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EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME COMMERCIAL BRANDS OF GREEN AND BLACK TEA IN UYO METROPOLIS OF AKWA IBOM STATE, NIGERIA

Uwemedimo Francis Umoh*[1], Emmanuel Iweh Etim[2], Imo Essiet Jacobs[1], Danladi Ngyan Bala[1] and John Akpan Udobang[3]

[1]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State.

[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State.

[3]Department of Clinical Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Uyo, Uyo, Akwa Ibom State.

***Corresponding Author: Dr. Uwemedimo Francis Umoh**

Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State.

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ABSTRACT

This research work was to evaluate some available commercial brands of green and black tea in Uyo metropolis in Akwa Ibom State of Nigeria to authenticate their quality at the point of consumption. Five (5) brands of green tea coded G1-G5 and three brands of black tea coded (B1-B3) were purchased randomly from some supermarkets in Uyo metropolis. Twenty five (25 g) of each of the tea brands were weighed, macerated with aqueous ethanol (70%). The filtrates were collected, concentrated and stored in the refrigerator. The antioxidant evaluation was carried out through the assessment of the tea packets, DPPH scavenging assay, iron chelating assay, estimation of total phenolic and total flavonoid contents. Result revealed that some of the teas were not properly labeled and for DPPH scavenging activity, B2 had a value of 82.4% at 100 µg/mL thereby demonstrating the highest scavenging activity for the black teas while G3 with 84.9% at 100 µg/mL demonstrated highest scavenging effect for the green teas. For iron chelating assay, B3 demonstrated the highest activity at all concentrations (20 µg/mL- 100 µg/mL) while G1 and G3 were highest for the green teas. In total phenol estimation assay, B1 showed the highest phenolic content for black teas while G4 was highest for green teas. Total flavonoid estimation revealed B1as having the highest flavonoid contents for black teas and G4 for the green teas. The results from this study authenticate the quality of these teas and confirm their suitability for consumption.

KEYWORDS: Antioxidants, evaluation, teas and quality.

INTRODUCTION

Tea is one of the most popular beverages worldwide. They are normally produced from the young leaves of *Camellia sinensis* L. and cultivated in more than 30 countries of the world. In considering the total amount of tea produced and consumed globally, 78% are black tea, 20% are green tea while 2% are white tea and the reason for this trend may not be unconnected to the cost, accessibility and affordability (Tariq *et al*., 2010). These tea grades are produced from different manufacturing processes ranging from panning, little or no oxidation to yield the white teas; partial oxidation for the green teas and total oxidation for the black teas (Graham, 1992).

Green teas contain mainly catechins of epigallocatechin gallate (EGCG), Epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) whereas thearubigins and theaflavins are the major components of black teas (Haslam, 2003).

Tea polyphenols are notable for free radical scavenging and antibacterial properties. In general, it is believed that these attributes are tied down to the extent of fermentation hereby placing the tea types in the following sequence of activity with white tea leading, followed by green tea and finally the black teas. The aim of this research was to evaluate some of the available brands of green and black teas in Uyo metropolis of Akwa Ibom State, Nigeria to authenticate their quality at consumption.

MATERIALS AND METHODS

Procurement of tea brands: Three (3) commercial brands of black tea (Lipton yellow label, Genex garden fresh black tea andTop tea) and five (5) of green tea (Certified organic green tea, Legend tea and herbs, Healthy hour green tea, Qualitea premium green and Loyd green) were randomly procured from five notable supermarkets in Uyo metropolis, Akwa Ibom State,

Nigeria and labeled B1-B3 and G1-G5 for the black teas and green teas, respectively.

Assessment of the labeling on the tea packets: The individual tea packs were assessed for the addresses of the manufacturers, manufacturing date, expiry date and batch number.

Preparation of Extracts: The various tea brands (25g) each were marcerated with 70% ethanol for 72h after and filtered first by decanting and then with the use of non-absorbent cotton wool. The filtrates were collected in conical flasks and concentrated to dryness *in vacuo* using rotary evaporator and the dried ethanol extracts of the teas preserved in refrigerators.

Antioxidant Assay

2,2-diphenyl-1-picryhydraxyl (DPPH) radical assay: The procedure here was similar to the one reported by Guangrong *et al*., 2008. Stock concentrations of 1mg/mL each of the ethanol extracts of the teas were prepared and further dilutions were carried out to obtain stocks of 20-100 µg/mL. 2 mL of the various concentrations of the teas were added to 2 mL of 0.3 mM DPPH in methanol. These solutions were vortexed, incubated in a dark chamber for thirty minutes at ambient temperature. A UV-spectrophotometer at 517nm was used to measure the absorbances against a DPPH blank and the percentage scavenging effects of the teas were calculated using using the formular below:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100\%$$

Iron chelating activity assay: The absorbances (510 nm) measured here was that of reaction mixtures of the teas which contained 1mL phenanthroline, 2 mL ferric chloride and 2 mL of the ethanol extracts of the teas at different concentrations (20-100 µg/mL), incubated for 10 minutes at ambient temperature. A blank absorbance was also measured without the teas and the percentage chelating activity calculated (Sharififar *et al*., 2009).

Estimation of total flavonoid contents: 5ml of 2% Aluminium trichloride ($ALCL_3$) in methanol was mixed thoroughly with equal volumes of tea solutions (Meda *et al*., 2005). UV-visible spectrophotometer (517 nm) readings of the mixtures were taken after 15minutes against a blank which consisted of 2ml of the different tea solutions and 2ml methanol without $ALCl_3$. Total flavonoid contents were calculated using garlic acid standard curve (20-100 µg/mL) and expressed in mg per rutin equivalent per 1g of the tea solutions.

Determination of total phenol: Folin Ciocalteau reagent was used in a manner similar to the report of Meda *et al*., 2005. A mixture of 2.5 mL of 10% Folin Ciocalteau reagent, 2 mL of sodium carbonate (Na_2CO_3) 2%W/V and 0.5 mL of the tea solutions were incubated at ambient temperature for thirty (30) minutes and followed by the measurements of the absorbances using a UV- spectrophotometer at 510 nm.

RESULTS AND DISCUSSION

The result of the assessment of the tea packets (Table 1) revealed that all the brands of black teas (B1-B3) procured were properly labeled with the manufacturer's addresses, manufacturing date, expiry date and batch numbers while only one brand of the green teas (G4) was accurately labeled to reflect the appropriate characteristics of good labelling (Moore, 2012; Veronin, 2011). The green tea G1did not have a specific manufacturer's address, manufacturing date and batch number; G2 and G3 were devoid of addresses of their manufacturers while G5 lacked a batch number. It becomes important to say that two out of the three brands of black teas procured for this study were locally produced in Nigeria while one was produced off shore in Sri Lanka. Also, all the brands of green teas were imported brands thereby inferring that many of the green teas consumed in Uyo metropolis of Akwa Ibom State, Nigeria, seem to be imported brands. Proper labeling is an important aspect of production of pharmaceutical products hence this research advocates the employment of good manufacturing practices by production/packaging companies of teas in order to aid in tracing of tea products and also to present these teas as standard pharmaceutical products for the overall benefit to end users (Jigisha *et al*., 2012; Sinija and Mishra, 2008).

Table 1: Result of Label Assessment.

Code	Brand name	Manufacturer	Address	Manufacturing date	Expiry date	Batch number
B1	Lipton Yellow Label	Unilever Nig. PLC	RC 113, Agbara Industrial Estate Layout, Agbara, Ogun state	March 21, 2016	March 21, 2017	161173
B2	Genex Garden Fresh Black Tea	Qualitea Ceylon (PVT) Ltd	No. 14 Station Road, Wattal Columbo, Sri Lanka	November, 2015	November, 2018	D15304
B3	Top Tea	Promasido (Nigeria) Ltd	3A Cowbell Way, Isolo Industrial Estate, Lagos, Nigeria	February, 2016	July, 2017	408A
G1	Certified Organic green Tea	Wensian Wu@163.com	China	NIL	Year 2020	NIL
G2	Legend Tea and Herbs	Legend Tea International	NIL	July, 2015	July, 2018	NG/07/2015
G3	Healthy Hour Green Tea	Xiamen Kangdali	China	January 20, 2016	January 20, 2019	1125
G4	Qualitea (Premium Green)	Quality Ceylon (PVT) LTD	No. 18 Station Road, Wattala, Columbo, Sri Lanka	November, 2015	November, 2018	D15304
G5	Loyd Green Tea	Mokate S. A	US: Katowicka 2659 43450 Ustron, Poland	August 19, 2015	August 19, 2017	NIL

DPPH scavenging activity: The result of the DPPH scavenging activity of the teas showed that both the black and green teas (Fig. 1 and 2) demonstrated considerable scavenging of DPPH radical. This scavenging activity was dose-dependent as the higher concentrations of the teas showed higher activity which are represented as percentage inhibitions when compared to ascorbic acid, a standard antioxidant. All the brands of the black teas (B1-B3) used for this study demonstrated excellent scavenging ability in the percentage inhibition range of 82%-83.6% while the green teas G1-G5 also exhibited good DPPH scavenging activity in the range of 69.8%-84.9%. DPPH is a stable organic free radical with absorption at 517nm and also looses this absorption whenever it accepts an electron or a free radical specie thereby resulting in a colour change from purple to yellow (Arash *et al*., 2015). However, ascorbic acid (Figure 1) used as a positive control was higher in activity (92%) at a concentration of 100µg/mL than both the black and green teas.

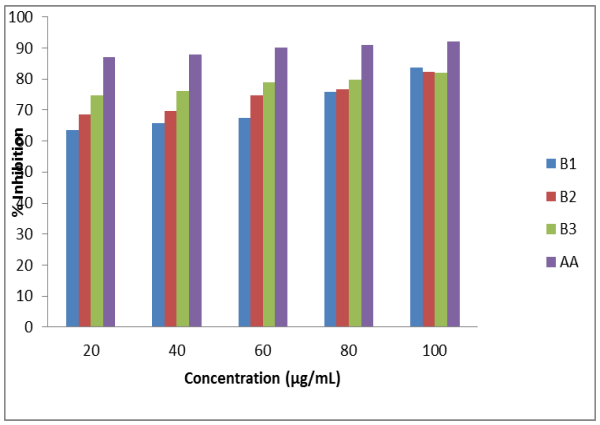

Fig. 1: Result of DPPH scavenging activity of black teas & ascorbic acid.

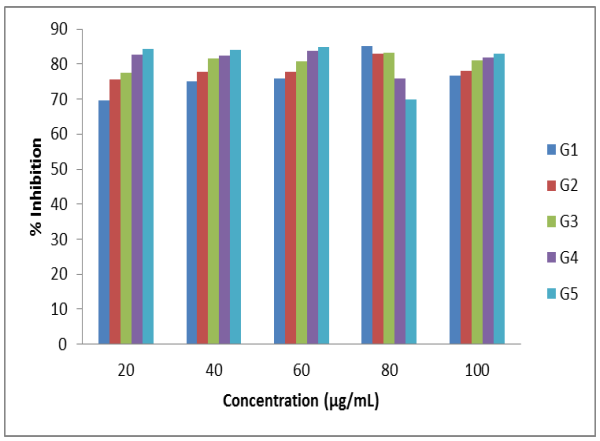

Fig. 2: Result of DPPH scavenging activity of green teas.

Iron chelating activity: The result of iron chelating potentials of the teas (Fig. 3 and 4) revealed that both tea groups demonstrated significant iron chelating effect in a dose-related manner (20-100µg/mL) when compared to ascorbic acid, a prototype iron chelating agent. It is known that the transition metal iron, has the potential to move single electrons even from the non reactive radicals hence, the main mechanism to avoid Reactive Oxygen Species (ROS) generation that is associated with redox active metal catalysis entails chelating of metal ions. The abilities of the teas in this study to chelate iron infers the interference of the teas with the formation of ferrous and O-phenanthroline and suggests that the teas captured ferrous ion before phenanthroline thereby decreasing the observed red colours (Umoh *et al*.,2016).

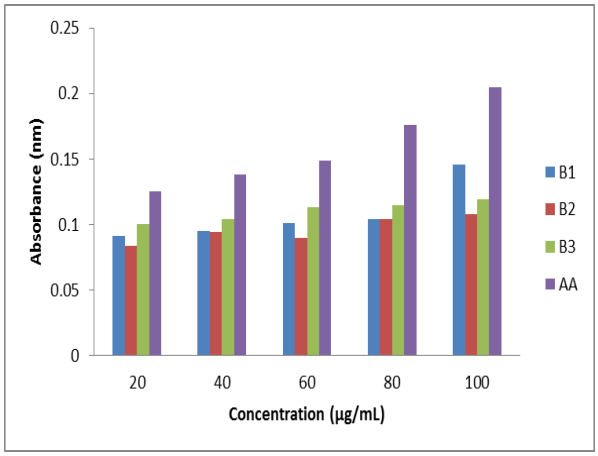

Fig. 3: Result of Iron chelating activity of black teas and ascorbic acid.

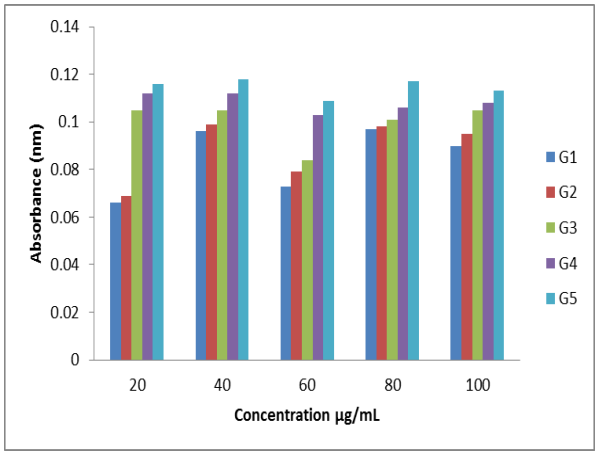

Fig. 4: Result of Iron chelating activity of green teas.

Total phenolic content: The result of the total phenolic contents of the teas as represented in Table 2 showed that both tea types had varying contents of phenolic compounds (5.33-10.59 for black teas and 2.40-7.96 for green teas) which were measured by Folin Ciocateau reagent in terms of rutin equivalents. The black tea B3 and G4 were highest in phenolic content in both black and green tea types, respectively. Phenolic compounds are those compounds with multiple hydroxyl group (OH) attachments to benzene ring(s) and accounts for the antioxidant properties of most antioxidant agents. Therefore, the abilities of these tea brands to exhibit excellent DPPH scavenging activity and chelate iron radical may be related to the content of polyphenols in them (Sharififar *et al*., 2009).

Table 2: Result of total phenolic content of teas.

Teas	Conc. (mg/mL)	Mean Absorbance (nm)	Total Phenolic Content (mg/g)
B1	100	28.04 ± 0.001	10.59
B2	100	17.93 ± 0.001	6.75
B3	100	14.19 ± 0.001	5.33
G1	100	6.46 ± 0.001	2.40
G2	100	15.12 ± 0.001	5.68
G3	100	8.08 ± 0.001	3.01
G4	100	21.12 ± 0.001	7.96
G5	100	10.08 ± 0.001	3.77

Total flavonoid content: The result of the total flavonoid contents of the commercial brands of both back and green teas (Table 3) revealed the total flavonoid contents of 33.68 mg/g, 16.19 mg/g, 20.99 mg/g and 18.99 mg/g, 19.19 mg/g, 20.37 mg/g, 34.79 mg/g, 18.59 mg/g measured in terms of rutin equivalents for black and green teas, respectively. Flavonoids are known phenolic compounds that are highly effective in the scavenging of most oxidizing moieties (free radicals) by suppressing reactive oxygen formation, chelating trace elements involved in free radical production, scavenging reactive species and up-regulating/protecting antioxidant defenses mechanisms (Bravo, 1998) thereby bringing about the health benefits associated with teas (Ezekiel, 2014: Bouayed and Bohn, 2010). Also, the flavonoid content in these teas accounts for the high phenolic contents (Arash *et al*., 2015). This result therefore supports the high DPPH and iron chelating activities of these teas.

Table 3: Result of total flavonoid content of teas.

Teas	Conc. (mg/mL)	Mean Absorbance (nm)	Total Phenolic Content (mg/g)
B1	100	1.73 ± 0.001	33.68
B2	100	0.85 ± 0.001	16.19
B3	100	1.09 ± 0.001	20.99
G1	100	0.99 ± 0.001	18.99
G2	100	1.00 ± 0.001	19.19
G3	100	1.06 ± 0.001	20.39
G4	100	1.78 ± 0.001	34.79
G5	100	0.97 ± 0.001	18.59

CONCLUSION

The results from this study conclude that all the commercial brands of teas procured demonstrated good antioxidant potentials and that the improper labels on some of them did not affect their antioxidant effects. More so, the high phenolic and flavonoid contents in these teas support their antioxidant abilities.

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