

Evaluation of the antioxidant capacity and content of polyphenols obtained from tea (*Camellia sinensis*) of four brands sold in Colombia by extraction at room temperature

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Abstract

Herbal medicines have long been used to treat chronic diseases such as cancer, neurodegeneration and diabetes, usually in the form of herbal teas, also called tisanes [1]. Tea, second only to water, is the most highly consumed beverage worldwide and its medicinal and health properties, long known to early Chinese civilizations, have been widely explored [2]. The consumption of green tea in Colombia is a recent trend and the market is continuously growing, then the most common commercially available types of green tea were tested in this study; Oriental, Lipton, Hindú and Jaibel. The main objective of this study was to determine and compare the amount of polyphenols present in tea samples considering extraction in water at room temperature and stirring every 30 seconds for 5 minutes. The antioxidant capacity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay and the oxygen radical absorbance capacity (ORAC) assay. Concentrations ranging from 22.36 ± 0.98 to 41.29 ± 0.86 mg Trolox equivalent / g dry sample for DPPH assay and 22.95 ± 1.31 to 46.25 ± 2.05 mg Trolox equivalent / g dry simple were determined for ORAC assay. It was also found that the amount of total phenols in tea samples ranging from 2.53 ± 0.25 to 14.63 ± 0.53 mg gallic acid equivalent (GAE) / g dry sample and total flavonoid content concentration was obtained from 2.67 ± 0.20 to 7.08 ± 0.38 mg catechin equivalent / g dry simple. The antioxidant activity and total flavonoid content were highly correlated for both DPPH ($r^2 = 0.9911$) as ORAC ($r^2 = 0.9968$). Tea extracts from the Oriental brand had the highest polyphenol content showing greater biological activity, contrary to Jaibel tea brand which recorded the lowest concentrations in all analyzes.

Key words: Antioxidant activity, *camellia sinensis*, green tea, room temperature.

Introduction

Green tea (*Camellia sinensis*) is well known for various health benefits associated with risk reduction of a wide range of chronic diseases, such as cancer, diabetes, and cardiovascular diseases [3]. In the past few years, special attention has been paid to the antioxidant activities of the polyphenolic compounds present in green tea due to their pharmaceutical properties. The performed studies report that green tea extract (GTE) shows many health beneficial properties, particularly against the damage caused by pollution, stress, cigarette smoke and other toxins, prevention of cardiovascular diseases, cancer, diabetes [4].

Tea is rich in polyphenolic compounds, namely the catechins, (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) are thought responsible for such beneficial effects [5].

The methods of preparing the beverage also vary worldwide: in China, tea leaves are steeped in hot water (70–80 °C for green tea) for 20–40 s, and the same tea leaves are usually repeatedly steeped (seven times). The Japanese usually prepare green tea by steeping leaves in hot water for about 2 min and using them for 2–3 infusions. In the United Kingdom, Ireland and in Canada, black tea is mostly prepared using boiling water and consumed with milk and often sugar. Americans are large consumers of iced tea which is made from hot tea cooled with ice. In recent times in Taiwan, especially in summer, cold water (4 or 25 °C) steeping is a new popular way for making tea [6].

Several studies have been done on the various brewing conditions to maximise the extraction of these components from green tea. The results have defined optimal water brewing conditions including the temperature being maintained at 80 °C for 30 min and a ratio of tea to water of 1:20 g/mL [7]. However, these optimisation studies focused on loose-leaf green tea and were carried out under laboratory conditions. These conditions are very different from household brewing habits, where tea is simply brewed in boiled water and left at room temperature for a short time (3 min) before being consumed [8].

This study is aimed to investigate whether the antioxidant capacity of different types of tea could be affected by steeping tea bags in cold water during short time of steeping and taking into account household habits.

Methods

Tea Samples

A set of four commercial brands of green tea (Oriental, Lipton, Hindú) and combinations of green tea with certain

fruits and aromatic flowers (Jaibel with *Hibiscus sabdariffa* and lemon). They were used in the experiment for comparison of cold tea infusions. These tea samples were purchased at the local supermarket and analyzed in triplicate according with the temperature of infusion.

Sample preparation

In order to simulate beverage brewing, each brand of tea were prepared using an aqueous extraction procedure, to study the effect of different extraction condition on the total phenols, flavonoids and antioxidant activity.

Cold tea infusions were prepared by adding 150 mL of potable water at room temperature to teabag that was weighed (table 1) and leaving the infusions to stand at room temperature (27–28 °C) for 5 minutes, agitating manually every minute and controlling its temperature every 30 seconds. See figure 1.

Three extractions of each tea brands (Oriental, Lipton, Hindú and Jaibel) were performed for three consecutive days for cold extract. Just three extractions for each of the four brands were retained by type of infusion.

Samples were diluted at 10000 ppm in distilled water and stored at 4 °C for subsequent analysis.

Total phenol content (TPC)

Total phenol content of the different tea samples was determined according to the Folin-Ciocalteu method described by V. L. Sigleton, R. Orthofer and R.M. Lamuela-Raventós [9]. The samples were diluted 1:10 in distilled water, 160 µL of each sample, and standar were added to polystyrene cells of 3000 µL. Then 1420 µL Folin reagent (1:10) and 1420 µL of Na₂CO₃ 7.5% were added and homogenized, After mixing, the samples were left for 120 minutes at room temperature in the dark, the absorbance was read at 758 nm according with the spectral scanning (SHIMADZU UV-1700 spectrophotometer). All blanks contained distilled water and tea, the phenolic compounds were quantified using calibration curves of Gallic acid and expressed as mg gallic acid equivalent / g sample (mg GAE/g sample).

Kinetics of the reaction was conducted for a period of two hours with tea samples and standards to determine the stability of the complex formed according with time.

Total flavonoid content (TFC)

The TFC was determined according to D.O Kim, K.W. Lee, H.J. Lee and C.Y. Lee [10]. The solution is a mixture of

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sodium nitrite solution followed by aluminum trichloride with sodium hydroxide and distilled water, which is mixed well and the absorbance is measured against a freshly prepared reagent blank. The extract was reacted with aluminum chloride for determination of flavonoid content. A pink color appears after a few minutes which indicate the presence of flavonoids according with J. Zhishen, T. Mengcheng, and W. Jianming [11]. Briefly, 600 μ L of distilled water was added to 150 μ L of each sample of hot and cold extraction tea and stock solution of catechin into polystyrene cells of 3000 μ L, 10 μ L of 5% sodium nitrate (NaNO₃) was added. The mixture was incubated in the dark for 5 minutes. A 45 μ L volume of aluminum chloride (AlCl₃, 10%) was added to the mixture and incubated for a further 6 minutes in the dark. A 300 μ L of NaOH, 1M, was added to the resulting mixture followed by addition of 360 μ L of distilled water. The absorbance was read at 505 nm according with the spectral scanning (SHIMADZU UV-1700 spectrophotometer). The samples were diluted 2:10 in distilled water, each blank contains tea extract and distilled water.

The flavonoids contents were quantified using calibration curves of Catechin and expressed as mg Catechin equivalent/g sample (mg CE/g sample).

Kinetics of the reaction was conducted for a period of one hour with tea samples and standards to determine the stability of the complex formed according with time.

Antioxidant activity

DPPH assay

DPPH assay was carried out in photometric cells of 3mL taking into account changes in the method of O. P. Sharma [12]. A volume of 300 μ L tea cold extract (all brands were 10 diluted times) and/ or trolox standard with their respective controls; positive control was hydroquinone 1000 ppm; negative control was methanol 96%, were added to photometric cells followed by 2700 mL of a methanol solution of DPPH at 30 ppm except the blank sample. The samples were incubated in the dark at room temperature for 30 minutes (according to the kinetics of the reaction was determined for the methanolic solution of DPPH and each of the tea samples analyzed: Oriental, Hindú, Jaibel and Lipton) and read at a wavelength of 517 nm by a spectrophotometer (SHIMADZU UV-1700

spectrophotometer). The scavenging capacity was calculated as: % A.A= $[A_{c(-)} - A_{tea} / A_{c(-)}] * 100$, where $A_{c(-)}$ is the absorbance of the control and A_{tea} is the absorbance of the tested sample. Trolox was used as standard. Free radical scavenging capacities of tea were expressed as mg Trolox Equivalent/ g sample. (mg TE/ g sample).

Kinetics of the reaction was conducted for a period of one hour with tea samples and standards to determine the stability of the complex formed according with time.

ORAC assay

The antioxidant activity of tea samples were also analyzed by ORAC (Oxygen Radical Absorbance Capacity) according to the method of K. M. Gillespie [13]. Dilutions of tea extracts ranging from 80 to 50 diluted times in PBS at 75 mM having tea cold extracts (Oriental and Lipton: 80 diluted times; Hindú and Jaibel: 50 diluted times). The diluted samples were added to a 96 wells with a solid white plate followed by 187 μ L of 80 nM fluorescein diluted in PBS (phosphate buffered saline) at 75 mM. After 15 minutes incubation in the dark at 37°C a solution of 140 mM AAPH diluted in 75 mM PBS is prepared and added to each well of the plate; AAPH is responsible for starting the decay of fluorescein so it is important to have a fast and strict control of the addition, recording data every 120 seconds until decay of fluorescein, using an emission wavelength of 493 nm and a filter excitation 515 nm using a fluorescence spectrophotometer (Varian, Cary Eclipse, version 1.1 (135)). The net AUC (area under the fluorescence decay curve) for each sample/standard was obtained by subtracting the area of the blank sample (PBS). Antioxidant activity was expressed as mg Trolox equivalent/ g sample (mg TE/g sample) using the linear regression value obtained from the trolox calibration curve.

Results

Total phenol content (TPC)

The total phenolic content of the 4 brands green tea are shown in figure 2 and table 2, were quantified using calibration curves of gallic acid (1-30 mg/L) performed every day of the assay. The total phenolic compounds were found between 2.53 – 14.63 mg gallic acid Equivalent /g sample. The highest levels was measured in Oriental brand (14.63 \pm 0.53 mg equivalent gallic acid / g sample),

similar amounts were also obtained in Lipton (10.38 ± 0.55 mg gallic acid equivalent / g sample) and Hindú (9.89 ± 0.66 mg gallic acid equivalent / g sample), while Jaibel contained the lowest amount (2.53 mg gallic acid equivalent / g sample).

Total flavonoid content. (TFC)

The total flavonoid content of the tea extract were low compared with TPC, and ranged from $2.67 \pm 0.20 - 7.08 \pm 0.38$ mg catechin equivalent / g sample, (see figure 3 and table 2) and quantified using calibration curve of catechin (1-30 mg/L) performed every day of the assay. Oriental and Hindú had the highest levels compared to other brands (Oriental: 7.08 ± 0.38 mg catechin equivalent / g sample, Hindú: 6.71 ± 0.36 mg catechin equivalent / g sample), for Lipton brand was obtained a content of 4.87 ± 0.26 mg catechin equivalent/ g sample, while Jaibel contained the lowest amount (2.67 ± 0.20 mg catechin equivalent / g sample).

Antioxidant Activity

The antioxidant activity of the tea infusions was evaluated using two independent assays, DPPH and ORAC. A calibration curve of trolox (3.5 - 240 μ M) allowed to compare antioxidant activity in different brands of tea expressed as μ mol Trolox equivalent / g dry sample. The results obtained from DPPH assay reported in the figure 4 and table 2, shown values ranged from $22.36 \pm 0.98 - 41.29 \pm 0.86$ mg Trolox equivalent / g dry sample, similar to the values obtained by ORAC assay reported in the figure 5, which have a range from $22.95 \pm 1.31 - 46.25 \pm 2.05$ mg Trolox equivalent / g dry sample. The Oriental and Hindú brand tea had the highest DPPH and ORAC values while the Jaibel brand showed the lowest.

Discussion

The degree of oxidation of the leaves defines the type of tea: white, yellow, green, oolong, pu-erh and black tea. Green tea is the least processed, resulting from a quick drying of the fresh leaves, with minimal oxidation, which make it richer in bioactive polyphenols comparatively to more processed teas, where these compounds are degraded during the process. The consumption of tea, especially green tea, has several well-established health benefits, namely the reduction of the incidence of oxidative

stress related diseases and cardiovascular disorders, for example [14].

The health benefits of green tea are mainly attributed to their high phenolic content, which make these beverages one of the major sources of health promoting polyphenols in our diet [1]. Extraction is the initial and the most important step in the recovery and purification of bioactive compounds from plant materials. In general, the conventional techniques for green tea extraction are heating, boiling, Soxhlet extraction and cold extraction, which are all limited by long extraction periods and low extraction efficiency [15]. The results obtained from the analysis of the antioxidant activity of tea infusions by E. Damiani and T. Bacchetti [16] showed that green tea exhibited a greater activity when steeped for 2 h in water at room temperature.

The different affinities of the extraction solvents for total tea leaf constituents in terms of their different extraction conditions, such as polarity of extracting solvents, and temperature play an important role while investigating the phytochemical profile and antioxidant functions of tea [17].

In this study the long extraction times at room temperature is not used, since the main objective was to simulate a household tea extraction in a way that would allow to obtain the polyphenolic compounds in green tea using short time period. Total phenolic content (TPC) of green tea are presented in table 2, the highest content was Oriental (14.63 ± 0.53 mg gallic acid equivalent/g sample) and the lowest was Jaibel (2.53 ± 0.25 mg gallic acid equivalent/g sample). In general the four brands showed a decreasing behaviour Oriental > Lipton \geq Hindú > Jaibel. An analysis of variance ANOVA (tukey's multiple comparison Test, significant $p < 0.05$), identified significant differences between Oriental, Lipton and Jaibel. (a, b, c) and no significant differences were found between Lipton and Hindú (b).

E. Venditti and T. Bacchetti compared the total phenol content levels in hot and cold teas using Folin-Ciocalteu's reagent. They found that TPC is always higher in hot teas than in cold teas for green tea extracts. The exception is with white tea, where TPC is significantly higher in the cold infusion than in the hot one. In addition, TPC, in white tea prepared with cold water steeping, is significantly higher than in all other teas prepared in the same way [6]. This indicates that these extracts analysed for tea is likely to be

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getting much smaller quantities due to the extraction method. Although this only applies to green tea. As one possible reason why in our study a lower content was obtained due to temperature conditions and extraction time. The differences found between brands may be due to manufacturing process used by each industry, particle size and other compounds as if Jaibel who contains hibiscus and lemon peel.

Total Flavonoid content (TFC) of green tea are presented in table 2, the highest content was Oriental (7.08 ± 0.38 mg catechin equivalent/g sample) and Hindú (6.71 ± 0.36 mg catechin equivalent/g sample), while the lowest was Jaibel (2.67 ± 0.20 mg catechin equivalent/g sample). In general the four brands showed a decreasing behaviour Oriental \geq Hindú > Lipton > Jaibel. An analysis of variance ANOVA (tukey's Multiple Comparison Test, significant $p < 0.05$), identified significant differences between Oriental, Lipton and Jaibel. (a, b, c) and no significant differences were found between Oriental and Hindú(a). See figure 2.

Luximon-Ramma [18] showed contents from 15 to 26 mg/g for total flavonoids in nine commercially black tea, considering extractions were performed at boiling temperature. With these data reported and compared to ours extraction continues to observe a lower content of flavonoids for green tea extracts at room temperature.

The antioxidant activity of the tea infusions was assessed using two independent assays, DPPH and ORAC. The DPPH method is based on the reduction of the relatively stable radical 1,1-diphenyl-2-picrylhydrazyl, to the formation of a non radical form in the presence of hydrogen donating antioxidant. The tea samples showed antioxidant activity by the reduction of purple colored DPPH to the yellow colored diphenylpicrylhydrazine derivatives at a wavelength of 517 nm [19].

The ORAC assay originally developed by Glazer and Ghiselli [20]. The ORAC assay offers several advantages over DPPH assay. It uses peroxy radicals that are better models of antioxidant reactions with oxidizing lipids and reactive oxygen species (ROS) in foods and in vivo, and it provides continuous generation of radicals on a realistic time scale (more like actual reactions in situ). The completely hydrogen atom transfer radical quenching mechanism presents a contrast and comparison to electron transfer in DPPH assays. The assay can be adapted to detect both hydrophilic and hydrophobic antioxidants generally or specifically by

altering the radical source, solvent, and target molecule, and has been routinely automated [21].

The antioxidant activity of green tea are presented in table 2, the highest content was Oriental (41.29 ± 0.86 mg Trolox equivalent/g sample) and Hindú (38.64 ± 1.72 mg Trolox equivalent/g sample) determined by DPPH assay, while the lowest was Jaibel (22.36 ± 0.98 mg Trolox equivalent/g sample). In general the four brands showed a decreasing behaviour Oriental \geq Hindú > Lipton > Jaibel. An analysis of variance ANOVA (tukey's Multiple Comparison Test, significant $p < 0.05$), identified significant differences between Oriental, Lipton and Jaibel. (a, b, c) and no significant differences were found between Oriental and Hindú (a). See figure 3.

The results obtained by ORAC allowed to see that the highest antioxidant activity was to Oriental tea (46.25 ± 2.05 mg Trolox equivalent/g sample) and the lowest was Jaibel (22.95 ± 1.31 mg Trolox equivalent/g sample), and their behaviour of all samples was Oriental > Hindú \geq Lipton > Jaibel. Antioxidant activity of all samples showed almost the same behaviour assessed by two methods.

According to the results obtained from the analysis performed, Jaibel tea brand showed the same results for both assays (TPC and TFC). Perhaps at this extraction conditions all the phenols presented in the solution are flavonoids. See table 2. Jaibel showed the lowest levels of phenolic compounds, flavonoids and antioxidant activity, possibly due to the addition of hibiscus and lemon peel that could mask other compounds [22].

Therefore from the above results, one can deduce that antioxidant activity in the various tea brands tested is correlated with their total flavonoid content. For DPPH assay the correlation is given by $r^2 = 0.9911$ and for ORAC assay the correlation is given by $r^2 = 0.9968$. In accordance with the linear correlations is possible to have some factors that could ease the form to get results about antioxidant activity having previously analyzed the total flavonoid content. Correlation between DPPH and TFC has the equation below:

$$\frac{TFC}{0.24} + 11.53 = A.A$$

Correlation between ORAC and TFC has the equation below:

$$\frac{TFC}{0.19} + 9.34 = A.A$$

The coefficients TFC are related to the total flavonoid content expressed in mg gallic acid equivalent/g sample and A.A to antioxidant activity expressed in mg Trolox equivalent/g sample.

To date, there appear to be no thorough studies on how antioxidant activity of teas may be affected by hot or cold water steeping and how this may be related to their polyphenol content. The results obtained contribute to gaining further knowledge on how the potential health benefits of this popular beverage may be maximized by the different methods of preparation.

Acknowledgments

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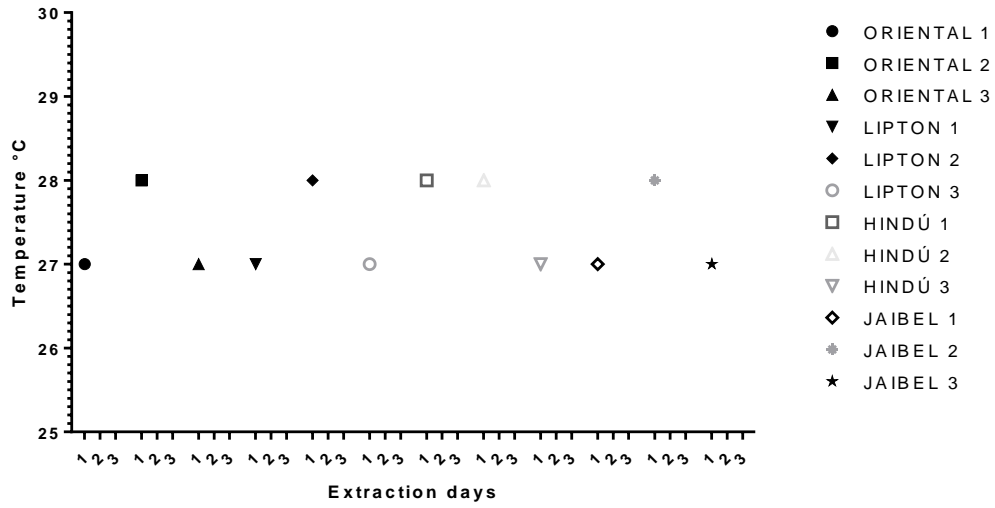


Figure 1. Temperature Control

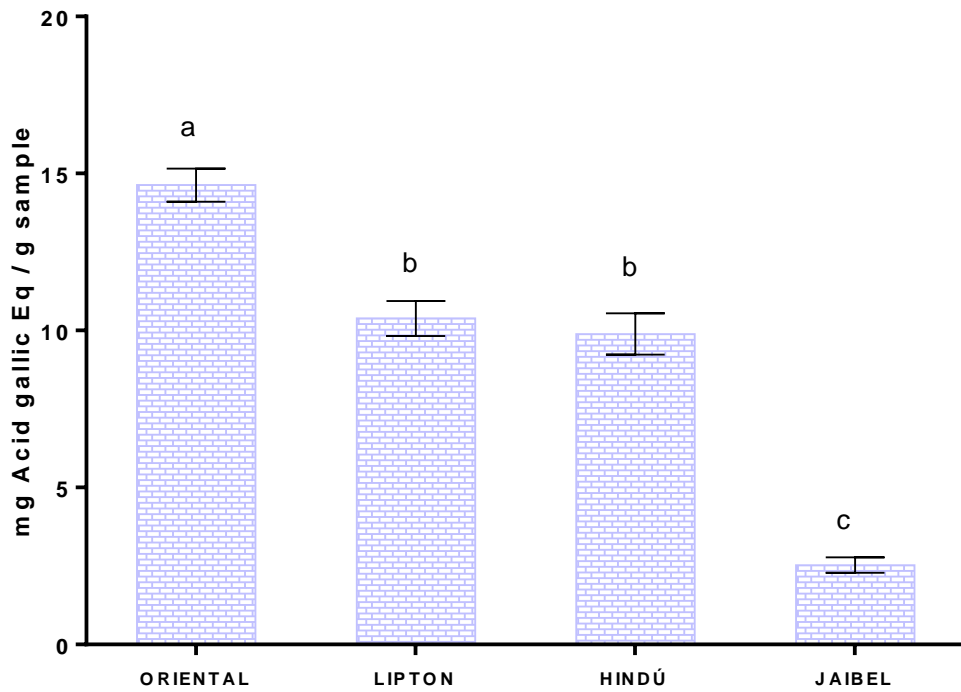


Figure 2. Total phenol content (TPC).

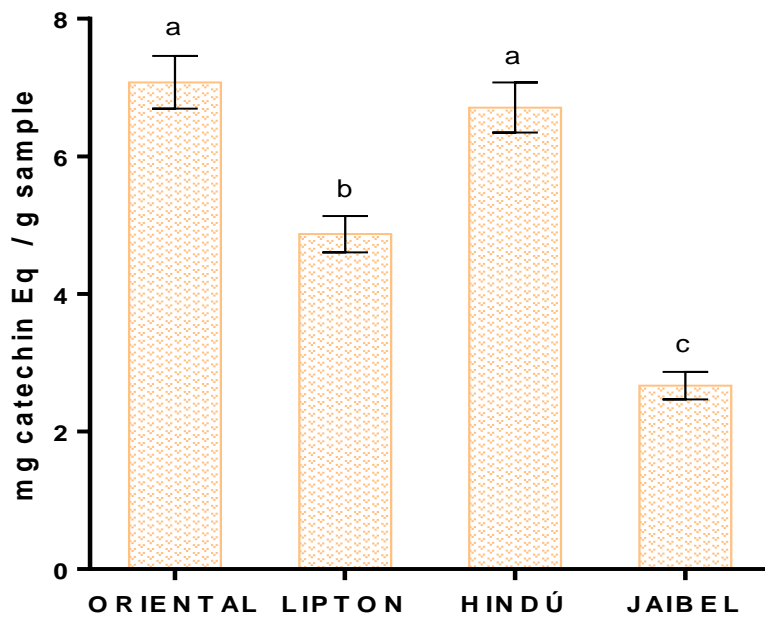


Figure 3. Total flavonoid content (TFC).

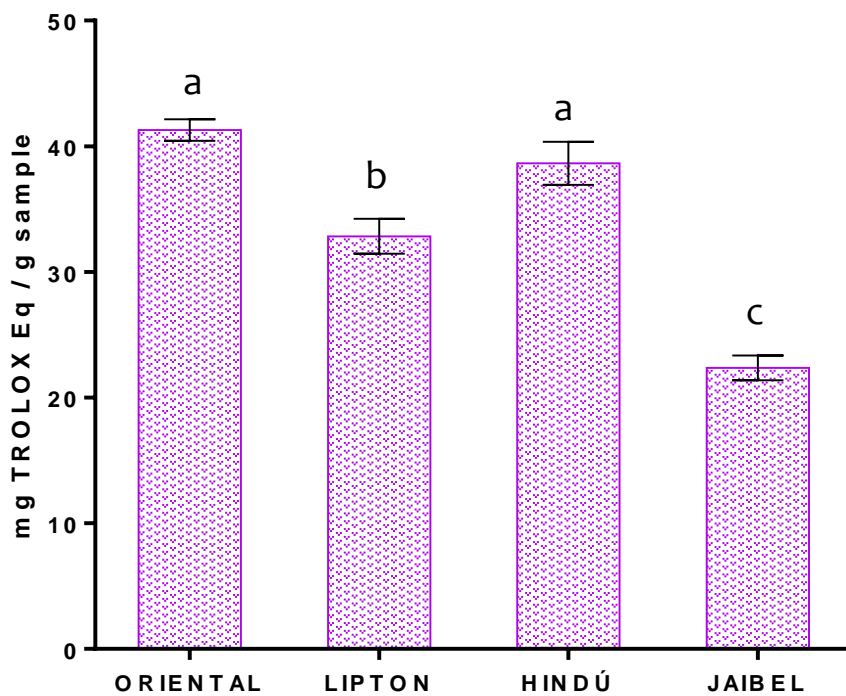


Figure 4. DPPH assay.

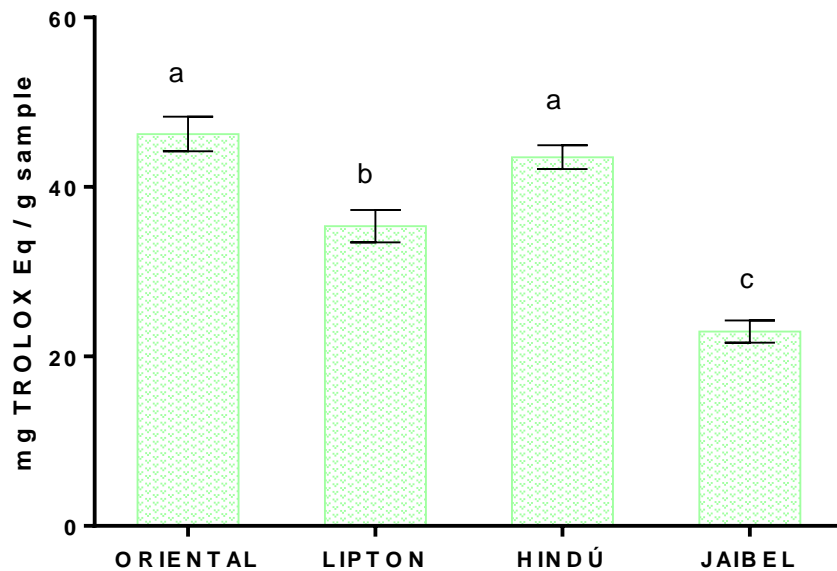


Figure 5. ORAC assay.

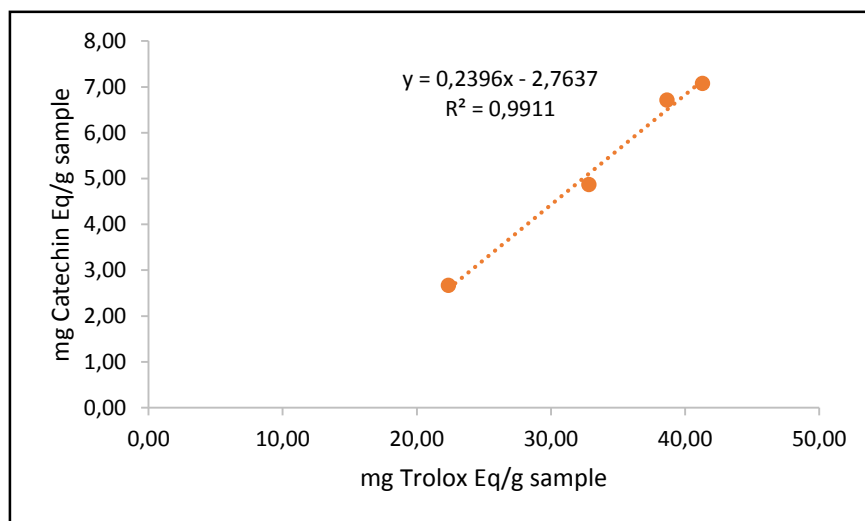


Figure 6. Correlation DPPH Vs TFC.

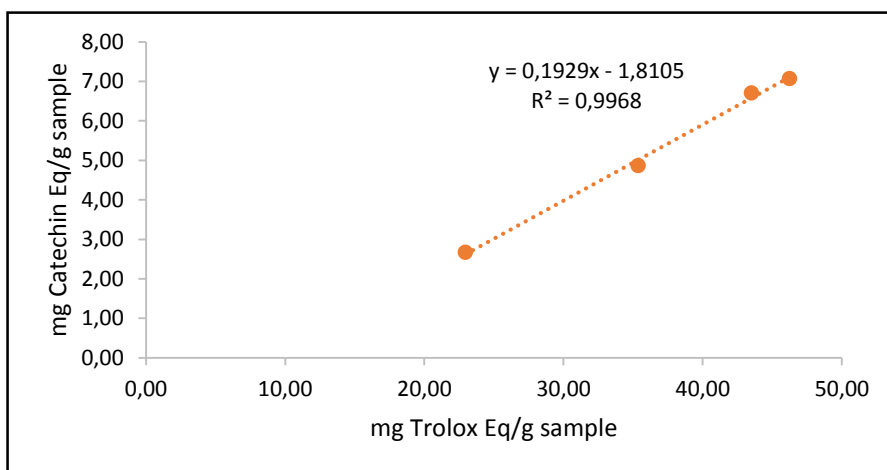


Figure 7. Correlation ORAC Vs TFC.

Table 1. Weight samples.

SAMPLE	DAY	WEIGHT (g)
ORIENTAL	1	2.0625
	2	1.9737
	3	1.9244
LIPTON	1	2.3728
	2	2.3989
	3	2.4916
HINDÚ	1	1.5817
	2	1.5918
	3	1.6317
JAIBEL	1	2.0323
	2	1.7301
	3	2.0854

Table 2. Summary, TPC, TFC, DPPH and ORAC results of green tea at room temperature.

	Total phenol content (TPC)	Total flavonoid content (TFC)	DPPH assay	ORAC assay
Sample	mg GAE / g sample	mg /g sample eq CAT	mg Trolox eq/ g sample	mg Trolox eq/ g sample
ORIENTAL	14.63 ± 0.53	7.08 ± 0.38	41.29 ± 0.86	46.25 ± 2.05
LIPTON	10.38 ± 0.55	4.87 ± 0.26	32.84 ± 1.40	35.37 ± 1.90
HINDÚ	9.89 ± 0.66	6.71 ± 0.36	38.64 ± 1.72	43.52 ± 1.40
JAIBEL	2.53 ± 0.25	2.67 ± 0.20	22.36 ± 0.98	22.95 ± 1.31