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COMPARATIVE STUDIES ON THE ANTIOXIDANT POTENTIALS OF FOUR DIFFERENT TYPES OF TEA; AN INSIGHT INTO A BETTER CHOICE OF HEALTHY BEVERAGE

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Abstract

The phytochemical constituents and *in vitro* antioxidant potentials of green, black, un-caffeinated, and herbal teas were evaluated and results compared. Green tea was found to be significantly (p < 0.05) higher in both total flavonoid content (TFC) (215.61±48.83 QE/mg) and total phenol content (124.81±79.05 GAEmg/ml) than others while un-caffeinated (411.55±9.21 GAEmg/ml), green (406.83±22.71 GAEmg/ml) and herbal (402.74±13.2 GAEmg/ml) teas exhibited significantly higher total tannin content (TTC) than black tea (325.14±108 GAEmg/ml). The un-caffeinated (184.32±33.62 QE/mg) and herbal (167.25±31.25 QE/mg) teas showed significantly higher TFC than the black tea (142.32±22.73 QE/mg). A positive correlation (r = 0.448) was observed between the TFC and ferric reducing antioxidant power (FRAP) assay as well as between TFC and 2,2-diphenyl-2-picrylhydrazyl DPPH (r = 0.347) radical scavenging activities of the tea samples. Results suggest that tea samples contain phytochemicals of which green tea exhibited least radical scavenging activity while black tea showed the highest antioxidant potentials highlighting their roles in neutralizing the excess free radicals arising from metabolic activities in living systems.

Keywords: Antioxidant activity, Phytochemical, Polyphenols, Tea.

Introduction

Oxygen an indispensable life element [1], is used in the mitochondria during cellular redox reactions for ATP production and in the process, free radicals are produced [2]. Reactive oxygen species (ROS) is a collective term used for a group of oxygen-centered oxidants, which are either free radicals or molecular species capable of generating free radicals endogenously or exogenously. ROS are both toxic and beneficial [1]. At low or moderate levels, they exert beneficial effects on cellular responses and immune function, but at high concentration, they generate oxidative stress, a deleterious process that can damage all cellular structures [1]. The human body counteracts oxidative stress by using antioxidants either naturally produced in situ, or through externally supplied foods and/or supplements [1]. Plants and animals contain various antioxidants, such as glutathione, vitamin C, vitamin E. polyphenols as well as enzymes such as catalase. superoxide dismutase and peroxidases [3]. It is a common knowledge that the antioxidants in fruits, vegetables, teas and wine are the main factors responsible for the observed efficacy of these foods in reducing the incidence of chronic diseases [4]. Processed tea, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant Camellia sinensis [5]. Though most of the tea could be classified as nonfermented/aerated green tea, semi-fermented (oolong) tea and fermented black tea [6], two types of tea products are widely consumed; green and black tea. The tea beverage is considered medicinal since ancient times because of its polyphenols and there is already growing evidence that tea polyphenols reduce the risk of heart diseases, neurological disease, cancer and obesity in humans [7]. Tea has been associated with anti-allergic [8] and antimicrobial properties [9]. Moreover, some studies epidemiological have associated consumption of tea with a lower risk of several types of cancer including those of the stomach, oral cavity, esophagus and lungs, therefore, tea appears to be an effective chemo-preventive agent for toxic chemicals and carcinogens [5,10]. Many plant phenolics have antioxidant properties that are even much stronger than vitamins E and C. Some synthetic antioxidants have been suspected to cause or prompt negative health effects and hence the need to substitute them with naturally occurring antioxidants sourced from plants [11, 12]. Because of this quest, the study evaluated the total polyphenols contents and in vitro antioxidant activities of four samples (black, green, uncaffeinated and herbal tea) using DPPH radical scavenging ability and the ferric reducing antioxidant power.

Methods

Collection of tea samples

Twenty commercially available teas grouped into four types (green, black, un-caffeinated and herbal teas) were purchased at shop-rite in the city of Enugu, Enugu State, Nigeria and various other local grocery stores in the city of Nsukka, Enugu State, Nigeria. The teas were divided into four groups as follows:

Group 1: Five different brands of black tea denoted by the letter B and numbers 1-5 (BT1=Ahmad Tea, BT2 = LOYD lemon tea, BT3 = Lipton tea, BT4 = Dilmah and BT5 = Shenghai Bozehng).

Group 2: Five different brands of un-caffeinated tea denoted by the letter U and numbers 1-5. (UT1 = Tetley tea, UT2 = Chamommile tea, UT3 = Lemon and Ginger tea, UT4 = Pepermint tea and UT5 = Red bush tea).

Group 3: Five different brands of green tea denoted by the letter G and numbers 1-5. (GT1 = Hillway tea, GT2 = Moringa tea, GT3 = Green life CRT tea, GT4 = Legend tea and GT5 = Tianshi tea.

Group 4: Five different brands of herbal teas denoted by the letter H numbers 1-5. (HT1 = Slim Dieters tea, HT2 = Ginseng tea, HT3 = Typhoid tea, HT4 = Fat Burner tea and HT5 = Anti-Diabetes tea).

A quantity, 10 g each was soaked in 100 ml of distilled water at 100°C and kept below 80°C to brew for 10 min. The mixture was decanted and filtered using Whatman No.1 filter paper. The resulting filtrate was used for the phytochemical and antioxidant assay.

Test for Tannin

Folin-Ciocalteau method of Mythili et al. [13] was used for the determination of the total tannins content (TTC) of the tea extracts. Gallic acid was used as standard. The total tannins content was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts [14].

Test for Flavonoid

The total flavonoid content of the aqueous extracts was determined photometrically using the aluminium chloride ($AlCl_3$) assay method of Ghasemzadeh et al. [15]. The absorbance was measured against a prepared reagent blank in triplicate at 430 nm. The total flavonoid content was expressed as quercetin equivalents in mg/100 g of

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Test for total phenolic contents

Total phenolic contents of the extract were determined using the Folin-Ciocalteu reagent as reported by Gholivand and Piryaei [16]. Gallic acid was used as standard.

Determination of antioxidant activity using DPPH radical scavenging model

The antioxidant activities of the extracts were measured by radical scavenging ability, using the stable radical, DPPH as reported by Aliyu et al. [17]. The standard synthetic antioxidant, butylhydroxytoluene was used as the positive control. The percentage antiradical activity (AA %) of the extracts was calculated using the formula proposed by Ghasemzadeh et al. [15] as shown below:

$$AA\% = \left(100 - \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control}}\right) \times 100\right)$$

Where, Abs sample, Abs blank, and Abs control are the absorbance values of the extract, blank, and control samples, respectively.

Ferric reducing antioxidant power assay

The ferric reducing power (FRAP) of the extracts were assayed based on the blue colouration that developed due to the reduction of ferric iron to the ferrous form as described by Loizzo et al. [18].

Statistical Analysis

The data were analysed using IBM Statistical Product and Service Solutions (SPSS), version 18.0 and graph pad prism, version 6. The results were expressed as mean \pm standard deviation (SD). Significant differences of the data were established by one-way analysis of variance (ANOVA). Mean values with (p<0.05) were considered statistically significant.

Results

Qualitative phytochemicals of different types of tea

Qualitative and quantitative identification of phytochemicals in the tea samples shows that flavonoid and tannin were found to be present in substantial concentrations (Table 1).

Antioxidant activity of groups 1, 2, 3 and 4 by 2,2-Diphenyl-2-Picrylhydrazyl (DPPH)

Figure 1 shows the IC_{50} (the concentration of the brewed tea that was able to scavenge half of the DPPH radical) values of the extracts. Black tea 2

(BT2) had the least IC₅₀ value of (538µg/ml), while black tea 5 (BT5) has the highest IC50 value of (825µg/ml). Figure 2 also shows the results of the antioxidant assay by DPPH model of group 2 (uncaffeinated tea samples). From the result, the uncaffeinated tea 3 (UT3) has the least IC_{50} value of 598µg/ml and un-caffeinated tea 2 (UT2) have the highest IC₅₀ value of 773 μ g/ml. The antioxidant assay by DPPH model of group 3 (green tea samples) are shown in Fig. 3. The result shows that green tea 3 (GT3) has the least IC₅₀ value of 471μ g/ml and green tea 1 (GT1) has the highest IC_{50} value of 894µg/ml. Fig 4 shows the result of the antioxidant assay by DPPH model of group 4 (herbal tea samples). Herbal tea 1 (HT1) has the least IC₅₀ value of 343 μ g/ml and herbal tea 5 (HT5) have the highest IC₅₀ value (824 $\mu g/ml$).

Antioxidant activity of black tea and un-caffeinated tea samples by ferric reducing antioxidant power (FRAP) model

The antioxidant assay by FRAP model of group 1 (black tea samples) is shown in Fig. 5. The IC₅₀ of the black tea samples were: black tea 3 (BT3) (491.22µg/ml) black tea 2 (BT2) (493.17µg/ml) black tea 5 (BT5) (494.71µg/ml), black tea 1 (BT1) (496.57µg/ml) and black tea 4 (BT4) (497.68µg/ml). The BT4 has the highest IC₅₀ and hence, least ferric ion chelator of this group. Figure 6 shows the results of the antioxidant assay by FRAP model of group 2 (un-caffeinated tea). From the result, the IC₅₀ of the un-caffeinated tea 1 (UT1) has the least IC₅₀ value of (598.14 µg/ml), un-caffeinated tea 3 (597.71µg/ml), un-caffeinated tea 5 has the highest IC₅₀ value of (602.20µg/ml).

Antioxidant activity of groups 3 and 4 by ferric reducing antioxidant power (FRAP) model

Figure 7 shows the results of the antioxidant assay by FRAP model of group 3 (green tea samples). The result shows that green tea 1 (GT1) has the least IC₅₀ value (614.83 µg/ml). Green tea 5 (GT5) has IC₅₀ value of 615.29 µg/ml, green tea 4 (GT4) (616.00 µg/ml) and green tea 2 (GT2) (624.02 µg/ml). The green tea 3 (GT3) have the highest IC₅₀ value of 624.02 µg/ml. The results of the antioxidant assay by FRAP model of group 4 (herbal tea samples) is shown in Fig. 8. The result shows that herbal tea 1 (HT1) has the least IC₅₀ with a value of 576.19 µg/ml. The IC₅₀ values of other herbal tea samples were herbal tea 5 (HT5) 578.00 µg/ml, herbal tea 2 (HT2); 580.62 µg/ml, herbal tea 3 (HT3) 585.11 µg/ml. Herbal tea 4 (HT4) has the highest IC₅₀ value of 587.68 µg/ml.

A comparison of the phytochemicals and antioxidant activities of different brands of tea

comparison of the phytochemicals А and antioxidant activities of different brands of tea is shown in Table 3. The total flavonoid content (TFC) tea (215.61±48.83 of green QE/mg) was significantly (p < 0.05) higher when compared to black tea (142.32±22.73 QE/mg), herbal tea (167.25±31.25 QE/mg) and un-caffeinated tea (184.69±33.62 QE/mg). However, there was no significant (p > 0.5) difference between the TFC of herbal tea and un-caffeinated tea. The total tannin content (TTC) of black tea (325.14±108 GAE/mg) was significantly (p < 0.05) lower than uncaffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). However, there was no significant (p > 0.5) difference among the TTC of uncaffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). The total phenol content (TPC) of green tea (124.81±79.05 GAE/mg) was significantly (p < 0.05) higher than black tea (51.81±8.90 GAE/mg), un-caffeinated tea (63.87±35.76 GAE/mg) and herbal tea (15.78±13.02 GAE/mg). However, there was no significant (p >0.5) difference between the TPC of black tea, uncaffeinated tea and herbal tea. The results of the ferric ion reducing power (FRAP) of green tea $(618.64\pm 233.63 \mu g/ml)$ was significantly (p < 0.05) higher than un-caffeinated tea (598.50±378µg/ml), herbal tea (581.52±271.47 µg/ml) and black tea μg/ml). The (494.66±208.82 DPPH radical scavenging activities of herbal tea (610.22±242.02µg/ml) were the least. There was no significant (p > 0.05) difference among the DPPH radical scavenging activity of black tea (702.88±104.10µg/ml), un-caffeinated tea (691.72±79.9µg/ml) and green tea (745.64±161.36µg/ml).

Discussion

In this study, the phytochemical constituents and antioxidant potentials of herbal tea, black tea, uncaffeinated tea and green tea samples were determined. The results of the qualitative phytochemical screening, as shown in Table 1, showed that flavonoids and tannins were present in all the tea samples. This observation is in line with that of Lee et al. [5]. Who also detected the presence of flavonoids and tannins in green tea. Flavonoids and phenolic acids are the most important groups of secondary metabolites of plants and their presence in tea is to be expected [19]. Flavonoids were detected in all the tea types. The total flavonoid content (TFC) of green tea $(215.61\pm48.83 \text{ QE/mg})$ was significantly (p < 0.05)higher than un-caffeinated tea (184.69±22.73 QE/mg), herbal tea (167.25±31.25 QE/mg) and black tea (142.32±22.73 QE/mg). The presence and concentration of flavonoids present in the tea will differ depending on the variety of leaf, growing environment, processing, manufacturing, particle size of ground tea leaves and infusion preparation. The major polyphenolic compounds in green tea are flavan-3-ols, a class of flavonoid which remains intact even after processing. Processing of black tea involves the condensation of catechin and orthoguinones (oxidation of the β ring di- and trihydroxylated catechins) by polyphenol oxidase to form the α -flavins (TFs) [20]. This could be responsible for the lower TFC observed in the present study. Reports showed that un-caffeinated tea is not processed from a single plant but a blend of herbs that have flavonoid [21]. Flavonoids being ubiguitous have been reported to be present in chamomile, red bush and peppermint from which un-caffeinated tea is produced [22]. This may be responsible for the higher TFC observed in uncaffeinated tea compared to black tea. Also, for the flavonoid content of tea to be absorbed, the flavonoid must be accessed by hot water infusion and the subsequent action of digestive juices. The absorption of the flavonoid liberated from tea will depend on its physicochemical properties such as molecular size and configuration, lipophilicity, solubility, and pKa [23]. Ethanol could also increase their absorption by improving their solubility. Antioxidant activity of flavonoid is concentration dependent; hence, their health benefits are dependent on the amount that is bioavailable after consumption. Tea flavonoids are beneficial to health. They have anti-proliferative, anti-inflammatory, antithrombogenic and anti-bacteria properties [24].

Tea "tannins" are chemically distinct from other types of plant tannins such as tannic acid. Tannic acid is absent in tea extract [25]. The results of the total tannin content (TTC) of the tea samples showed no significant (p > 0.05) difference among the TTC of uncaffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). However, the TTC of these three tea samples were significantly (p < 0.05) higher than that of black tea (325.14±108 GAE/mg). This could be attributed the differences in the fermentation processes of the various tea types. Processing of black tea converts the tea polyphenols to theaflavin and thearubigin. Samanta et al. [26] reported the presence of these polyphenolic compounds (theaflavin and thearubigin) in black teas. Tannins do not function solely as primary antioxidants (i.e., they donate hydrogen atom or electrons); they also function as secondary antioxidants. They have the ability to chelate metal ions such as Fe (II) and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation [27]. Tannins contribute an astringent or bitter taste to teas, in addition to the antioxidant property; tannins have been reported to possess anticarcinogenic, anti-mutagenic potentials and antimicrobial properties [28].

The total phenolic content (TPC) of the aqueous tea extract, as shown in Table 2, revealed that the TPC of green tea (124.81± 79.05 GAE/mg) was significantly (p < 0.05) higher than those of uncaffeinated tea (63.67±35.76 GAE/mg), black tea (51.81±8.90 GAE/mg) and herbal tea (15.78±13.02 GAE/mg). This could be attributed to the differences in the processing methods. Processing of black tea, un-caffeinated tea and herbal tea involve the oxidation of their polyphenols to other compounds. In contrast, green tea processing does not affect the polyphenols, hence, high polyphenols content in green tea. Tian et al. [29] reported that green tea polyphenols constitute up to 30% of the leaf's dry weight. Tea polyphenols are rich natural source of antioxidant. It has broad-spectrum and specific creative effects in antioxidant, antiatherosclerosis. resistance to dental caries, antitumor, anti-radiation, ant-aging, antimicrobial and in reducing blood pressure, hematic fat and blood sugar and even in anti-HIV [25]. The radical scavenging activity of the extract is expressed in IC_{50} (the percentage of the extract that was able to scavenge half the concentration of a radical). The lower the IC_{50} of a substance against a radical, the better radical scavenging power of that substance. The 2,2 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the tea samples, as shown in Figures 10-14, indicated that the antioxidant potential of a herbal tea (610.22±8.79%) was significantly (p<0.05) higher than un-caffeinated tea (691.72±79.94%), black tea (702.88±3.79.94%) and green tea (745.64±4.93%). In this study it was observed from the manufactures description that some of the brands of herbal tea were made by the combinations of tea types, from different plant such as Camellia Sinensis, cassia tea, ginseng, apigenin, and tangerine and this could be the reason for the observed higher antioxidant capacity. Furthermore the low radical scavenging activity observed in green tea may be due to the antioxidant activity of

flavonoid, which was detected in high concentration in green tea. Yen et al. [30] and Procházkováa et al. [31] also reported that flavonoids pro-oxidant activity is concentration dependent. The possible pro-oxidant effects of flavonoids may be important in vivo if free transition metal ions are involved in oxidation processes [31]. Flavonoids are capable of reducing Cu (II) to Cu (I) and thus initiate the formation of radicals [32]. In healthy human body, metal ions appear largely sequestered in forms that are unable to catalyze free radicals (e.g in ferritin or caeruloplasmin) [33]. However, injury to tissues may release iron or copper and catalytic metal ions have also been measured in atherosclerosis lesions [34]. However, there was no significant (p > 0.05)difference between the DPPH radical scavenging power of un-caffeinated tea, black tea and green tea. All the tea samples demonstrated good radical scavenging activity. This could be due to the presence of the secondary metabolites found in the tea samples. The results of the ferric reducing antioxidant power (FRAP) assay revealed that the IC_{50} of green tea ($618.64 \pm 4.60\%$) was significantly (p < 0.05) higher than black tea (494.66±2.58 %), uncaffeinated tea (598.50±3.43%) and herbal tea (581.52 \pm 4.81%). The black tea had the least IC₅₀ and thus, more potent in ferric reducing antioxidant power. Catechin oxidation involved in black tea preparation leads to the formation of catechin dimers, known as theaflavins and thearubugins. These compounds are responsible for the colour and taste and also key factors in the antioxidant activity [5]. The ability to reduce ferric iron to their ferrous state shows effective radical scavenging characteristics of the various teas. Herbal and uncaffeinated tea contains apigenin and other polyphenolic compounds which may be responsible for the antioxidant activity of the tea samples [5].

A positive correlation between the antioxidant capacity and the phytochemical constituent of the tea samples was observed. There was a positive correlation between the TTC and FRAP of the tea samples. Tannins have the ability to chelate metal ions such as Fe (II) and interfere with one of the reaction steps in the Fenton reaction and thereby oxidation [27]. However, a negative retard correlation between tea TTC and DPPH radical scavenging activity was observed. This could be because at certain concentration, tannin acts as prooxidant other than antioxidant. A positive correlation was also observed between the TFC and FRAP, and DPPH radical scavenging activity of the tea samples. Similarly, a positive correlation was observed between the total phenol content (TPC) and DPPH

radical scavenging activity of the tea samples. Same was observed between TPC and FRAP. The antioxidant activities of tea samples are due to the polyphenol content. Though catechins are oxidized to theaflavins and thearubugin during processing of some tea types, the products also possess radical scavenging activity. Korari et al. [20] reported that conversion of catechins to theaflavins during black tea processing does not affect the radical scavenging potency of black tea. The findings from this study agree with this opinion. These observations are consistent with those of Leung et al. [35] that reported that black tea possess relatively same antioxidant potency as catechins present in green tea. This is because theaflavins and thearubugin can donate hydrogen electrons from the hydroxyl groups in their structure to stabilize free radicals. In addition to the capturing (quenching) of free radicals, the tea catechins can chelate metal ions such as iron and copper, preventing their participation in Fenton and Haber-Weiss reactions [36].

Conclusion

These findings demonstrated that the green tea, black tea, un-caffeinated tea and herbal tea samples are rich in important phytochemicals such as flavonoids and tannins, and possess antioxidant potentials. However, the tea types vary in their contents of antioxidants and in their antioxidant potentials. Based on the FRAP assay, black tea had the highest antioxidant potential while green tea had the least. Conversely, based on the DPPH assay, black tea, un-caffeinated tea and green tea had equal antioxidant potential while herbal tea had the highest antioxidant potentials.

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Table 1.	Qualitative	phytochemical	screening	of
different	types of tea			

Teas	Flavonoid	Tannin
BT ₁	+++	++
BT ₂	+++	++
BT ₃	++	++
BT_4	++	++
BT ₅	+	+
UT1	++	++
UT ₂	+	+
UT ₃	++	+
UT ₄	+	+
UT ₅	+	+
GT_1	++	++
GT ₂	++	+
GT ₃	++	++
GT ₄	+++	+
GT ₅	++	++
HT ₁	++	++
HT ₂	+	+

+ = detected in low quantity; ++ = detected in moderate quantity, +++ = detected in high quantity $HT_4^=$ not detected ++ +

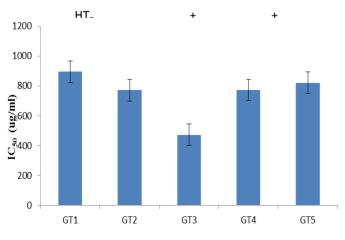


Figure 3. The antioxidant activity of Green teas by 2,2-Diphenyl-2-Picrylhydrazyl (DPPH). GT=Green tea

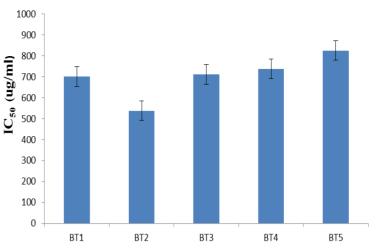


Figure 1. The antioxidant activity of different types of black tea by 2,2-Diphenyl-2-Picrylhydrazyl (DPPH). BT = Black tea

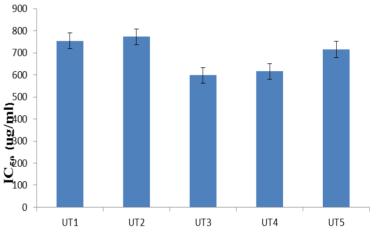


Figure 2. Antioxidant activity of un-caffeinated teas by 2,2-Diphenyl-2-Picrylhydrazyl (DPPH) model. UT = Un-caffeinated tea

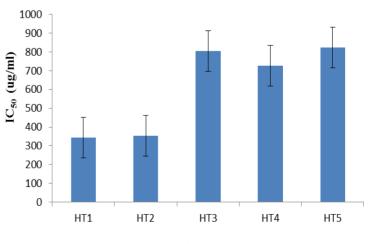


Figure 4. Antioxidant activity of herbal teas by 2,2-Diphenyl-2-Picrylhydrazyl (DPPH).HT = Herbal teas

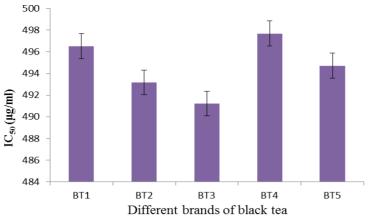


Figure 5. Antioxidant activity of black teas by Ferric Reducing Antioxidant Power (FRAP). BT = Black tea

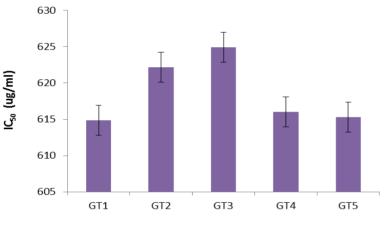


Figure 7. The antioxidant activity of green tea by Ferric Reducing Antioxidant Power (FRAP). GT=Green tea

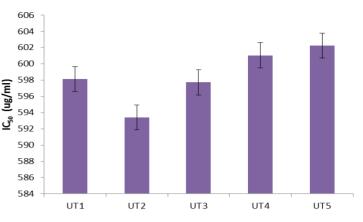


Figure 6. : The antioxidant activity of un-caffeinated tea by Ferric Reducing Antioxidant Power (FRAP).UT = Uncaffeinated tea

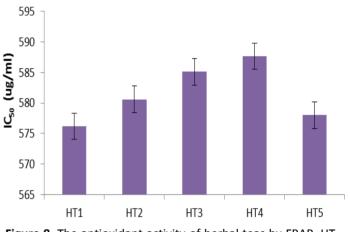


Figure 8. The antioxidant activity of herbal teas by FRAP. HT = Herbal tea

Teas	TFC(mg/ml)	TTC(mg/ml)	TPC(mg/ml)	FRAP(µg/ml)	DPPH(µg/ml)
Black Tea	142.32±22.73ª	325.14±108ª	51.81±8.90ª	494.66±208.82ª	702.88±104.10 ^b
Un-caffeinated Tea	184.69±33.62 ^{ab}	411.55±9.21 ^b	63.87±35.76ª	598.50±378.25 ^c	691.72±79.94 ^b
Green Tea	215.61±48.83 ^b	406.83±22.71 ^b	124.81±79.05 ^b	618.64±233.63 ^d	745.64±161.36 ^b
Herbal Tea	167.25±31.25 ^{ab}	402.74±7.95 ^b	15.78±13.02ª	581.52±271.47 ^b	610.22±242.02ª

Data are mean \pm SD ; Values with different superscript in a column are significant at p < 0.05. TFC = Total flavonoid content; TTC = Total tannin content; TPC = Total phenol content. FRAP = Ferric reducing antioxidant power; DPPH = 2,2-Diphenyl-2-picrylhydrazyl