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 renowned for its therapeutic properties due to its phenolic compounds. Extracts from plants with high polyphenol content possess antioxidant properties that neutralize free radicals involved in the oxidation of various molecules. This study investigated the total phenolic and flavonoid content, 1,1-diphenylpicrylhydrazyl (DPPH) radical scavenging activity (RSA), and ferric reducing antioxidant power (FRAP) of commercially available green tea leaves for potential use in the green synthesis of silica nanoparticles. The antioxidant activity of green tea leaves demonstrates its reducing power, which is crucial for utilizing its extract in nanoparticle synthesis. Gallic acid and quercetin served as standards in the Folin–Ciocalteu and aluminum chloride methods, respectively, to measure total phenolic and flavonoid concentrations. To determine antioxidant content ascorbic acid and gallic acid were standards for the DPPH free radical scavenging and FRAP assays. Fourier Transform Infrared (FT-IR) spectra was performed to evaluate the green tea extract. The results revealed green tea leaves exhibited high phenolic, flavonoid content and significant antioxidant activity. The presence of few notable vibrational bands in FT-IR graph also confirmed the existence of chemical substances that are associated with antioxidant qualities (polyphenols, flavonoids). Indicating that the green tea leaf extract can act as an effective reducing and capping agent in the synthesis of silica nanoparticles.

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

1. INTRODUCTION

Tea plant, (*Camellia sinensis*) is indigenous to Asia and a member of the Theaceae family. It is cultivated extensively all around the world and used to make the commercialized product green tea [1]. Green tea is an intricate blend of polyphenols which are vital biologically active substances [2]. The leaves, which serve as the primary ingredient in the creation of various varieties of tea, are abundant in polyphenols, they additionally contain a range of commonly known proteins, amino acids, alkaloids, and phytochemicals such as caffeine, vitamins C and E, mineral compounds (such as potassium, fluoride, and aluminum), phenolic acids, condensed tannins, and hydrolyzable tannins, trace elements (such as zinc, magnesium, and folic acid), lipids, pigments, and aromatic compounds [3]. Of these, the two main types of polyphenols present in green tea are flavanols and flavonols. 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, whereas the replacement of gallic acid at the same position defines catechin gallates [3]. Favonols, also known as catechins, consist of hydroxyl groups and aromatic rings, which grant them strong antioxidant properties [4]. Flavonoids are polyphenols characterized by a 15-carbon skeleton, with flavonols as one of their subgroups [5]. Together, they account for approximately 16-30% of the weight of the fresh tea leaf when it is dried [6,7].

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) [8]. The catechins, which are colorless, astringent, and water-soluble, are the major species that are rapidly oxidizable. Antioxidants are known as steady substances that furnish electrons to free radicals, stabilizing them and preventing their reactivity with other molecules [9]. "Free radical" refers to molecules that have no electron pair in their atomic orbital, making them very reactive and unstable [10]. Oxidative stress results from an unequal ratio of antioxidants to free radicals within cells or tissues, leading to damage to lipids, proteins, and nucleic acids [11]. To achieve stability, free radicals can either receive or donate electrons (oxidants or reductions) to other molecules, transforming the molecule into a free radicle [12].

Numerous assays are available to measure antioxidant activity, total flavonoid concentration, and total phenolic content [13]. Several studies have investigated the relationship between total flavonoids, phenolics, and antioxidant activity, suggesting a potential linear correlation between these variables [14]. Different methods are employed to assess the antioxidant capacity and overall reducing ability of organisms like plants and algae [15,16]. These techniques belong to the categories of electron transfer and hydrogen atom-based techniques. Tests based on electron transfer include DPPH, Folin-Ciocalteu, and FRAP [17,18]. 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 [19]. The phenolic compounds' scavenging ability may stem from the active hydrogen-donating capacity of the hydroxyl groups [20]. Additionally, the radical scavenging activity is more significantly influenced by high molecular weight, the number and proximity of aromatic rings, and the type of substitution of hydroxyl groups, as opposed to certain functional groups [21].

There are several ways to synthesize nanoparticles, including chemical, biological, and physical techniques. Reducing and capping agents are essential for the synthesis of nanoparticles [22]. High radiation, highly concentrated, dangerous, and extremely poisonous chemicals are utilized as environmental-harming reducing and stabilizing agents in chemical and physical processes [23]. 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 [24]. During the synthesis from corresponding aqueous salts, a redox reaction occurs [22]. Reducing compounds found in a variety of organism extracts transmit electrons to

metal ions, resulting in the formation of nanoparticles [25]. Plants with higher levels of total reducing compounds are expected to yield increased concentrations of nanoparticles [26].

2. MATERIAL AND METHODS

The following reagents were used: Methanol (HiMedia), Sodium carbonate anhydrous (HiMedia), Gallic acid monohydrate (HiMedia), Sodium nitrite (HiMedia), Sodium Hydroxide (HiMedia), L-Ascorbic acid (HiMedia), Hydrochloric acid (HiMedia), Ferric chloride hexahydrate (HiMedia), Sodium acetate anhydrous (HiMedia), 2,4,6-Tripyridyl-S-Triazine (Central Drug House), 2,2-Diphenyl-1-Picrylhydrazyl (Sigma-Aldrich), Folin & Ciocalteus Phenol (Sisco Research Laboratories), Acetic Acid Glacial (Sisco Research Laboratories), Aluminium Chloride Hexahydrate (Sisco Research Laboratories), Quercetin Dihydrate (Sisco Research Laboratories). All reagents used were of Analytical grade. The plant material used was a commercial brand of green tea (GT), obtained from the 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. For the preparation of methanolic extract 2 gram of powdered green tea leaves was added in 100 ml methanol and kept at room temperature in the dark for 24 hr. The extract was filtered through Whatman No.1 filter paper, and stored in refrigerator for further use.

2.1 Total Phenolic Content (TPC)

The green synthesis of nanoparticles is facilitated by phenolic compounds due to their high antioxidant activity, which makes them effective reducing agents. The total phenolic content (TPC) of the sample was assessed [27]. 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. The average TPC was expressed in milligrams of gallic acid equivalents (GAE) per gram of dried sample.

2.2 Total Flavonoid Content (TFC)

Flavonoids are essential as reducing and electrostatic agents in the synthesis of nanoparticles from their precursors, facilitating the eco-friendly production of nanoparticles (NPs) [28]. The total flavonoid content (TFC) was measured using the aluminum chloride test method [29]. 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 a 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 free radical scavenging activity was evaluated by measuring the percentage decrease in the initial color concentration [30]. 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. The 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 using 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 [31]. 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

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. Similarly, the other findings also confirmed the presence high content of polyphenols in GT leaf extract [32,33]. Research has shown that dried plant materials exhibit higher levels of antioxidant activity and antioxidants like polyphenolics compared to fresh plant materials [34]. This is because the drying process can break down cellular components, allowing phenolic compounds to be released from the food matrix more quickly [35,36]. In dried samples, destructive enzymes are inactivated due to low water activity, resulting in significant concentrations of phenolic compounds [37]. Polyphenols are the primary compounds responsible for antioxidant activity,

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 [38].

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 other finding confirming the presence of flavonoid in the green tea also plays a significant role in its antioxidant activity [39]. Bioflavonoids, such as catechin and its derivatives, have exceptional antioxidant properties [40]. Catechins are a class of phenolic and flavonoid molecules that are chemically unstable [41]. Catechins easily oxidize in solution, losing hydrogen atoms and forming oxidized quinone products as well as a semiquinone radical intermediate [22,25].

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, a free radical, by donating electrons. This reaction neutralizes the free radical, converting it into a non-radical form [26]. The assay results indicated that green tea has a DPPH radical scavenging capacity of 36.45±2.45 mg AAE/g. 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) [42]. 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 [43].

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 the previous studies that the FRAP levels were found to be significantly augmented in green tea [42]. FRAP is a degree of compounds' ability to behave as electron donors and green tea contains numerous chemicals capable of transferring electrons [43,44]. The presence of reductones is associated with its reducing power. Reductones function as antioxidants by donating a hydrogen atom to break the chain of free radicals [45]. 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+} [46].

3.5 FT-IR Analysis

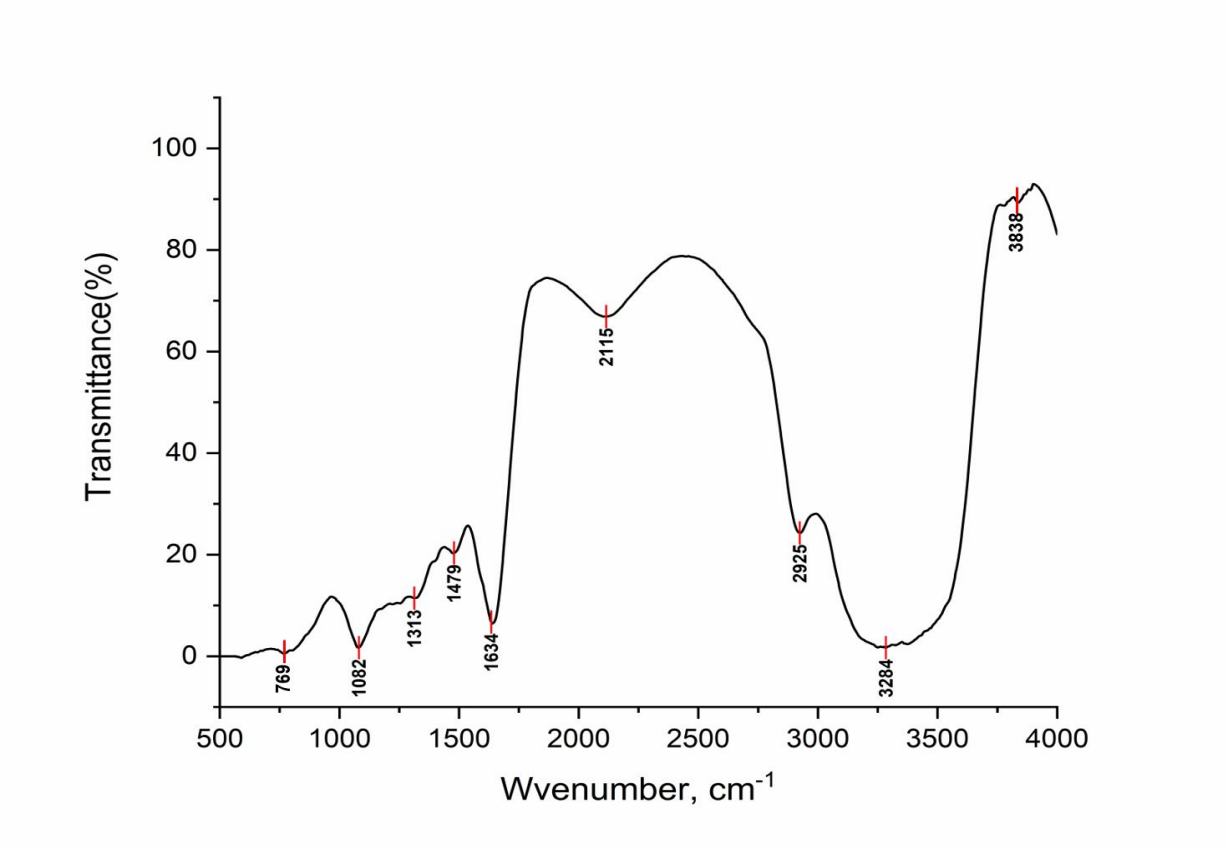

Fig.1: FT-IR Analysis of Green Tea Extract

The FTIR spectra of GT extract revealed a weak band at 769 cm^{-1} is the result of out of plane vibrations attributed to aromatic C−H bonds [47]. The absorption bands in the position of 1082 cm^{-1} can be caused by C–OH stretching which belongs to amino acid or alcohol [48]. The band at 1313 cm^{-1} is caused by the C–N stretch of amide–I in protein [49]. A C−C stretch (aromatic) band was also detected at 1479 cm^{-1} [50]. The stretching vibration of the C=C (aromatics) and C=O (carbonyl) bonds found in flavonoids, polyphenols and catechins gallic acid, catechin, or L-theanine is attributed to the strong defined band at 1634 cm^{-1} [50,48]. The source of the 2115 cm^{-1} vibrations was C-O stretching [46]. At 2925 cm^{-1}, a band associated with the vibration of the C−H stretch or O−H stretch of a carboxylic acid bond was detected [51]. The broad band seen in GT extracts in the 3284 cm^{-1} region is indicative of the OH stretching of phenolic and flavonoid components [52,53]. The stretching of the hydroxyl group and O-H bond is responsible for the peaks that occur at 3838 cm^{-1} [54]. Therefore, it can be seen from the FTIR spectrum that the GT sample contains high levels of proteins, carboxylic acid, 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.

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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