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ESTIMATION OF PHENOLIC CONTENT IN BLACK AND GREEN TEA

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Abstract

Background: *Tea is one of the most consumed beverages in the world and is rich in natural compounds called phenols. These phenolic compounds act as antioxidants that help protect the body from damage caused by free radicals. Measuring the phenol content in black and green tea helps understand their nutritional and health benefits.* ***Objectives:*** *To evaluate the total phenolic content in various commercial black and green tea samples.* ***Methodology:*** *Using the Folin–Ciocalteu (F–C) colorimetric assay, a widely recognized method for quantifying phenolics based on their reducing capacity. Tea infusions were prepared in methanol, followed by extraction and reaction with F–C reagent in the presence of sodium carbonate. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer, and results were compared against a caffeic acid calibration curve to express phenolic concentrations as caffeic acid equivalents.* ***Results:*** *The study revealed that green tea generally possessed higher phenolic content than black tea, with Aziz Green Tea exhibiting the highest concentration (35.739 mg/L), while Millat Green Tea showed the lowest (12.474 mg/L). The higher phenolic content in green tea is linked to minimal oxidation during processing, which preserves bioactive compounds.* ***Conclusion:*** *These findings confirm that green tea possesses greater antioxidant potential compared to black tea and results also support ongoing efforts to promote polyphenol-rich diets for the prevention of chronic diseases.*

INTRODUCTION

Phenols are chemical compounds that have an aromatic hydrocarbon group (ring of carbons) joined to a hydroxyl group (-OH). These come in the form of simple phenols and polyphenols with many carbon rings Figure 1. They serve a wide range of purposes in both our bodies and the environment since they may take on a variety of forms and sizes. The health-promoting phenols that are present in food are the ones that are being covered here.

There are two types of healthy polyphenols found in food: flavonoids and non-flavonoids. One of the many micronutrients present in our food is polyphenol. They may help prevent degenerative illnesses including dementia, cancer, and cardiovascular disease.

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To function correctly, phenols must be digested, metabolized, and altered inside the body. In the process of digesting phenols, phenol sulfotransferase (PST) is the primary enzyme. You may infer from its name that this enzyme is very sulfur dependent. Thus, the body's metabolism of phenol and sulfation intersects in a significant way. To begin, let me discuss sulfation.

Sulfation can either activate or deactivate a large variety of biological molecules, and any alteration in the body's sulphate supply may have detrimental effects. A class of sulfotransferase enzymes, such as PST, transfer a sulphate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a molecule. This process is known as sulfation. Upon the transfer of that sulphate group, the compound becomes active.

Symptoms of phenol overload include; Waking in the middle of the night, Trouble falling asleep, Night sweats, Aggression, Hyperactivity, Dark circles under the eyes, Inappropriate laughter (often at nighttime), Self-stimming behavior, Head banging or self-injury (from headache), Diarrhea, sometimes constipation

Medications that target viruses and bacteria can kill them or at least half their multiplication without harming the body's healthy cells and tissues. So far, several substances that exhibit these properties have been identified. Phenols are strong antiviral and antibacterial agents in this setting as well. Phenolics, for example, halfed the development and spread of hepatitis C virus (HCV), a major blood-borne pathogen responsible for cirrhosis and hepatocellular cancer (HCC), in primary human hepatocytes, thus preventing infection (Hsu et al., 2015). Gallic acid and its derivatives have demonstrated the ability to prevent the proliferation of periodontopathic and cariogenic bacteria (Kang et al., 2008). Salmonella showed susceptibility to the pronounced antibacterial and antiviral properties of gallic acid and methyl gallate (Choi et al., 2008). They contend that these phenolics' binding to the viral proteins may prevent the virus from invading cells. Shown that (+)-epigallocatechin 3-O-gallate suppresses virus type-1 infection (Lin et al., 2000). Furthermore, phenolics including tannins, isoflavones, and stilbenes prevented the development of bacteria like Salmonella, Clostridium, Bacillus, and E. coli as well as fungi, yeasts, and viruses (Alvesalo, 2006; Cabrera, 2006; Morinaga, 2005).

The Folin-Ciocalteu (F-C) test has become the gold standard for phenolic chemical identification and quantification in a wide variety of foods and biological materials since it is easy to replicate and was first developed for use in wine analysis (López et al.,2024). Several techniques exist for determining the total phenolic content and antioxidant capabilities of fruits and vegetables through chemical processes that involve the transfer of one electron (SETs) or hydrogen atoms (HATs). As an illustration, the oxygen radical's absorbance capacity test relies on HAT, whereas the ferric reducing antioxidant power (FRAP) and F-C tests use SET reactions. Nonetheless, TEAC tests use both the SET and HAT methods. The findings of these several tests don't always line up with one another; this is especially true when looking at the SET and HAT together. In a separate study that compared several methods, the antioxidant capacity of plant extracts was linked to the estimated concentration of phenolic compounds using the F-C methodology. The total phenolic content was also shown to be strongly associated with the ferric-reducing potential as measured by the FRAP assay (R = 0.906; Dudonne et al., 2009). Because of its sensitivity and ability to measure a large variety of phenolic compounds, the F-C test is suitable for analyzing complex phenolic combinations prevalent in fruits, vegetables, and other foods. (Poly) phenols are a large class of chemical compounds that are distinguished from other antioxidants in plants by the presence of a phenol functional group, which consists of a hydroxyl (−OH) group immediately linked to an aromatic ring. Research into the F-C reaction's structure-activity relationships with the most common dietary phenolics has indicated that phenolic compounds' antioxidant activity is strongly influenced by their structural features.

This study aims to provide a comprehensive description of the chemistry of the F-C test by focusing on the reagent, the redox reaction that happens throughout the experiment, and the relationship between the fundamental elements of the principal phenolic compounds in the diet and their antioxidant activity. Similarly, a high level of adherence to a Mediterranean food plan is associated with total

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phenolic consumption when F-C measurement is used (Munoz et al., 2020).

Caffeic acid (3,4-dihydroxycinnamic acid) is one of the most abundant hydroxycinnamic acids, a subgroup of phenolic acids, found widely in plant-based foods and beverages. It is recognized for its strong antioxidant properties and plays a vital role in plant defense and human health (Silva et al., 2014). Structurally, caffeic acid consists of a catechol moiety (a benzene ring with two hydroxyl groups) attached to a propenoic acid side chain, which contributes to its redox activity and biological effectiveness Figure 2. Caffeic acid is commonly found in coffee, fruits (such as apples and berries), vegetables, olive oil, wine, and herbs. The dietary intake of caffeic acid and its derivatives has been linked to numerous health benefits, including anti-inflammatory, anticarcinogenic, antimicrobial, and cardioprotective effects (Clifford & M.N 2000)

Analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry, and UV-visible spectrophotometry are widely used for the quantification of caffeic acid in plant and food samples. In many studies, caffeic acid is used as a standard compound or expressed as an equivalent (CAE) in the determination of phenolic content (Wojdyło, Oszmiański, & Czemerys, 2007).

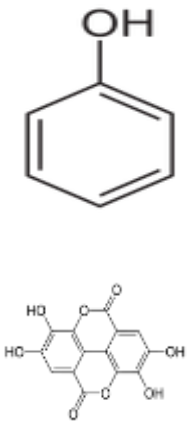


Figure 1: Phenol and polyphenol

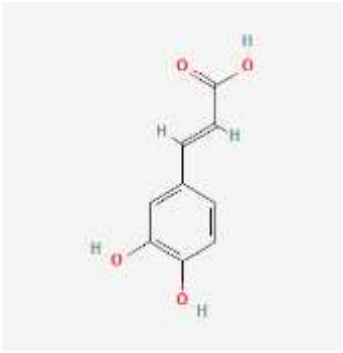


Figure 2: Caffeic Acid

MATERIALS AND METHODS

Samples Collection

Total 10 samples were selected to estimate the phenols concentration from black tea and green tea having five different brands of both tea such as black tea brands are (Millat, Arza, Tabal, Kohinor, Suppreme) while green tea brands are (Aziz, Diamond, Ziarat, Tabal, Millat).

Extraction

Phenols are first extracted into ten different brands of tea. Each sample (6g) was dipped in 40ml of methanol and stirred for one hour using a magnetic stirrer (table -1). A blue complex was produced by treating the filtrate solutions with the Folin-C reagent, which is a combination of molybdate and heteropolyphosphotungstate. The methanol-based solvent was subsequently evaporated using a rotary evaporator. The concentration of reactive phenolic chemicals in a sample is directly correlated with its blue hue intensity. To find the phenolic content, we compare the sample solution's absorbance at 765 nm to a calibration curve that contains caffeic acid as a reference.

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Table 1: Sample Extraction

Brands Name (Solute)	Weight (g)	Methanol (Solvent) ml
Arza Black Tea	6	40
Tabal Black Tea	6	40
Kohinor Black Tea	6	40
Suppreme Black Tea	6	40
Millat Black Tea	6	40
Diamond Green Tea	6	40
Ziarat Green Tea	6	40
Tabal Green Tea	6	40
Millat Green Tea	6	40
Aziz Green Tea	6	40

Apparatus

- Weighing balance
- Beaker – 100ml
- Measuring Cylinder
- Magnetic Stirrir
- Filter Paper
- Rotary Evaporator
- Aluminium Foil
- UV-Vis spectrophometer. — Using 1 cm quartz cuvettes, it is possible to measure absorbance at 765 nm.

Reagents

- Ten different brands of tea (black and green tea)
- Methanol
- Distilled water
- Folin Reagent
- Sodium carbonate solution
- Caffeic acid
- Blank solution

Preparation for Test Solutions

Preparation of calibration standard phenol solutions (Caffeic Acid Solutions):

Accurately weigh (g) five different concentrations of caffeic acid and transfer into a 100mL of beaker. Add about 10mL of distilled water and 10ml of methanol mix well in a magnetic stirrer until the solids are dissolved. All these solutions were covered with aluminium foil Figure 3.



Figure 3: Caffeic Acid Solution

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The estimated amounts of caffeic acid in each calibration standard solution are displayed in Table 2.

Table 2: Caffeic Acid Solution Preparation

Calibratiion standard solution	Volume of beaker, ml	Approximate concn of caffeic acid, mg/L
1	20	1
2	20	2
3	20	5
4	20	10
5	20	20

Preparation of sample test solution.

Transfer approximately 10 milligrams of each of the ten test materials into a 100-milliliter beaker by precise weighing. Pour in approximately 1 milliliter of distilled water. Stir in enough water to make it a diluent mixture. You can get the absorbance within the range of the calibration curve by adjusting the weight of the test material according to the total phenolic content.

Determination

Calibration Standard Solution (Caffeic acid solution)

Prepare a series of five beakers, each containing 70mL of water. Into each beaker, add 1ml of the following different concentration of calibration standard solution: (1mg, 2mg, 5mg, 10mg, 20mg) and Pour 1.5 mL of Folin & Ciocalteu's phenol reagent into each beaker. After 1–8 minutes, add 15 mL of sodium carbonate solution and stir throughly. Keeps for one hour when kept in darkness.

Blank Solution

We took 1ml distilled water, 70ml H_2O and 1.5ml Folin reagent leave it for (1-8) minutes then added 15ml sodium carbonate solution. Blank solution absorbance value is 0.0384 at 765nm.

Measurement

Measure the absorbance at 765 nm after calibrating the spectrophotometer with a blank. Repeat the process of transferring 1 mL of each reaction to a cuvette. Make sure to measure the solutions used as standards for calibration before taking readings from the test solutions samples Figure 4.



Figure 4: UV-Visible Spectrophotometer

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Sample test solution

Ten individual beakers should be filled with 70 mL of water and 1.5 mL of Folin & Ciocalteu's phenol reagent. Put 1 milliliter of each of the ten sample test solutions into a separate beaker and let them sit for one to eight minutes. Thoroughly combine 15 milliliters of sodium carbonate solution with each beaker. For one hour, keep the beaker in a dark place. Afterwards, 765 nm was used to measure absorbance changes caused by UV light.

Table 3: Sample Solution Absorbance

Concentration g/ml	Absorbance At 765nm	Blank solution value (0.0384) substracted from absorbance value
Black Tea Tabal 0.01g	1.639	1.600
Supreme Black Tea 0.01g	1.588	1.549
Millad Black Tea 0.01g	1.586	1.5476
Kohinor Black Tea 0.01g	1.595	1.556
Arza Black Tea 0.01g	1.535	1.496
Diamond Green Tea 0.01g	1.479	1.440
Tapal Green Tea 0.01g	1.684	1.645
Millat Green Tea 0.01g	1.185	1.146
Aziz Green Tea 0.01g	1.732	1.693
Ziarat Green Tea 0.01g	1.624	1.585

Table 4: Caffeic Acid Solution Absorbance

Concentration (mg/ml)	Absorbance
0.05	0.9785
0.1	1.0898
0.25	1.4215
0.5	2.0006
1	3.1816

RESULTS AND DISCUSSIONS

The experimental results of estimating the total phenolic content (TPC) of black and green tea samples are presented in this chapter. We took five different brands of black tea such as (Millat, Arza, Tabal, Kohinor, Suppreme) similarly green tea brands are (Aziz, Diamond, Ziarat, Tabal, Millat). The Folin-Ciocalteu technique was used to calculate the TPC, and a standard curve of caffeic acid was used to quantify it. An amalgam of phosphotungstic and phosphomolybdic acids is known as the Folin-Ciocalteu reagent. It forms a blue complex that may be measured by spectrophotometry when it combines with phenolic chemicals in an alkaline media. Phenolic chemicals decrease the Folin reagent in an electron-transfer-based reaction, and the intensity of the blue color corresponds to the overall amount of phenolic content (Singleton et al., 2009). To facilitate comparisons across samples, the data is usually presented in units of caffeic acid equivalents (CAE). Each gram of dried tea leaves is converted to milligrams of caffeic acid equivalents (mg CAE/g) to show the results. An industry-standard calibration curve was constructed using solutions of caffeine acid at different concentrations (0.05, 0.1, 0.25, 0.5, 1 mg/mL). A UV-Vis spectrophotometer was used to measure absorbance at a wavelength of 765 nm. Upon noting a linear relationship between concentration and absorbance, the following regression equation was derived in Table 4

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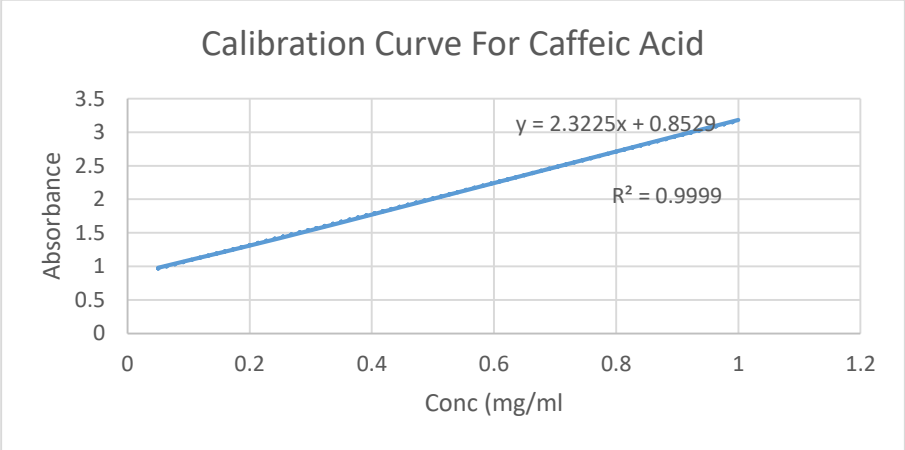


Figure 5: Caffeic Acid Graph

The TPC of the black and green tea samples was calculated using the calibration curve. The results were average after each sample was examined three times.

Total phenolic content in test material as is—Calculate the total phenolic content of the test material, in % w/w as is, as follows:

Total phenols (% w/w, as is) = [(A - b)/m] x [(V x D)/ (W x 1000)] x 100

In this context, A stands for the sample test solution's absorbance at 765 nm, b for the y-intercept of the calibration curve, m for the slope of the curve, W for the test material's weight in milligrams, V for the sample test solution's volume in milliliters, D for the dilution factor (20), and 1000 for the conversion from milliliters to L. Keep in mind that the values are given in terms of caffeic acid comparables.

Table 5: Black Tea Caffeic Acid Equivalent

Concentration mg/ml	Caffeic Acid Equivalents
Tabal Tea 10mg	31.779 mg/L
Supreme Tea 10mg	29.601 mg/L
Millad Tea 10mg	29.601 mg/L
Kohinor Tea 10mg	29.898 mg/L
Arza Tea 10mg	27.324 mg/L

Table 6: Green Tea Caffeic acid Equivalent

Concentration mg/ml	Caffeic Acid Equivalents
Diamond Tea 10mg	24.948 mg/L
Tapal Tea 10mg	33.759 mg/L
Millat Tea 10mg	12.474 mg/L
Aziz Tea 10mg	35.739 mg/L
Ziarat Tea 10mg	31.185 mg/L

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The maximum phenolic concentration (35.739 mg/L) was found in Aziz Green Tea, while the lowest was found in Millat Green Tea (12.474 mg/L). The phenolic content of green tea was often higher than that of black tea, indicating more variation between green tea brands.

This study employed the Folin-Ciocalteu (F-C) technique to estimate the total phenolic content (TPC) of green and black tea. Green tea has a substantially greater phenolic content than black tea, according to the data. These results support the generally held belief that green tea retains more bioactive chemicals because it undergoes less oxidation during processing, and they are in line with earlier research.

An aromatic hydrocarbon group is directly joined to a hydroxyl group (-OH) to form phenols, a family of chemical substances. Polyphenols are substances that contain many phenolic groups. They are known for their anti-inflammatory, anti-carcinogenic, and antioxidant qualities and are found in a variety of plant-based foods and drinks, including tea (Pandey et al., 2009).

Tea contains flavonoids, such as catechins, theaflavins, and therubigins, which are the primary polyphenols. Aflavins and arubigins are oxidised compounds found in black tea, but green tea is high in catechins, particularly epigallocatechin gallate (EGCG) (Balentine et al., 2007). The study's conclusions show that green tea has a higher TPC than black tea. This is a result of the production procedures. To prevent oxidation and inactivate the enzymes polyphenol oxidase, green tea is made by steaming or pan-frying the leaves shortly after harvest. On the other hand, fully fermented black tea has reduced quantities of unoxidized phenolics because catechins oxidise to generate arubigins and flavonoids (McKay et al., 2002).

Green tea's increased phenolic content predicts enhanced antioxidant potential since polyphenols are important contributors to antioxidant activity. This bolsters the idea that drinking frequent green tea drinking may offer more health advantages, such as a lower risk of cancer, cardiovascular disease, and neurological illnesses (Arts et al., 2005)

CONCLUSION

This research measured and compared the total phenolic content (TPC) in different brands of black and green tea using the Folin–Ciocalteu method. Results showed that green tea contained more phenolic compounds than black tea. Aziz Green Tea had the highest TPC (35.739 mg/L), while Millat Green Tea had the lowest (12.474 mg/L). The variation is due to processing—green tea keeps more natural compounds because it undergoes less oxidation. The study highlights green tea as a healthy beverage rich in antioxidants that support heart and overall health.

Author's contribution

Conceptualization: NK
Methodology: SA
Formal analysis: SB, HA
Writing review and editing: NK, JK, SN
All authors have read and agreed to the published version

Conflict of Interest

All authors declare no conflict of interest

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