Determination of Polyphenols, Reducing Potential and FT-IR Analysis of Green Tea (*Camellia sinensis* L.) Leaves Extract for Nanoparticles Synthesis

ABSTRACT

Green Tea (*Camellia sinensis* L.), is well known for its therapeutic qualities, owing to the presence of phenolic compounds. Plant extracts with significant polyphenol content have antioxidant properties that help in quenching the free radicals that are involved in oxidation of various molecules and compounds. In this study, the total phenolic, flavonoid, 1,1-diphenylpicrylhydrazyl (DPPH) - radical scavenging activity (RSA), and ferric reducing antioxidant power (FRAP) of commercially available green tea leaves was investigated for further green synthesis of silica nanoparticles. The antioxidant activity of green tea leaves is an indicator of its reducing power, an important parameter for using its extract in the synthesis of nanoparticles. Gallic acid and quercetin were used as standards in the Folin–Ciocalteu and aluminium chloride procedures, respectively, to calculate the total phenolic and flavonoid concentrations. On the other hand, ascorbic acid and gallic acid were set as standards for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing assay power procedures used to measure the antioxidant content. Fourier Transform Infrared (FT-IR) spectra was obtained to evaluate the green tea extract. The absorbance (%) of a few notable vibrational bands that are associated with chemical substances that have antioxidant qualities (polyphenols, flavonoids) was the main emphasis. The green tea leaves were found to contain high phenolic content and high antioxidant activity, indicating that the green tea leaf extract can act as a suitable capping substrate in the synthesis of silica nanoparticles ensuring the stability of the formed nanoparticles.

Keywords: Camellia sinensis L., green tea extract, phenol, flavonoid, antioxidant activity, FT-IR

1. INTRODUCTION

Tea plant (*Camellia sinensis*), which is employed in the production of green tea, is a member of the Theaceae family that is native to Asia but is extensively grown across the world [1]. Green tea is an intricate blend of polyphenols which are vital biologically active substances. The leaves, which serve as the primary ingredient in the creation of various varieties of tea, are abundant in polyphenols, they

also contain a variety of widely recognized phytochemicals, proteins, amino acids, alkaloids (such as caffeine), vitamins C and E, mineral compounds (such as potassium, fluoride, and aluminium), phenolic acids, condensed tannins, and hydrolyzable tannins, trace elements (such as zinc, magnesium, and folic acid), lipids, pigments, and aromatic compounds [2]. They are also high in purine alkaloids and saponins. Among these, Flavanols and flavonols are the primary categories of polyphenols that can be found in green tea. Together, they account for approximately 16-30% of the weight of the fresh tea leaf when it is dried [3,4]. These flavonols, also known as catechins, consist of hydroxyl groups and aromatic rings, which grant them strong antioxidant properties. Flavonoids are polyphenols characterized by a 15-carbon skeleton, with flavonols as one of their subgroups. Flavonoids and their glycosides, chlorogenic acid, gallic acid, coumarylquinic acid, and theogallin are some of the additional polyphenols that are found. Gallocatechins are catechins characterized by having three hydroxyl groups at position 3 of the ring, while catechin gallates are defined by the substitution of gallic acid at the same position [2].

The flavan-3-ols, specifically epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), epicatechin (EC), and epicatechin-3-gallate (ECG), have a significant role in the antioxidant properties of green tea (GT). The catechins, which are colourless, astringent, and water-soluble, are the major species that are rapidly oxidizable. Antioxidants are known as steady substances which furnish electrons to free radicals, stabilizing them and preventing their reactivity with other molecules [5]. The term "free radical" describes molecules that are unstable and highly reactive because they lack an electron pair in their atomic orbital [6]. Oxidative stress results from an unequal ratio of antioxidants to free radicals within cells or tissues, leading to damage in lipids, proteins, and nucleic acids [7]. To achieve stability, free radicals can either receive or donate electrons (oxidants or reductions) to other molecules, transforming the molecule into a free radicle [8].

Numerous assays are available to measure antioxidant activity, total flavonoid concentration, and total phenolic content. Several studies have investigated the relationship between total flavonoids, phenolics, and antioxidant activity, suggesting a potential linear correlation between these variables. Different methods are employed to assess the antioxidant capacity and overall reducing ability of organisms like plants and algae [9-12]. These methods fall into the hydrogen-atom and electron transfer-based categories. Examples of electron transfer-based tests include FRAP, DPPH, and Folin-Ciocalteu [13,14]. In these redox reactions, antioxidants in the sample donate electrons to the metal ion in the Folin-Ciocalteu reagent or oxidants like the DPPH radical. Therefore, the complete reducing ability of a sample can be ascertained by evaluating changes in absorbance at a specific wavelength against a standard curve. The phenolic compounds' scavenging ability may stem from the active hydrogen-donating capacity of the hydroxyl groups [15]. Additionally, the radical scavenging activity is more significantly influenced by high molecular weight, the number and proximity of aromatic rings, and the nature of hydroxyl group substitution, rather than by specific functional groups [16].

There are several ways to synthesize nanoparticles, including chemical, biological, and physical techniques. Reducing and capping agents are essential for the synthesis of nanoparticles. High radiation, highly concentrated, dangerous, and extremely poisonous chemicals are utilized as

environmental-harming reducing and stabilizing agents in chemical and physical processes. The green synthesis method, which uses plant extracts to create nanoparticles, is a very cost-effective, ecologically friendly, and sustainable procedure. Because green synthesis is a one-step process, the nanoparticles produced have adequate size, greater stability, and a diversity of different natures. During the synthesis from corresponding aqueous salts, a redox reaction occurs. Extracts from various organisms contain reducing agents that transfer electrons to metal ions, leading to the production of nanoparticles [17-19]. Plants with higher levels of total reducing compounds are expected to yield increased concentrations of nanoparticles.

2. MATERIAL AND METHODS

The plant material used was a commercial brand green tea (GT), obtained from market and used as the sample for each test. To enhance extraction efficiency, the dried GT leaves were crushed using an autoclaved mortar and pestle.

2.1 Total Phenolic Content (TPC)

The green synthesis of nanoparticles is carried by phenolic compounds because they have higher antioxidant activity and antioxidants are excellent at reducing metal ions. The total phenolic content (TPC) of the sample was measured [20]. 0.5 mL of methanolic extract was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% Na_2CO_3 solution. The mixture was then incubated at 45°C for 45 minutes. After incubation, the absorbance of samples at 765 nm was measured using methanol as the blank. The TPC was determined by extrapolating from a calibration curve created using a gallic acid solution. The estimation of phenolic compounds was done in three sets. TPC was expressed in milligrams of gallic acid equivalents (GAE) per gram of dried sample.

2.2 Total Flavonoid Content (TFC)

When creating metal nanoparticles from their metal salt precursors, flavonoids are helpful as reducing and electrostatic agents. This allows for the green production of NPs [21]. The total flavonoid content (TFC) was ascertained through aluminium chloride test technique [22]. 0.2 mL of 5% $NaNO_2$ was combined with 2 mL of methanolic extract. After five minutes, 0.2 mL of 2% $AlCl_3$ was added to the mixture and allowed to stand for six minutes. The entire volume was then adjusted to 5 mL after adding 2 mL of 1N NaOH and the mixture was incubated at room temperature for 15 minutes. Methanol was used as blank. The absorbance was measured at 510 nm. Quercetin served as the standard reference for quantifying total flavonoids. The total flavonoid content of each extract is expressed in (mg QE) per g of dry weight.

2.3 2,2-Diphenyl-picrylhydrazyl (DPPH) Radical Scavenging Assay

The percentage loss of the initial color concentration method was used to determine the free radical scavenging activity [23]. The solution consisted of methanol (3 mL), tea extract (0.5 mL), and DPPH solution (0.3 mL, 0.5 mM) in methanol. The mixture was incubated in the dark at 25°C for 45 minutes. Methanol solution was used as the control blank. The percentage inhibition of DPPH was calculated by measuring the absorbance at 517 nm.

The following formula was used to calculate the DPPH radical scavenging capacity:

% scavenging capacity = [(Abs Control) – (Abs sample)/(Abs Control)]*100

Where Abs Control = Absorbance of the control

Abs sample = Absorbance of the sample

This radical scavenging capacity was stated in ascorbic acid equivalents per gram of dry tea (AAE/g), determined by extrapolation from a calibration curve of L-ascorbic.

2.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant strength of green tea was estimated by the following method. The FRAP reagent was prepared by mixing 40 mM HCl in a 10:1:1 ratio with 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride hexahydrate, and 10 mM 2,4,6-Tris(2-pyridyl) (TPTZ) s-triazine. For every 50 µL of green tea sample, 100 µL of FRAP reagent was used. In the same way, ddH_2O (50 µL) was used in place of the tea sample to prepare a blank. This mixture was incubated for 10 minutes at 25°C i.e. room temperature. Following incubation, absorbance was measured at 593 nm using UV-Visible Spectrophotometry [24]. The FRAP value is in milligrams of GAE per gram of dried sample. This value is calculated based on the standard curve of gallic acid.

2.5 FT-IR Analysis of Green Tea Extract

20 grams of GT were added in 500 mL of deionized water to create the tea extract. To extract the tea, the mixture was cooked to 80°C in a water bath. The extract was then cooled and filtered using Whatman filter paper. The filtered solution was gathered and stored in a sterile, dry beaker for FT-IR spectroscopy analysis.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content (TPC)

The total polyphenol content was determined using the Folin-Ciocalteu method. The assay results indicated that the dried leaves of GT contained 11.51±0.36 mg GAE/g phenolic content levels expressed as gallic acid equivalent. Similar findings were reported by Ramírez-Aristizabal et al. (2017) and Chakraborty et al. (2015) high content of polyphenols was observed in GT leaf extract [25,26]. Research has shown that dried plant materials exhibit higher levels of antioxidant activity and antioxidants like polyphenolics compared to fresh plant materials [27]. This is because the drying process can break down cellular components, allowing phenolic compounds to be released from the food matrix more quickly [28,29]. In dried samples, destructive enzymes are inactivated due to low water activity, resulting in significant concentrations of phenolic compounds [30]. Polyphenols, the primary family of chemical substances contributing to antioxidant action, consist of one or more hydroxyl groups attached to an aromatic ring. The highest concentration of polyphenols was found in GT due to the presence of flavan-3-ols such as catechin (C), EGCG, ECG, EGC, and EC [31].

3.2 Total Flavonoid Content (TFC)

The total flavonoid content of green tea was measured using the aluminium chloride colorimetric technique. The assay results showed that green tea leaves contained 4.73±0.31 mg QE/g of flavonoids. Our results showed alignment with data obtained by Ramírez-Aristizabal et al. (2017) and Fawwaz et al. (2022) for flavonoid content [25,32]. Bioflavonoids, such as catechin and its derivatives, have exceptional antioxidant properties [33]. According to Higdon and Frei (2003), catechins are a class of phenolic and flavonoid molecules that are chemically unstable [34]. Yuan et al. (2009) and Honzel et al. (2008) reported that catechins easily oxidize in solution, losing hydrogen atoms and forming oxidized quinone products as well as a semiquinone radical intermediate [17,18].

3.3 2,2-Diphenyl-picrylhydrazyl (DPPH) Radical Scavenging Assay

One of the most often used methods for assessing antioxidant activity is the radical scavenging method known as DPPH. In this technique, antioxidant compounds react with the DPPH solution, which acts as a free radical. By donating electrons, these compounds can neutralize the free radical, thereby converting the atom with an unpaired electron into a non-radical form [19]. The assay results indicated that green tea has a DPPH radical scavenging capacity of 36.45±2.45 mg AAE/g. Studies by Ramírez-Aristizabal et al. (2017) and Singh et al. (2022) emphasised that the green tea have free Radical Scavenging capacity [25,35]. GT's powerful antioxidant properties are attributed to the three adjacent hydroxyl (OH) groups on the β-ring of its catechins, such as gallocatechin gallate (GCG), EGCG, and gallocatechin (GC). These catechins are more efficient at scavenging free radicals compared to those with only two adjacent OH groups, such as epicatechin (EC) and catechin gallate (CG). Green tea has substantially higher levels of EGCG and EGC [36].

3.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The modified ferric ion reducing antioxidant power (FRAP) method was utilized to assess the total antioxidant power of green tea. The results revealed significant FRAP levels in green tea was 13.86±0.48 mg GAE/g. Our results also supported study by Singh et al. (2022), which have reported that the FRAP levels were found to be significantly augmented in green tea [35]. FRAP is a degree of compounds' ability to behave as electron donors (Chan et al., 2007) and green tea contains numerous chemicals capable of transferring electrons [37]. The presence of reductones is associated with its reducing power [38]. According to Gordon (1990), reductones function as antioxidants by donating a hydrogen atom to break the chain of free radicals. GT contains various polyphenols, with higher levels attributed to flavan-3-ols such as C, EGCG, ECG, EGC and EC. Phenolic compounds exhibit strong reducing power and can donate an electron to convert ferric ion Fe^{3+} to ferrous ion Fe^{2+} [39].

3.5 FT-IR Analysis of Green Tea Extract

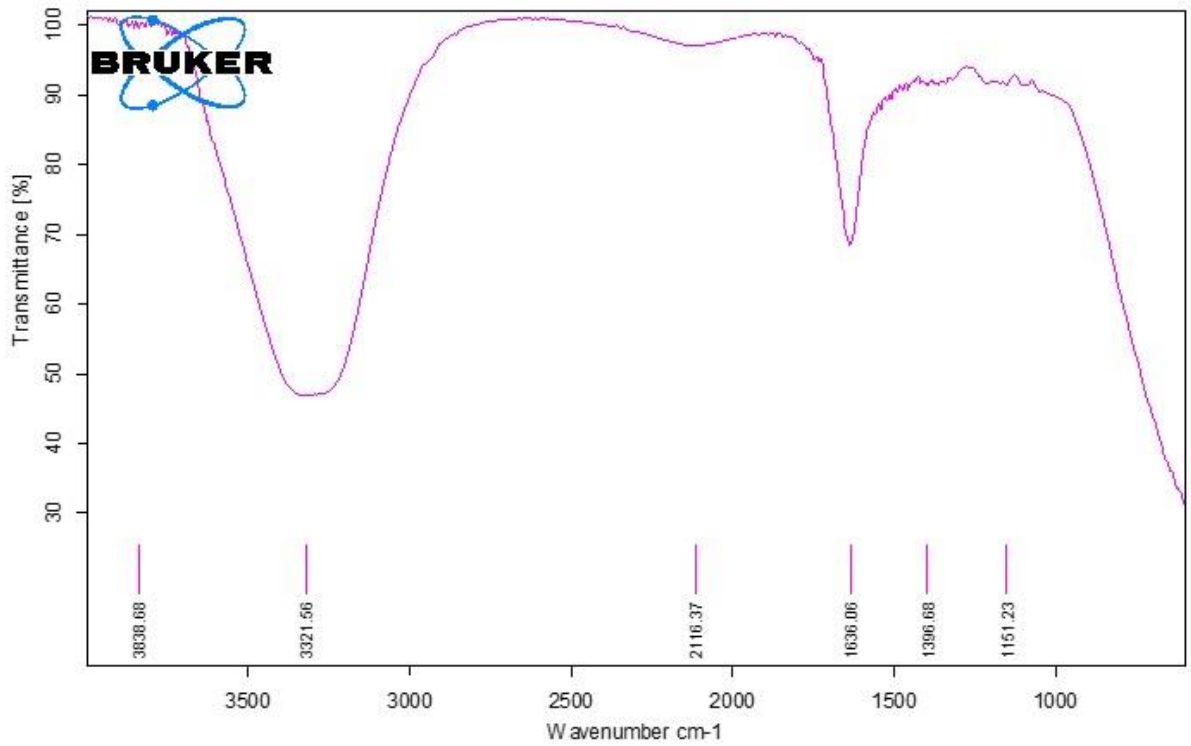

Fig .1 : **FT-IR Analysis of Green Tea Extract**

The FTIR spectra of GT extract reveal absorption bands for L-theanine that appear at 1151 cm-1. Tea plants are known to contain L-theanine, a special type of amino acid that is water soluble and non-protein based [40,41]. The band at 1396 cm–1 is caused by the C–N stretch of amide–I in protein [42]. The stretching vibration of the C=C (aromatics) and C=O (carbonyl) bonds found in flavonoids, polyphenols and catechins is attributed to the strong defined band at 1636 cm−1. The source of the 2116 cm-1 vibrations was C-O stretching [43]. The broad band seen in GT extracts in the 3321 cm–1 region is indicative of the OH stretching of phenolic and flavonoid components [44,45]. The stretching of the hydroxyl group and O-H bond is responsible for the peaks that occur at 3838 cm^{-1} [46]. Therefore, it can be seen from the FTIR spectrum that the GT sample contains high levels of proteins, amino acids and polyphenols. Flavonoids or phenolic compounds may act as reducing agents, and proteins may act as capping or stabilizing agents to prevent agglomeration and give stability [32, 33].

4. CONCLUSION

This study showed that the phenolic and flavonoid content of green tea (*Camellia sinensis* L.) leaf extract demonstrated strong antioxidant activity. The antioxidant properties were FRAP assay, TPH, TFC, DPPH radical scavenging assay. The extract's FTIR spectra displayed bands that corroborated the presence of these chemical components. Thus, it can be concluded that green tea possesses notable antioxidant activity, as well as substantial total phenol and flavonoid content, that will act as reducing agents leading to the production of nanoparticles, indicating the suitability of GT leave

extract for the further synthesis of nanoparticles. We believe that this work could stimulate the synthesis of various nanoparticles, that have a wide range of applications.

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