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COMPARATIVE STUDY OF PHENOLIC AND ANTIOXIDANT ACTIVITY OF CAFFEINATED AND NON-CAFFEINATED INFUSIONS

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ABSTRACT

Tea is the second most consumed beverage aside from water and has gained much attention due to its health-promoting benefits. This study aimed to quantify the levels of phenolic and antioxidant content in various caffeinated (Green tea, White tea) and non-caffeinated (Hibiscus Sabdariffa tea) infusions and identify the most suitable infusion for consumption. UV spectroscopy analytical method was used for the quantification of phenolics and antioxidants. The results of present study indicate the total phenol and total flavonoid content of teas are high, and Hibiscus Sabdadiffa show considerable amount that of caffeinated teas. The study therefore contributes to the evaluation of antioxidant activity provides evidence that non-caffeinated infusions are equally good.

KEYWORDS: Green tea; White tea; Hibiscus Sabdariffa; antioxidants; UV Spectroscopy.

INTRODUCTION

Tea is the second most consumed beverage aside from water and has gained much attention due to its health-promoting benefits, including antimutagenic, anticancer and antiapoptotic, neuroprotective, hypoglycemic and antihyperglycemic, antioxidant, antimicrobial, and inflammatory effects. These biological activities are associated in part to the antioxidant activity of chemical compounds present in teas, especially flavonoids and phenolic acids.[1-2]

Processed tea, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant *Camellia sinensi*. Two types of tea products are most widely consumed; green and black tea. In both cases, it is the chemical composition of the tea shoots and the reactions that occur during processing that determine the nature of the finished product and its quality. Though most of the tea produced in the world can be classified as non-fermented/aerated (green) tea, semi-fermented (oolong) tea and fermented (black) tea, processing has diversified to the production of specialty teas e.g. white tea, flavored teas, organic teas, decaffeinated teas, herbal teas, scented teas and various other blends. The manufacturing techniques of the above types of tea products, which may either be orthodox or non-orthodox, vary considerably and have a pronounced impact on the formative and degradative patterns of various cellular components. The conventional orthodox method which consists of rolling the leaf on a rolling bed, stretching and tearing the leaf has in some cases been replaced with non-orthodox methods or curl, tear and crush (CTC) which have a quicker and more severe leaf disruption leading to production of smaller fragments and consequently more oxidation.

In the preparation of green tea, the withered leaves are steamed and then dried relatively rapidly after plucking to minimize chemical and enzymatic reactions. This stops the polyphenol oxidase [PPO] enzyme [EC 1.10.31] catalyzed oxidation of tea leaf catechins.

White tea is a rare specialty tea that gets its name from a specific tea plant variety, as well as a particular post-harvest processing method that raises small silvery hairs on the dried buds. White tea contains a higher proportion of the buds that are covered with fine “silvery” hairs that impart a light white color to the tea. The brew from white tea is pale yellow in color with no “grassy” undertones sometimes associated with green tea. True white tea is lightly fermented, rapidly steamed and dried leaving the leaves “fresh”. Unlike black, green and oolong teas, white tea is not rolled or crushed but it is steamed rapidly and air-dried to preserve most of the polyphenols. This unique processing produces a rare and expensive but highly refreshing drink. The differences between the various processes of manufacture result in differences in the polyphenol profile between black, green, oolong and white tea.[3-5]

Though all tea variety’s had wide health benefits they do have some side effects due presence of caffeine. They include headache, nervousness, sleep problems, vomiting, diarrhea, irritability, irregular heartbeat, tremor, heart burn, dizziness, ringing in the ears,

convulsions, and confusion. The fatal dose of caffeine may cause tired blood (anemia), anxiety disorders, bleeding disorders, heart conditions, Diabetes, Diarrhea, Irritable bowel syndrome (IBS), Glaucoma, High blood pressure, Liver disease, Weak bones (osteoporosis).[6]

Hibiscus sabdariffa L. is a perennial herbaceous plant found to the tropical and subtropical zones of both hemispheres. The species is grown for its fibers and calyces, of which there are three types: green, red and dark red. The red calyces are the most commonly used type. Due to their high content in acids, vitamin C and particularly anthocyanins, the red calyces are the most used part of the plant. They are eaten as a vegetable and are used in tonic drinks and traditional medicine. The health benefits include antihypertensive properties, aid in weight loss, anti-inflammatory properties, mild anti-bacterial properties, immune booster and diuretic properties (7-12). The present study aims to do comparative study for antioxidant properties in aqueous and methanolic extract of green, white and *Hibiscus sabdariffa L.*

EXPERIMENTAL METHODOLOGY

Materials

Tea powders of Green tea, White tea were obtained from local market. The calyx of *Hibiscus sabdariffa L* (HS) were obtained from local market and authenticated by the Department of Botany Hindu Collage, Machilipatnam. Pure sample of Tannic acid and Quercetin were obtained from SD Fine chemicals, Mumbai.

Preparation of infusions

The aqueous infusion of green tea and white tea was prepared using boiling water as directed on pack. The obtained preparation was filtered for further experimentation. The infusion *HS* of was prepared by boiling 1 gm of dried calyx with 50ml of water for 15mins. The obtained preparation was filtered for further experimentation.

The methanolic infusion of green tea, white tea and was prepared by soaking 1gm powder in 100ml of methanol for 1hr. The obtained preparation was filtered for further experimentation. The infusion *HS* was prepared by soaking 1gm of dried calyx in 100ml of methanol for 1hr. The obtained preparation was filtered for further experimentation.

Estimation of total Phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. To 1mg/ml solution of crude extract 3ml of distilled water, 0.5ml of Folin–Ciocalteu reagent added and mixed thoroughly for 3min, followed by the addition of 2ml of 20%(w/v) sodium carbonate. The mixture was allowed to stand for a further 1hr in the dark, and absorbance was measured at 650nm. The total phenolic content was calculated from the calibration curve, as mg of Tannic acid equivalent per gram dry weight.

Estimation of total flavonoid content[13]

The total flavonoid content of crude extract was determined by the aluminum chloride ($AlCl_3$) colorimetric method. The 50µl of crude extract (1mg/ml ethanol) made up to 1ml with methanol, mixed with 4ml of distilled water and 0.3ml of 5%$NaNO_2$ solution, then incubated for 5min. To above mixture 0.3ml of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for 6min. Then, 2ml of 1M NaOH solution was added, and the final volume of the mixture was made to 10 ml with distilled water. The mixture was allowed to stand for 15min. and absorbance was measured at 510nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg Quercetin equivalent per gram dry weight.

Determination of Antioxidant activity by in vitro methods[14]

Hydrogen peroxide scavenging (H_2O_2) assay

A solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (50mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230nm using a spectrophotometer. Extract (20–60µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230nm is determined after 10min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

Where, A_i is the absorbance of control and A_t is the absorbance of test.

Reducing power method (RP)

The reducing power was determined by the method of Oyaizu. Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm. The 1ml of test sample solution, was mixed with phosphate buffer (2.5ml) and potassium ferrocyanide (2.5ml). The mixture was incubated at 50°C for 20min. To the mixture Trichloroacetic acid (2.5ml) added, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700nm. Ascorbic acid (20µg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates in reducing power.

Ferric reducing power assay

The 100 µL of the extract (100–500µg/ml) was mixed with 2.5ml of phosphate buffer(pH 6.6) and 2.5ml of 1%

potassium ferricyanide and incubated at 50°C for 20min. Then, 2.5ml of 10% trichloroacetic acid was added, and the tubes were centrifuged at 10,000rpm for 10min. Then, 5ml of the upper layer were mixed with 5.0ml distilled water and 1ml of 0.1% ferric chloride and the absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control.

RESULTS AND DISCUSSION

Determination of Total Phenolic Content

The total phenolic content of the infusion was calculated from the calibration curve. The calibration curve was developed by taking Tannic acid solution of 2µg/ml, to 10µg/ml concentration. The absorbance was measured at 725nm. The result was presented in table 1 and Figure 1.

Table 1: Calibration Curve Results for Total Phenolic Content.

S. No.	Concentration (µg/ml)	Absorbance
1	2.00	0.101
2	4.00	0.200
3	6.00	0.328
4	8.00	0.397
5	10.00	0.498
Slope		0.0496
Intercept		0.0075
Regression Equation		0.0496x+0.0075
Correlation Coefficient		0.9932

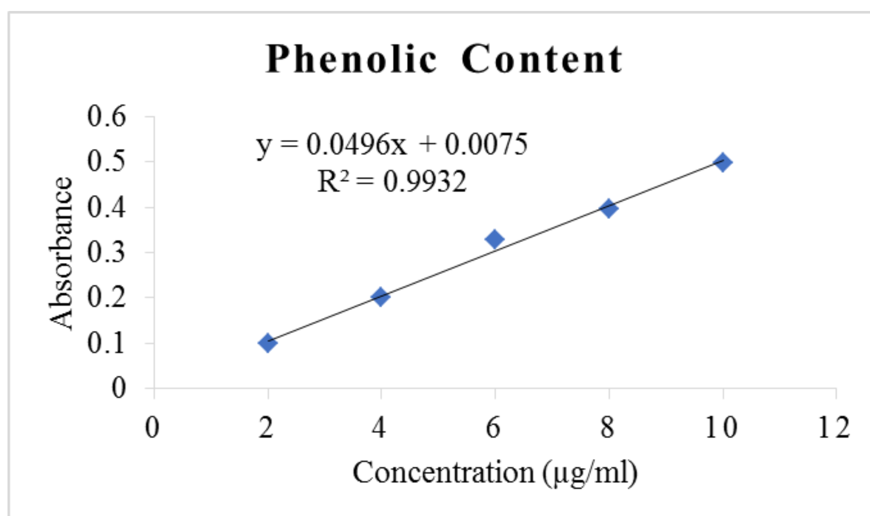

Figure 1: Calibration Curve for Total Phenolic Content.

The total phenolic content of the aqueous and methanolic infusions of White tea, Green tea & *HS* were calculated from the calibration curve (r^2 =0.99302). The values are given as tannic acid equivalents. The concentration of all samples was fixed as 1µg/ml and analysis was carried as triplicate. The total phenolic content was found to be more in aqueous white tea infusion. The results were presented in table2. The phenolic content in aqueous and methanolic extract decreased in following order: White Tea > Green Tea > HS

Table 2: Total Phenolic Content of Infusions.

S. No.	Sample	Aqueous Infusion Mean ± SD	Methanolic Infusion Mean ± SD
1	White tea	0.180 ± 0.008	0.180 ± 0.0053
2	Green tea	0.176 ± 0.007	0.167 ± 0.0017
3	*HS*	0.109 ± 0.027	0.084 ± 0.0026

Determination of total flavonoid content

The total flavonoid content of the infusion was calculated from the calibration curve. The calibration curve was developed by taking quercetin solution of 2µg/ml, to 10µg/ml concentration. The absorbance was measured at 510nm. The Correlation Coefficient (r^2) was found to be 0.99317. The calibration data was presented in Table 3 and figure 2.

Table 3: Standard Curves Table for Total Flavonoid.

S. No.	Concentration (µg/ml)	Absorbance
1	2.00	0.148
2	4.00	0.242
3	6.00	0.367
4	8.00	0.483
5	10.00	0.637
Slope		0.061
Intercept		0.0097
Regression Equation		0.061x+0.0097
Correlation Coefficient		0.9934

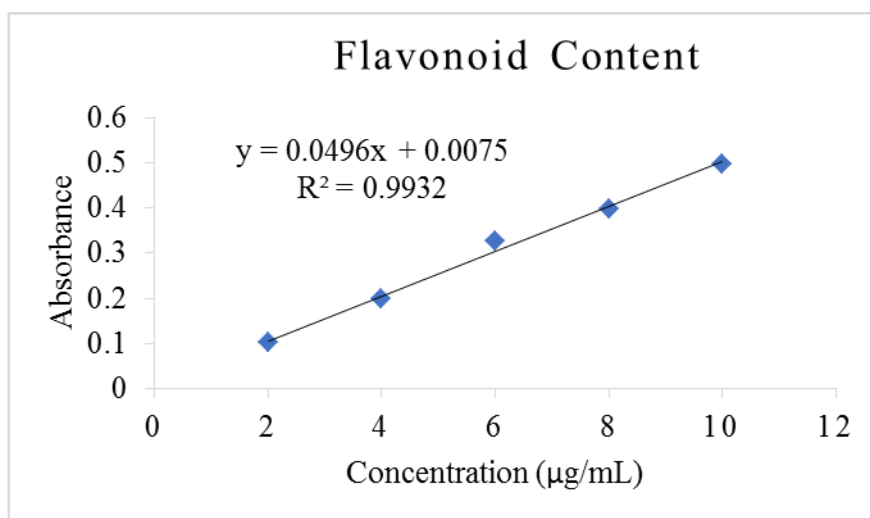

Figure 2: Calibration Curve Results for Total Flavonoid Content.

The total flavonoid content of the aqueous and methanolic infusions of white tea, green tea & *Hibiscus Sabdariffa L* were calculated from the calibration curve (r^2 =0.99317). The values are given as quercetin equivalents. The concentration of all samples was fixed as 1µg/ml and analysis was carried as triplicate. The total flavonoid content was found to be more in aqueous white tea infusion. The result is presented in table 4. The phenolic content in aqueous and methanolic extract decreased in following order: White Tea > Green Tea > Hibiscus Sabdariffa.

Table 4: Total Flavonoid Content of Infusions.

S. No.	Sample	Aqueous Infusion Mean ± SD	Methanolic Infusion Mean ± SD
1	White tea	0.0093 ± 0.0006	0.0097 ± 0.0006
2	Green tea	0.0093 ± 0.0006	0.009
3	*HS*	0.032	0.0337 ± 0.0012

Determination of Antioxidant Activity

Method 1: Ferric reducing power assay

The reducing power of the extracts was compared to ascorbic acid is shown in Table 5 and figure 3. The reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. In this investigation, the reductive capabilities infusions when compared to standard ascorbic acid methanolic infusion of green tea showed high reductive ability. The lowest activity was exhibited by aqueous white tea.

Table 5: Antioxidant activity of infusion by ferric reducing power assay.

S. No.	Sample	Absorbance (Mean)	% Inhibition
1	Control	0.089	--
2	Ascorbic Acid	0.003	96.62
3	Aq. White Tea	0.068	23.970
4	Aq. Green Tea	0.043	51.685
5	Aq. *HS*	0.053	40.075
6	Me. White Tea	0.035	60.300
7	Me. Green Tea	0.009	89.513
8	Me. *HS*	0.056	36.704

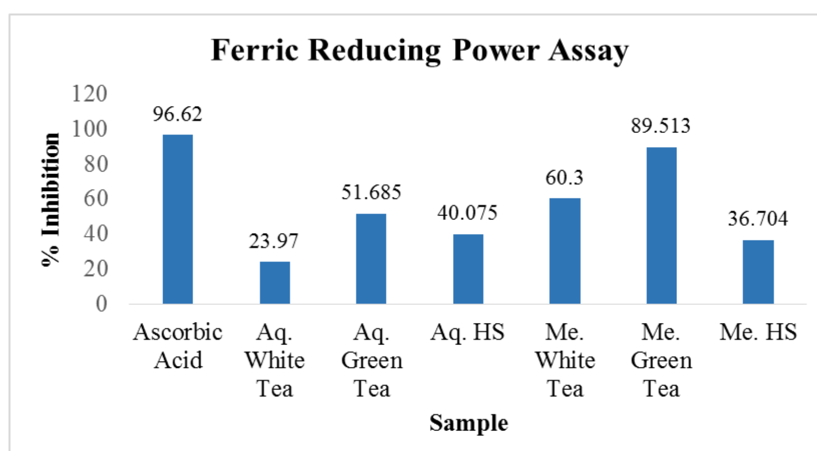

Figure 3: Antioxidant activity of infusion by ferric reducing power assay.

Method 2: Hydrogen peroxide scavenging assay

The scavenging ability of water and methanol extracts of infusion on hydrogen peroxide is shown Table 6 and figure 4 compared with ascorbic acid as standard. Of all infusions aqueous infusion of green tea showed high reductive ability. The lowest activity was exhibited by methanolic white tea.

Table 6: Antioxidant activity of infusion by Hydrogen peroxide scavenging assay.

S. No.	Sample	Absorbance	% Inhibition
1	Control	0.095	--
2	Ascorbic Acid	0.009	90.546
3	Aq. White tea	0.021	77.591
4	Aq. Green tea	0.012	87.395
5	Aq. *H. Sabdariffa*	0.018	81.092
6	Me. White tea	0.044	54.132
7	Me. Green tea	0.017	82.143
8	Me. *H. Sabdariffa*	0.021	77.591

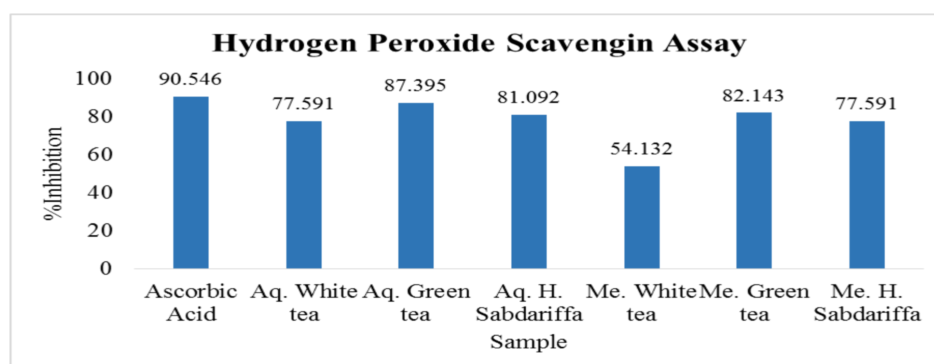

Figure 4: Antioxidant activity of infusion by Hydrogen peroxide scavenging assay.

Method 3: Antioxidant activity of infusion by nitric oxide scavenging activity

As in aerobic conditions NO is very unstable and producing intermediates (NO_2, N_2O_4, N_3O_4) reacts with oxygen. In this reaction the stable products nitrite and nitrate will also produce and peroxynitrite will produce by reacting with superoxide. Table 7 and Figure 5 explains the antioxidant ability of infusion by nitric oxide scavenging activity.

Table 7: Antioxidant activity of infusion by nitric oxide scavenging activity.

S. No.	Sample	Absorbance	% inhibition
1	Control	0.091	--
2	Ascorbic acid	0.009	90.110
3	Aq. White tea	0.022	75.458
4	Aq. Green tea	0.021	77.289
5	Aq. *H. Sabdariffa*	0.018	80.220
6	Me. White tea	0.022	75.458
7	Me. Green tea	0.020	78.388
8	Me. *H. Sabdariffa*	0.021	76.557

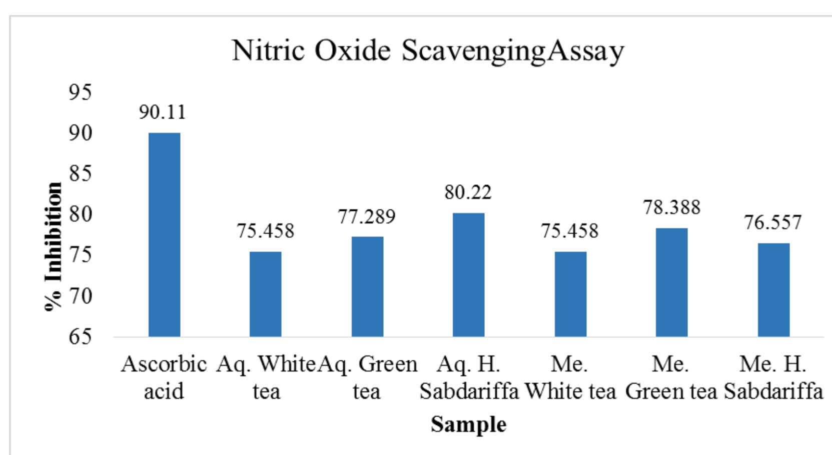

Figure 5: Antioxidant activity of infusion by nitric oxide scavenging activity

CONCLUSION

The result of present study indicates that total phenol and total flavonoid content of teas are high and Hibiscus Sabdariffa L show considerable amount that of teas. The results of tests with Hydrogen peroxide, Nitric oxide and Ferric reducing power of the extracts of tea and Hibiscus Sabdariffa L show that these extracts have a considerable radical scavenging activity, where by the activity of the Green tea is higher than that of White tea and Hibiscus Sabdariffa. It should however be noted that Hibiscus Sabdariffa has a slightly higher antioxidant activity than that of White tea. Although the study showed that the activity of Hibiscus Sabdariffa is lower than that of ascorbic acid and of tea, it provided an appraisal of the antioxidant potential. The study therefore contributes to the evaluation of antioxidant activity provides evidence that non-caffeinated infusions are equally good.

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