY

GTL> GTLp> GTN> GTT>GTQ.