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COMPARATIVE STUDY OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF FAMOUS GREEN TEA BRANDS IN INDIA

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ABSTRACT: Green tea, one of the most popular beverages in India and around the world, is made from dried leaves of the *Camellia sinensis* L. tea plant. The same flavour, such as lemon and honey from each brand, has been employed to prevent errors in chemical composition caused by variations in available flavours. Samples were prepared for infusion by heating distilled water to 100°C for ten minutes. The amount of tannin, flavonoid, and phenolic compounds in total antioxidants was calculated. Tanza (TZ) had the highest concentrations of flavonoids and phenolics, measuring at 8.560667 ± 0.093383 RE/ml and 45.4233 ± 0.274 GAE/ml of sample, respectively. Tetley (TL) had the highest tannin content, 39.194 ± 0.178037 TAE/ml of sample. The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay was used to measure the free radical scavenging activity of the samples. Vahdam green tea showed the highest activity, with $80.74333 \pm 0.298124\%$; this may be because to its organic cultivation. Green tea can be consumed as a supplement to enhance general health as it is a great source of free radicals.

INTRODUCTION: One of the most popular and culturally significant drinks in the world, green tea is valued for its many health advantages in addition to its unique flavour. It is made from, minimally processed leaves of the East Asian native *Camellia sinensis* plant, an evergreen shrub. The glossy, vivid green leaves of this tea plant are distinctive for their sensitivity and freshness, and having tiny hairs on the undersides. Additionally, the plant produces fragrant, delicate, white flower that usually grow alone or in little clusters of two to four. The tea plant yields tiny brownish-green fruits in addition to its leaves and flowers.

These fruits contain one to four seeds, which can be spherical or slightly flattened. The plant thrives on acidic, well-drained soils that are typically found in tropical, humid, or subtropical areas. The inherent antioxidants and polyphenols found in fresh leaves are preserved during the process of making green tea. The leaves are immediately heated after being collected, which is sometimes done by hand to guarantee that the youngest and most fragile leaves are chosen. To deactivate the oxidation-causing enzymes, this is usually accomplished by steaming (as in traditional Japanese procedures) or pan-firing (as in Chinese techniques).

The leaves are then meticulously rolled, moulded, and dried, which improves their appearance while retaining their flavour and nutritional content. Green tea maintains a high concentration of bioactive substances, such as flavonoids and catechins, which are recognized for their

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antioxidant qualities, as a result of this minimum processing. These health-promoting ingredients, along with the beverage's refreshing flavour and cultural legacy, are major factors in its growing appeal on a global scale^{1,2}.

Tea extracts, especially those from green tea, are widely used in cosmetics and personal care products due to their many advantages for skin and hair. These include pore-refining, skin-tightening, and cleansing qualities that help create a smoother, younger-looking appearance. Green tea is also well known for its ability to strengthen hair strands, lessen dandruff, and improve the general health of the scalp by acting as a natural conditioner. Tea has a rich cultural history in addition to its health and aesthetic benefits. Tea oil, a sweet, moderately aromatic flavouring oil used in many Asian dishes, is extracted from the seeds of the tea plant. It is a versatile component in the kitchen because of its light flavour and nutritional content. Tea has also historically been used in herbal and folk medicine. Because of its inherent caffeine concentration, it has been used as a stimulant for a long time. It has also been used to improve energy levels, lessen excessive perspiration from fevers, and ease intestinal discomfort. More recently, studies have shown that tea contains a number of bioactive substances that have anti-inflammatory, antibacterial, and immune-boosting properties. The high concentration of antioxidants in tea, especially in green tea, is one of its most renowned attributes. These antioxidants, which include polyphenols and catechins, aid in shielding the skin from UV radiation-induced harm, which may help avoid skin cancer and premature aging. Tea is known for its many health benefits, including being antioxidant, antimutagenic, anticarcinogenic, and antihypertensive, in addition to its pleasant flavour and fragrant aroma. Together, these qualities make tea an effective natural remedy for enhancing health and wellbeing^{3,4}.

A broad class of chemical substances known as antioxidants is essential for preserving cellular health because it prevents oxidation reactions and neutralizes reactive oxygen species (ROS) and dangerous free radicals. Naturally occurring by products of regular cellular metabolism, especially during aerobic respiration, are these reactive compounds. Even while ROS are necessary for

some cell signalling pathways, an excessive build-up of them can result in oxidative stress, which damages proteins, lipids, DNA, and other important macromolecules.

Antioxidants are therefore essential for maintaining homeostasis and cellular defense. ROS are mostly produced by aerobic metabolic processes in higher plants, particularly in the mitochondria, peroxisomes, and chloroplasts, results in the production of partially reduced forms of molecular oxygen, including hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals ($\bullet OH$). Although ROS cannot be prevented, plants have developed a sophisticated and very effective antioxidant defense mechanism to mitigate their negative effects. Together, enzymatic and non-enzymatic antioxidants make up this plant defense system, which scavenges reactive oxygen species (ROS) and shields cellular constituents from oxidative damage. Important enzymes including glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) are examples of enzymatic antioxidants. These enzymes accelerate processes that change reactive oxygen species (ROS) into less dangerous molecules like molecular oxygen and water. Conversely, a variety of naturally occurring metabolites and secondary chemicals make up non-enzymatic antioxidants. Glutathione (GSH) and ascorbate (AsA or vitamin C) are two of the most significant water-soluble antioxidant metabolites found in plants. In the redox cycle, these compounds directly contribute to the neutralization of ROS and the regeneration of other antioxidants. The antioxidant defense of the plant is also greatly aided by a number of secondary metabolites. In addition to being essential for detoxifying ROS, compounds including polyphenols, flavonoids, tannins, and terpenoids also offer defense against environmental stresses like salinity, dehydration, UV rays, and pathogen invasion.

In response to biotic and abiotic stressors, these substances frequently build up in plant tissues, increasing the resilience of the plant as a whole. Plant antioxidants are an essential line of defense against oxidative damage, protecting cells and allowing them to survive stressful situations. Since many of these plant-derived antioxidants are also good for human health when taken as food or

supplements, their varied chemical makeup and multipurpose functions highlight their significance in both plant physiology and human nutrition⁵.

The chemical 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) is a deep violet free radical that is remarkably stable at ambient temperature when it is oxidized. Because of its stability, DPPH is a perfect reagent for assessing antioxidants' capacity to scavenge free radicals in chemical and biological samples. Because of an unpaired electron in its chemical structure, which absorbs light effectively at about 517 nm in the visible spectrum, DPPH has a vivid violet tint. The DPPH radical is reduced to a non-radical form when an antioxidant is added to the solution by giving it an electron or a hydrogen atom. The free radical is neutralized by this reduction, which results in a noticeable shift from violet to pale yellow. A UV-Visible spectrophotometer can be used to quantify this colorimetric shift quantitatively, and the drop in absorbance at 517 nm is a sign of radical scavenging activity⁶.

The current study determines the antioxidant capacity of five well-known commercial brands of green tea, each with a honey and lemon flavour by examining their capacity to scavenge DPPH free radicals. The study also sought to determine the amounts of total phenolic chemicals, which are well-known for having potent antioxidant qualities. Since flavonoids play a major role in neutralizing free radicals, the amount of flavonoids in each brand was also measured. Because tannin plays a dual role in antioxidant activity and may have an impact on the nutritional value and palatability of tea, its concentration was measured. In order to ascertain which brand provides the greatest health-promoting advantages, the study aimed to examine these properties.

MATERIAL AND METHODS:

Chemicals and Instrument: DPPH free radical, ascorbic acid, sodium carbonate, gallic acid, rutin, tannic acid, sodium nitrite, sodium hydroxide, aluminum chloride, ethanol, folinocalate reagent, and well-known green tea brands with lemon and honey flavor (Vahdam, Lipton, Tanza, Tetley, and Te-a-me) **Fig. 1.** With the help of a Shimadzu UV-1800 spectrophotometer, the absorption values were measured.

Preparation of Green Tea Infusion: For ten minutes at 100 °C, one gram of green tea sample was infused in one hundred milliliter of distilled water. Before each use, new samples were created.

Total Phenolic Content: The Slinkard *et al.*⁷ technique was used to calculate the total phenolic content (TPC). 200 µl of Folin-Ciocalteu reagent, 1 ml ethanol, 3.16 ml distilled water, and 300 µl of plant extract (1 mg/ml) were added. After 8 minutes of incubation at room temperature, 600 µl of a 10% sodium carbonate solution was added. Test tubes were then covered with aluminum foil and incubated for 30 minutes at 40°C in a water bath. An equivalent volume of water was substituted for the green tea sample in the blank. Using a UV visible spectrophotometer, the absorbance value was measured at 765 nm. The standard curve was created using gallic acid. The linear regression equation of the gallic acid standard calibration diagram has been used to calculate the data. Gallic acid is chosen because it can be quantified and because it has a major role in the physiologically active chemical composition of green tea⁸. TPC was calculated as milligrams of gallic acid equivalent (GAE) per milliliter of sample.

Total Flavanoid Content: The Ahmed *et al.* Approach⁹, which determines the total flavonoid content (TFC), was followed. 3.4 ml of 30% aqueous ethanol, 150 µl of 0.5 M aq. sodium nitrite solution, and 150 µl of 0.3 M aluminum chloride solution were added to 300 µl of extract (1 mg/ml). After 5 minutes, 1 ml of a 1 M sodium hydroxide solution was added, thoroughly shaken, and a UV visible spectrophotometer was used to measure the absorbance at 506 nm. Rutin was used for the standard curve and an equal volume of water was used for the blank. TFC was calculated as mg of rutin equivalent (RE) per milliliter of sample.

Total Tannin Content: With a few slight adjustments, the Folin-Ciocalteu method¹⁰ was used to determine the tannins. A volumetric flask (10 ml) with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, and 1 ml of 35% sodium carbonate solution was filled with approximately 0.1 mL of the sample, which was then diluted to make 10 ml with distilled water. After giving the mixture a good shake, it was left at room

temperature for half an hour. A UV/Visible spectrophotometer was used to measure absorbance at 700 nm. Tannic acid was utilized for the standard curve and distilled water was used as the blank. The amount of tannin in the sample was stated as milligrams of tannic acid equivalent (TAE) per milliliter.

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free Radical Scavenging Assay: With a small adjustment, the Brand-Williams *et al.* approach⁶ was applied. After combining 2.4 mg of DPPH with 10 ml of ethanol, the DPPH radical stock solution was created and refrigerated for later use. By diluting the stock with ethanol to an absorbance value of 0.98 (± 0.02) at 517 nm, the working solution for the DPPH was created¹¹. After adding 200 μ l of green tea infusion to 3 ml of DPPH working solution, the mixture was incubated for 30 minutes at room temperature in the dark. At 517 nm, absorbance was measured.

$$\% \text{ Antioxidant activity or \% DPPH radical scavenging activity} = [(AbC - AbS) / AbC] \times 100$$

Where, AbC and AbS stand for the absorbance of the green tea sample and the control, respectively. Instead of using the green tea sample, 200 μ l of distilled water was utilized as a control.

Statistical Analysis: Using Microsoft Excel 2007, determine the regression (r^2) value and $\pm$ SEM.

RESULTS AND DISCUSSION: An analysis of the total antioxidant content shows that the maximum quantity of tannins, phenolics, and flavanoids were present in all five samples. Tanza (Tz) had the highest phenolic content **Fig. 2**, Tanza (Tz) had the most flavonoid content **Fig. 3**, and Vahdam (V), the healthiest, had the lowest tannin concentration **Fig. 4**. DPPH radical scavenging activity was significantly present in all five green tea samples. Vahdam (V) > Lipton (L) > Tanza (TZ) > Te-a-me (TE) > Tetley (TL) represented the highest activity **Fig. 5**.

Different scientists have documented the overall antioxidant content of green tea in literature. For instance, the total phenolic, flavonoid, and tannin content have been reported to be 313.31 ± 1.41 , 16.98 ± 0.27 , and 266.79 ± 2.59 , respectively¹². The impact of infusion time was also measured, and

after 30 minutes, the total phenolic compounds and tannin concentration were reported to be 7.71% and 4.49%, respectively¹³. The phenolic and flavonoid content of green tea from several tea plant clones was measured in aqueous and acidified extracts to assess the phytochemicals of the tea¹⁴. The antioxidant potential of many samples, such as medicinal herbs¹⁵, green tea¹⁶, fruit samples¹⁷, and many others, is measured by the DPPH radical scavenging assay. The antioxidant properties of green tea's catechins¹⁸, the function of green tea's polyphenols in preventing cancer¹⁹, and the antioxidant properties of matcha¹⁶, loose leaf, and bagged green tea were also studied. The flavanol group of polyphenols known as catechins, which can make up as much as 30% of the dry leaf weight, is remarkably abundant in fresh tea leaves. Additional polyphenols consist of flavanols and their glycosides, as well as derivatives such chlorogenic acid, coumarylquinic acid, and theogallin, which is specific to tea (3-galloylquinic acid). The other common methylxanthines, theophylline and theobromine, are present in very modest levels along with caffeine, which is present at an average of 3%. Tea is also the only source of the amino acid theanine (5-N-ethylglutamine). Manganese and aluminum build up in tea.

Apart from the typical range of enzymes found in plant cells, tea leaves also have an active polyphenol oxidase that facilitates the aerobic oxidation of catechins when the structure of the leaf cell is disturbed during the production of black tea. The distinct flavor and color of black tea are imparted by the condensation processes between the different quinones created by the enzymatic oxidations. These chemicals include bisflavanols, theaflavins, epitheaflavic acids, and thearubigens. With caffeine, the majority of these substances easily form complexes. Tannic acid is not present in tea. The majority of the extractable matter in black tea is made up of thearubigens, yet little is known about their makeup. Part of the complex is composed of proanthocyanidins. Their genesis could be attributed to tea peroxidase. Numerous volatile chemicals present in the fragrance fraction of black tea are formed in part by the catechin quinines^{20, 21}. In the current study, five different brands of green tea were examined using lemon and honey as a comparison flavor.

Vahdam green tea, out of the five samples, has the most health advantages because it is grown organically, which boosts the produce's nutritional value and minimizes tannin content while optimizing its ability to scavenge free radicals. Compared to processed tea from conventional agricultural systems, organic tea has higher levels of (+)-catechin (C), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin (EC), and (-)-epicatechin-3-gallate (ECG), as well as amino acids like proline and gamma-aminobutyric acid. Organic tea has lower levels of most free amino acids, including theanine²².

Tannin precipitate proteins, block digestive enzymes, and alter vitamins and minerals and regarded being detrimental to nutrition. Certain parts of the world have high rates of oesophageal and cheek malignancies, which have also been linked to tannin components. Nonetheless, some tannin molecules have been shown to lessen the mutagenicity of several mutagens in ideal levels. There have also been reports of anticarcinogenic properties for tannins. So, tannins when present in optimal ranges are found to be good for immune system²³.



FIG. 1: DIFFERENT SAMPLES OF GREEN TEA (HONEY AND LEMON FLAVOR)

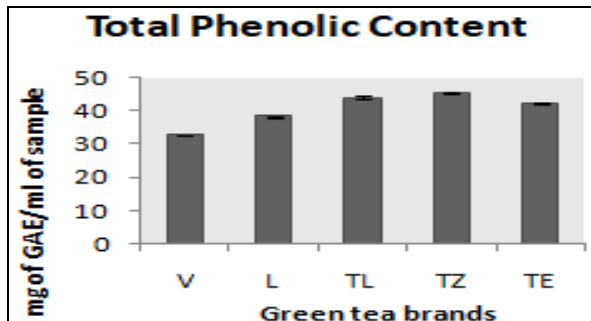


FIG. 2: TOTAL PHENOLIC CONTENT OF GREEN TEA BRANDS (V=VAHDAM, L= LIPTON, TL=TETLEY, TZ=TANZA, TE= TE-A-ME, GAE= GALLIC ACID EQUIVALENT)

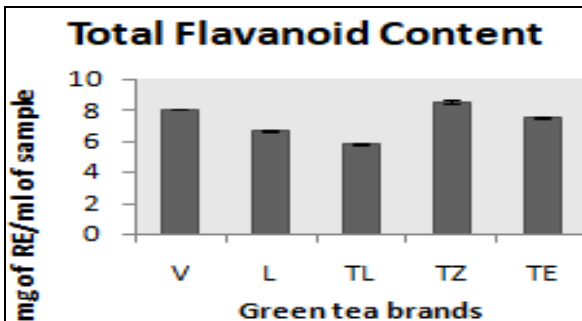


FIG. 3: TOTAL FLAVANOID CONTENT OF GREEN TEA BRANDS (V=VAHDAM, L= LIPTON, TL=TETLEY, TZ=TANZA, TE= TE-A-ME, RE= RUTIN EQUIVALENT)

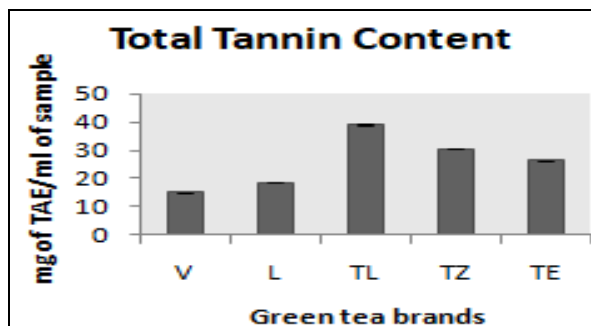


FIG. 4 TOTAL TANNIN CONTENT OF GREEN TEA BRANDS (V=VAHDAM, L= LIPTON, TL=TETLEY, TZ=TANZA, TE= TE-A-ME, TAE= TANNIC ACID EQUIVALENT)

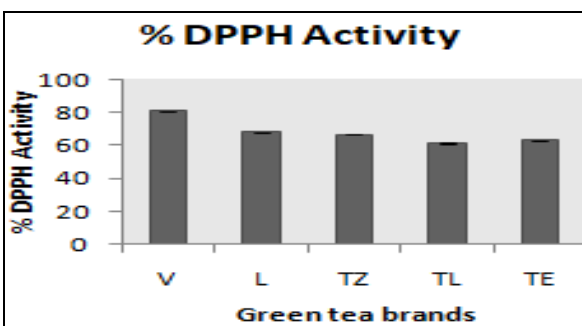


FIG. 5: % DPPH ACTIVITY OF GREEN TEA BRANDS (V=VAHDAM, L= LIPTON, TL=TETLEY, TZ=TANZA, TE= TE-A-ME, DPPH= 2,2-DIPHENYL-1-PICRYL-HYDRAZYL-HYDRATE)

CONCLUSION: The current investigation showed that all five of the examined green tea brands had notable antioxidant activity, suggesting that incorporating them into a daily diet may have health benefits. There were minor variations in several biochemical characteristics, even though the overall antioxidant concentration of the five brands was very similar. Vahdam green tea was the most successful of them all, exhibiting exceptional free radical scavenging capabilities. This increased effectiveness points to a stronger ability to counteract dangerous reactive oxygen species, which are known to damage cells and have a role in a number of chronic illnesses. Additionally, out of all the brands, Vahdam had the lowest tannin concentration. The lower tannin content further supports its acceptability for frequent ingestion, as excessive tannin levels may hinder nutrient absorption and cause stomach pain in sensitive people. According to these results, Vahdam green tea is a very good choice for a diet that prioritizes health since it offers the best possible balance between digestive friendliness and antioxidant advantages. In conclusion, because of its rich antioxidant profile, green tea in general can be considered a good natural supplement. Frequent consumption may improve metabolic health, strengthen the body's defenses against oxidative stress, and improve general wellbeing. Vahdam green tea in particular stands out as a great option for people looking to maintain or enhance their health through regular eating habits because of its low tannin content and robust antioxidant potential.

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CONFLICT OF INTEREST: NIL

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