ISSN 2277-2685
IJPSL/May. 2023/ Vol-13/Issue-2/1-15
B. Swapna *et. al* International Journal of Pharmacetical Sciences Letters

Biological Synthesis and Characterization of Silver Nanoparticles of *Camelia Sinenesis* and Evaluation of Its Biological Activity

B. Swapna[*1] K.B. Chandrasekhar[2,] D. Madhuri[3], G. Sumathi[4]

1. Research Scholar, Department of Chemistry, Jawaharlal Nehru Technological University Anantapuramu. Anantapur, Andhra Pradesh, India.
2. Vice Chancellor of Krishna University & Professor in Chemistry; Machilipatnam, Andhrapradesh. India.
3. Creative Educational Society's College of Pharmacy, Kurnool, Andhrapradesh.
4. Ananthalakshmi Institute of Technology, Anantapuramu.

Corresponding Author: B. Swapna

Email: 303swapna@gmail.com

Abstract

Green tea leaf aqueous extracts of varying volumes (5ml, 10ml, 15ml, 20ml, 25ml) are outlined for the green synthesis of silver nanoparticles using a 1Mm silver nitrate solution. UV-visible absorption spectroscopy, FTIR, SEM, and HPLC are utilized to evaluate synthesized nanoparticles. The synthesis was predicated on the phenomenon of surface plasmon resonance, as determined by UV- spectra at 200-400 nm, which confirmed the presence of silver nanoparticles. The Fourier-transform infrared (FTIR) spectrum confirms the presence of Amine, Alcohol, Aldehyde, Aromatic, C-H bending, and C-O bending functional groups as concentrations of green tea leaf extracts increase. The Scanning Electron Microscopy (SEM) analysis reveals a helical structure of silver nanoparticles ranging in size from 5 to 100 nm, which exhibits good antioxidant and antimicrobial properties against pathogens such as E. coli and *Staphylococcus aureus*, as well as good antimicrobial activity.

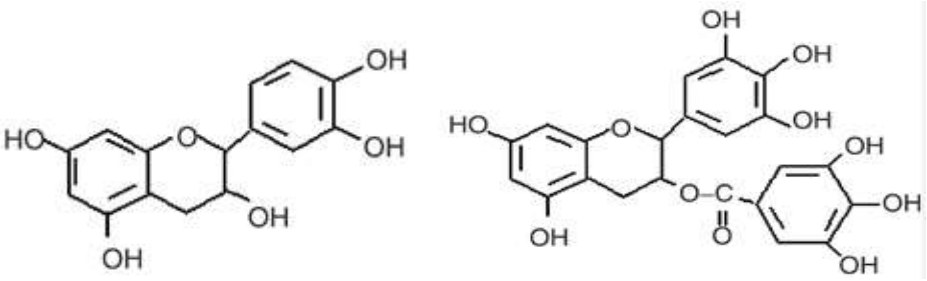
Key words: Green tea leaf aqueous extract, Silver Nanoparticles, UV-VIS, FTIR, SEM, HPLC, Antioxidant activity and antimicrobial activity.

1. Introduction

Globally, green tea is one of the most consumed beverages in the culinary industry. Its consumption has been firmly linked to preventive medicine, as numerous studies have reported significant health advantages. Such as digestion-enhancing, anti-inflammatory, antimicrobial, and antioxidant properties. Acceleration of the metabolic rate, regulation of triglyceride levels, and prevention of the distress caused by obesity, diabetes, cardiovascular disease, and cancer. (1,2). These benefits are a direct result of the polyphenol content of tea, which acts as an antioxidant by reducing oxidative stress in cells and lipid peroxidation, among other mechanisms of action. (3). Thus, new quality standards have become more stringent by requiring multiple and effective polyphenols characterization techniques, including high-performance liquid chromatography (HPLC), ultraviolet-visible, scanning electron microscopy (SEM), and Fourier transform infrared resonance spectroscopy (FTIR) (4, 5). These methods have demonstrated reliability and precision. However, operation costs and analyte management are expensive and time-consuming. Due to the presence of alkaloids (caffeine) and catechin derivatives, green tea has diverse pharmacological effects (6-8). Due to its antioxidant photoprotection, ageing and antiwrinkle, skin whitening, skin infection, hair growth, and antidandruff properties, green tea is extensively used in cosmetic formulations. Ongoing research aims to enhance or uncover the potential biomedical benefits of green tea. (15). The advantages of plant extracts in nanomaterials synthesis are as follows: (a) they are very inexpensive; (b) they are readily available; (c) there is no danger of contamination; and (d) extract preparation does not require expertise, intensive labor, or complicated equipment. In this context, a number of studies have demonstrated that plant extract-directed, antibiotics-incorporated nanomaterials and nanoclusters exhibit potent antimicrobial activity against a variety of microorganisms. (16-23). The analysis of scanning electron microscopy (SEM) reveals the helical structure of silver nanoparticles ranging in size from 5 to 100 nm, which exhibits excellent antioxidant and antimicrobial properties against pathogens such as E. coli and Staphylococcus aureus, as well as good antimicrobial activity.

Green tea (Camelia sinenesis) Unfermented tea is green tea. Tea is an infusion of leaves that has been ingested as a beverage for centuries and is valued for its medicinal qualities. Alkaloids, saponins, tannins, catechin, and polyphenols were identified through a phytochemical analysis of tea (24). Numerous microorganisms are susceptible to the antimicrobial effects of tea leaves. Green tea contains between 30 and 40 percent of polyphenols that can be extracted with water. According to previous research, four

polyphenol compounds, Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallocatechin (EGC), and Epicatechin (EC), are potent antioxidants (25). Recent interest has been drawn to the use of plant extracts as a reducing and encapsulating agent for metallic nanoparticles due to the protocol's speed, simplicity, environmental friendliness, and economic viability. (26-28). In contrast, biogenic or biological routes to synthesize metallic nanoparticles, such as silver nanoparticles, are becoming an economically viable alternative in the field of green chemistry (29,26,30). Biogenic synthesis of metallic nanoparticles is known to be straightforward, environmentally benign, cost-effective, and scalable. Phytochemicals and aqueous environments additionally supplant chemical compounds and organic solvents, respectively. (31). For the synthesis of metallic nanoparticles, plant extracts such as seed powder, fruit, bran, rind, bark, flower, and leaf are currently utilized. The presence of phenolic compounds, amino acids, and vitamins confers medicinal value upon plant extract. (27-32). The green tea plant (Camellia sinensis) is an abundant source of polyphenolic compounds. Green tea is primarily composed of the reducing and capping agents' epigallocatechin, epigallocatechin-3-gallate, epicatechin, and eoicatechin-3-gallate (33,34,35). This plant is a member of the Theaceae family and is abundant in bioactive phytochemicals that make it an effective antiseptic, anticancer, and antimicrobial agent. (36). Green tea's chemical composition is complex and incompletely defined. Polyphenols, specifically flavonoids such as catechins, catechin gallates, and proanthocyanins, are the most prevalent constituents of green tea. The fresh leaves contain caffeine (approximately 3.5% of the total dry weight or about 50mg/cup when brewed), the bromine (0.15-0.2%), theophylline (0.02-0.045%) and other methyl xanthene's, lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%), and free amino acids (1-5.5%), in addition to the unique amino acid theanine (4%), numerous flavor compounds are also present in much lower amounts. Many of the biological benefits of green tea have been attributed to the catechin fraction, which accounts for up to 30 percent of the leaf's dry weight. (37).



A) Epicatechin B) Epigallocatechingallate

Figure 1: Structure of Epicatechin and Epigallocatechingallate.

Antibacterial activity of nanoparticles against various bacterial isolates was demonstrated at concentrations that were non-toxic to mammalian cells. Using plant leaf extracts, this study confirms previous findings regarding the biosynthesis of nanometals with potent antibacterial properties and high biocompatibility.

Taxonomic profile:

Taxonomy of *Camelia sinenesis*	
Kingdom	*Plantae*
Phylum	*Spermatophyta*
Subphylum	*Angiospermae*
Class	*Dicotyledonae*
Order	*Theales*
Family	*Theaceae*
Genus	*Camelia*
Species	*Camelia sinenesis*
Kingdom	*Plantae*

2. MATERIALS AND METHODS

2(a) Plant collection

Fresh green tea leaves were procured from the Mangalore plantation for this experiment. Supermarket green tea leaves and green tea particles were purchased. Air-dried, chopped, and pulverized into powder were the fresh green tea leaves. Green tea leaves and green tea particles were directly brewed.

Figure 2: Dried green tea leaf

2(b) Aqueous extraction

In a 250ml sterile conical flask, 10g of each of the pulverized leaves were extracted by soaking for two days in 100 ml of distilled water. The filtrates were then concentrated using rotavapor, placed in universal flasks, and chilled to 4oC prior to use. As a preliminary investigation, aqueous extraction was conducted.

Figure 3: Aqueous Extraction of Pulverized green tea leaves.

2(c) Methanol Etraction:

Ten grams of powdered samples of each were successively extracted with 70% and 50% methanol to generate crude extracts containing a broad spectrum of active compounds. The extracts were made by macerating plant material with solvents for two days in a shaker. The respective extracts were filtered through Whatmann No. 1 filter paper and desiccated in rotavapor at temperatures below 450°C under reduced pressure to produce a dense residue.

2(d) Test Organism:

This experiment's pathogens were obtained from Kurnool Medical College. Both Escherichia coli and Streptococcus aureus are bacterial strains. The bacterial strains were maintained by

weekly transfer into nutrient broth, and the fungal strains were maintained in Sabouraud's dextrose broth.

2(e) Minimum inhibitory concentration:

The minimal inhibitory concentration (MIC) of crude extracts was determined using the broth dilution method. In sterile test containers, 5 milliliters of sterile nutrient were added to a loopful of test bacterial strains. Increasing concentrations of leaf extract were introduced to each test tube (20l, 40l, 60l, 80l, 100l). The contents of the containers are gently shaken to ensure that the leaves extract is thoroughly mixed. The test containers were incubated for 24 hours at 370C. A control tube containing no test organisms was maintained.

2(f) Synthesis of Silver nanoparticles:

Using a pipette, 60 ml of green tea extract was transferred to a volumetric flask, diluted to ml, and homogenized by hand. The resulting solution was made alkaline by adding a minute amount of potassium carbonate (K_2CO_3) until the PH reached 10 (pH=10). Then, 20 ml of the silver nitrate stock solution was added all at once. It can be seen below in **Figure 4**.

Figure 4: Synthesis of Ag-NP (Silver Nanoparticles) from reaction between $AgNO_3$ and green tea extract.

3. Characterization of silver Nanoparticles

Various techniques, including colour change, UV- Visible Spectroscopy, Scanning Electron Microscopy (SEM), Infrared Spectroscopy, and High-Performance Liquid Chromatography, were used to characterize the synthesized silver nanoparticles (HPLC).

3(a) Formation of Silver nanoparticles:

The colour change was observed visually in the Erlenmeyer flask containing green tea extract and AgNO3 solution.

3(b) UV-Visible Spectroscopy:

Uv-Visible Spectroscopy confirmed the reduction of silver ions in the colloidal solution. A small sample of Ag Nps was placed in a quartz cuvette and scanned between 200 and 400 nm using distilled water as a reference. After adding green tea extract to AgNO3 solution, the UV-Vis absorption spectrum of the sample was measured using a Perkin Elmer Spectrophotometer at various times (5, 10, 15, 20, 25 min) and concentrations (20l, 40 l, 60 l, 80 l, 100 l).

3(c) Scanning Electron Microscopy:

By means of scanning electron microscopy, the surface morphology of silver nanoparticles was observed. After 4 hours of reaction, the sample was prepared by centrifuging colloidal solution at 12000rpm for 4 minutes. The particle was dispersed in deionized water before being centrifuged once more. The procedure was repeated four times before being completed with an acetone cleaning. To create the suspension, purified silver nanoparticles were sonicated for 10 minutes. A very small amount of the sample was deposited on glass plates and then allowed to dry at room temperature to produce the thin coatings. The sample was exposed to light until it was thoroughly dry. The produced sample was analyzed with the Zeiss 700 Scanning Electron Microscope using SEM.

3(d) Fourier transform infrared (FTIR) Spectroscopy

Three-dimensional transform infrared (FTIR) spectroscopy: For the infrared Fourier transform spectroscopy analysis, the residual solution was centrifuged at 10,000 revolutions per minute for 10 minutes. To eliminate any residual free biomass, the resulting suspension was resuspended in 1 mL of sterile distillated water. After purification, the suspension was

freeze-dried to produce powder. Mixing dried Ag NPs with potassium bromide (KBr) and recording the spectra with a Perkin Elmer Spectrum.

3(e) High performance liquid chromatography (HPLC)

When dissolved in acetone, the sample exhibited a broad retention peak at 10.233 and 9.097 minutes. A climax of low intensity was observed at 2.21 and 11.398 minutes.

4. Results

4(a) UV Spectroscopy:

UV-Visible spectrometers are commonly used to confirm the formation of AgNps in a colloidal solution by observing metallic nanoparticles' surface phenomena. This optical property is sensitive to the nanoparticles' size, shape, concentration, and agglomeration state. (40) The change from a yellowish to a brownish hue indicates the formation of AgNps, as depicted in fig. The UV scan of the silver nanoparticles in green tea solution revealed a distinct Gaussian-shaped peak at 21nm.

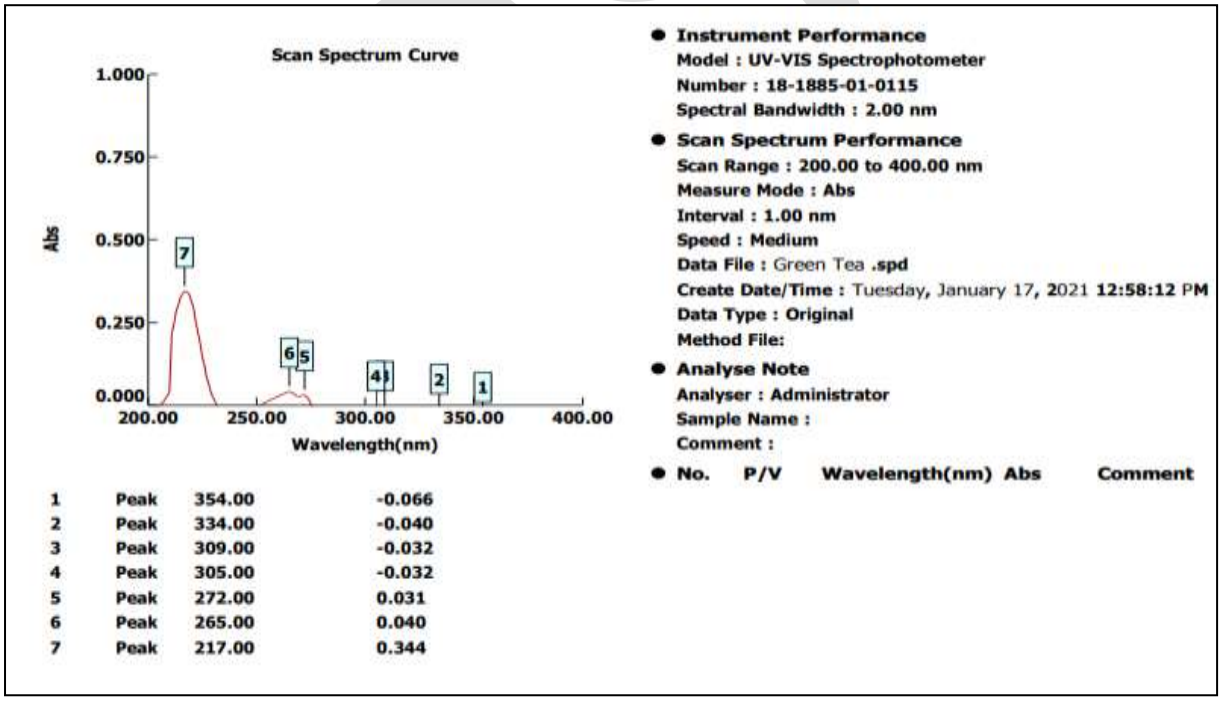


Figure 5: UV-Visible Spectra of AgNPs

4(b) FTIR analysis:

The FTIR spectra of desiccated green tea AgNPs and green tea extracts are illustrated. In **Figure 6**. By correlating the absorption bands to the corresponding compounds, the photochemical constituents involved in the reduction and capping of AgNPs were studied. Green tea extract exhibited distinct peaks at 3308, 2983, 2851, 1655, 1383, 1106, 1038,

and 969 cm -1. The broad band at 3308 cm -1 is attributable to the O-H stretching of alcohol in polyphenols and the N-H stretching of amines. The broad bands at 2983 cm -1 and 2851 cm -1 are the result of C-H stretching in alkanes and O-H stretching in carboxylic acids, respectively. The peaks at 1106 and 1038 cm -1 correspond to C-O-C stretching, and the peak at 969 cm -1 corresponds to C=C bending. Green tea aqueous extract phytochemical analysis reveals the presence of polyphenols including gallic acid (GA), Gallocatechin (GC), Catechin (CE), epigallocatechin, protein, flavonoid, saponin, and glycosides. Green tea AgNPs exhibit peaks at 2919, 1609, 1244, 1017, and 753 cm-1 in their FTIR spectra. The peaks at 2919 cm -1 and 1609 cm -1, respectively, correspond to (C-H) stretching and N-H bending. By comparing the FTIR spectra of green tea and green tea AgNPs, variations in their chemical constituents can be identified. The peaks at 1244 and 1017 cm-1 can be attributed to C-O stretching and C=O ketone stretching, respectively, while the peak at 753 cm-1 corresponds to C-H bending.

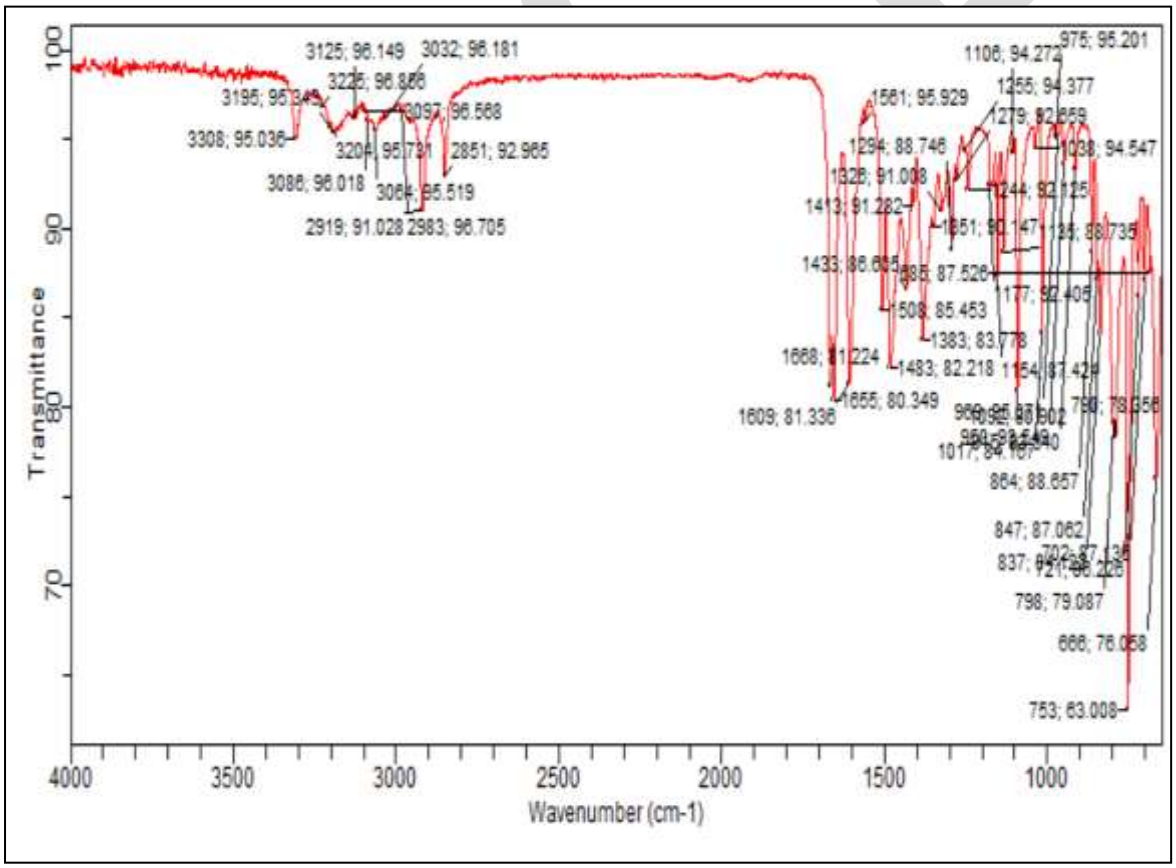


Figure 6: FTIR analysis of AgNPs

4(c) SEM analysis

The SEM images of silver nanoparticles in green tea with varying sizes 70 nm is predominately dispersed as aggregates. The nanoparticles were not in direct contact with one another within the aggregates, which can be attributed to the stabilizing effect of the extract's

encapsulating agents. It is known that these phytochemicals actively reduce and stabilize metal nanoparticles (41).

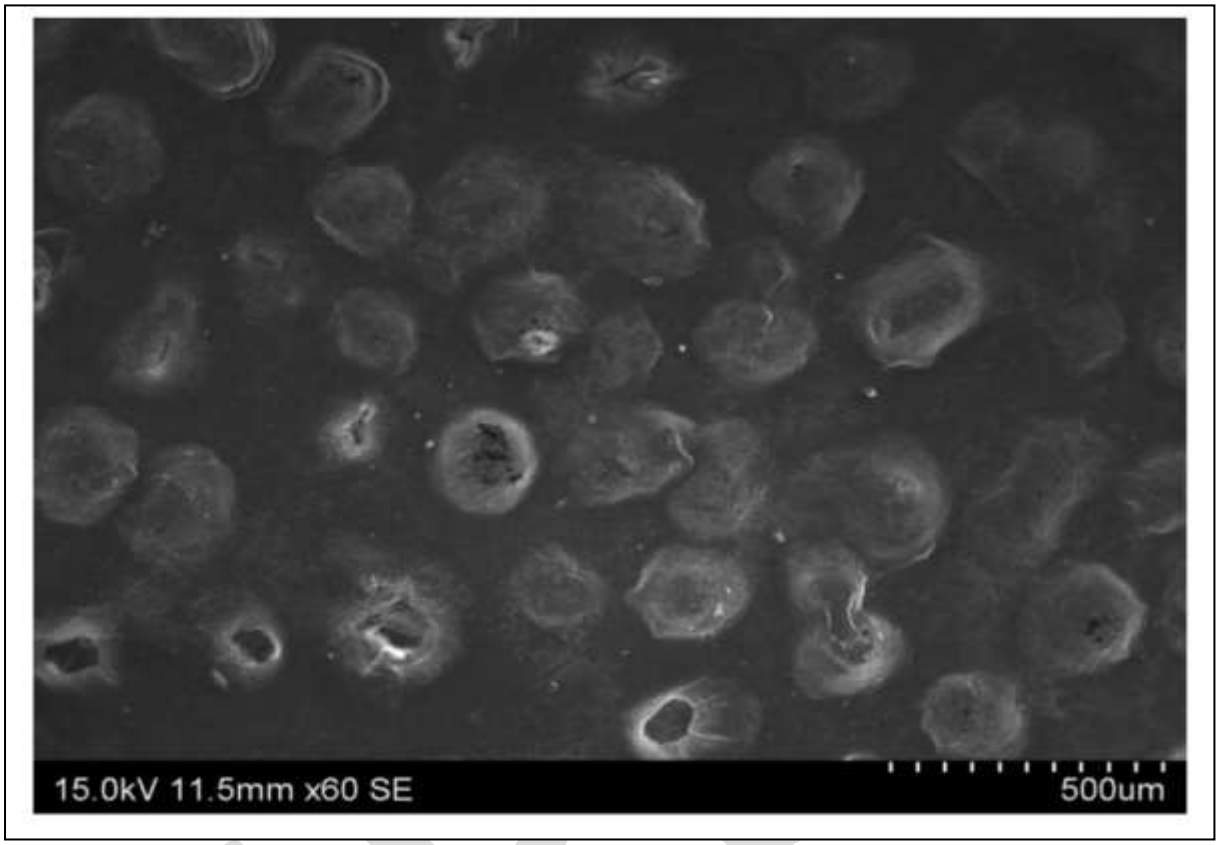


Figure 7: Scanning Electron Microscope analysis of AgNPs.

4(d) HPLC analysis:

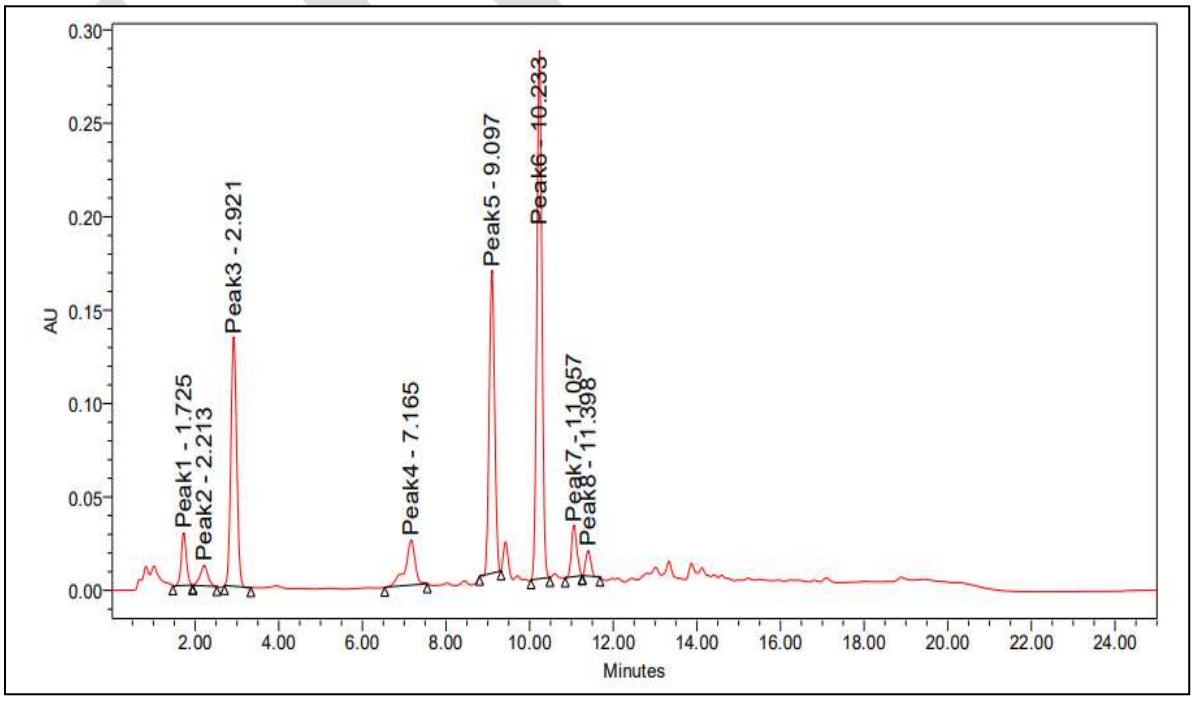


Figure 8: Graph representation of HPLC analysis

	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution
1	Peak1	1.725	236748	3.78	998	1.06	
2	Peak2	2.213	139814	2.23	667	1.03	1.67
3	Peak3	2.921	1288889	20.55	2079	1.07	2.28
4	Peak4	7.165	400239	6.38	6515	0.73	13.38
5	Peak5	9.097	1470991	23.46	22174	1.03	6.22
6	Peak6	10.233	2368264	37.76	33729	1.03	4.78
7	Peak7	11.057	248578	3.96	31643	1.14	3.40
8	Peak8	11.398	117835	1.88	36063	1.12	1.37

Table 2: Various peaks and their different characteristics

4(f) Antibacterial activity

Several studies have documented the antibacterial properties of green tea silver nanoparticles (42-45).

5. Discussion

The straightforward and practical photosynthetic method became an alternative to chemical and physical processes. In green synthesis, it was believed that the plant extract functions as a reducing and stabilizing agent for the production of metal nanoparticles. Such consistent and environmentally friendly technologies help to boost the synthesis and application of nanoparticles that are food for. (46). Various natural products, such as green tea (Camellia sinensis) (47), starch (48), lemon grass leaves extract, and leguminous shrub (Sesbania drummondii), have been used to synthesize green silver nanoparticles (49). Silver nanoparticles in the range of 70 nm can be produced with less leaf extract and without the addition of chemicals and physical processes such as centrifugation, sonication, and annealing (50). In this study, we describe a straightforward one-step method for the synthesis of silver nanoparticles by the reduction of aqueous silver ions with green tea extract at room temperature without the use of an additive that prevents silver nanoparticle aggregation. Through the reduction of silver ions, green-tea-capped silver nanoparticles have been created. When the aqueous extract and silver nitrate solution were combined, the colour of the extract

began to change. The dark brown colour of silver nanoparticles in aqueous solution was previously known. A yellowish-brown hue in the reaction vessels indicated the formation of silver nanoparticles (51). Due to the excitation of surface Plasmon resonance, a colour change occurred, which may be indicative of the formation of silver nanoparticles (48). The UV-Vis spectra revealed a maximal absorbance at 217nm, which increased with incubation time of silver nitrate with plant extract. Observations suggested that the reduction of Ag^{+} ions occurred extracellularly. It was previously reported that absorbance at approximatelynm is a characteristic of silver particles (52). The SEM analysis of the brown colour stable samples revealed the formation of silver nanoparticles, which were well dispersed in samples treated with silver nitrate. Since the silver colloidal particles possessed a negative charge due to the adsorbed ions, one can deduce that a repulsive force precluded particle aggregation. Numerous researchers have cited the biosynthesis of nanoparticles using plant extract as an example of a biosynthesis reaction. Synthesis of spherical silver nanoparticles at ambient conditions using a purified compound extracted from henna leaf (53). Using C. sinensis extract from green tea as reducing and stabilizing agents, gold and silver nanoparticles can be produced in aqueous solution under ambient conditions (52).

6. Conclusion

Using green tea extracts, we have proposed an environmentally benign method for the synthesis of silver nanoparticles. This basic, efficient, and environmentally friendly silver nanoparticle synthesis can be utilized in a variety of biomedical and biotechnological applications. The outcome demonstrated that green tea extract can be utilized in the synthesis of silver nanoparticles. UV-Visible spectroscopy confirms the formation of silver nanoparticles, and SEM measurements of the nanoparticles' crystalline nature and average particle size demonstrate the formation of nanoparticles without aggregation. The synthesis of silver nanoparticles utilized an environmentally friendly method that minimized the addition of hazardous residues to the environment. The synthesized nanoparticles were 5-100 nm in size, crystalline, and exhibited an absorption spectrum at 217 nm, as determined by a variety of techniques.

References

1. R.A. Riemersma, C. A. Rice-Evans, R.M. Tyrrell. M. N. Clifford, and M. E. J. Lean. "Tea flavonoids and cardiovascular health," *QJM _ Mon.J.Assoc. Physicians*, Vol. 94, no. 5, pp. 277-282, 2001.
2. N. Khan and H. Mukhtar, "Tea polyphenols for health promotion," *Life Sci*., vol.81, no.7,pp. 519-533,2007.
3. S. Maiti, A. Nazmeen, N. Medda, R. Patra, and T. K. Ghosh, "Flavonoids green tea against oxidant stress and inflammation with related human diseases," *Clin. Nutr. Exp*., vol.24, pp. 1-14, 2019.
4. J. Novak, M. Seruga, and S. Komorsky-Lovric, "Characterization of catechins in green and black teas using square-wave voltammetry and RP-HPLC-ECD," *Food Chem.*, vol. 122, no.4, pp. 1283-1289, 2010.
5. X. H. Zhang et al., "Comparison of three second-order multivariate calibration methods for the rapid identification and quqntitativeanalysis of tea polyphenols in Chinese teas using high-performance liquid chromatography," *J. Chromatogar*. A.vol. 1618, p, 460905,2020.
6. A. Perva-Uzunalic, M. Skerget, Z. Knez, B. Weinreich, F.Otto and S. Gruner, *Food chem.*, 2006,96, 597.
7. E. W. Chan, E. Y. Soh, P.P. Tie and Y. P. Law, *Pharmacogen Res*, 2011, 3, 266.
8. S. Pinnell, *J. Am Acad. Dermatol*, 2003, 48, 1.
9. X. Gao, L. Zhang, H. Wei and H. Chen, *Clin. Dermatol.*, 2008, 26, 367.
10. D. S. Kim, S. H. Park, S. B. Kwon, K. Li, S. W. Youn and K. C. Park, *Arch. Pharmacal Res*., 2004, 27, 334.
11. J. M. Song, K. H. Lee and B. L. Seong, *Antiviral Res.*, 2005, 68, 66.
12. N. Sueoka, M. Suganuma, E. Sueoka, S. Okabe, S. Matsuyama, K. Imai, K. Nakachi and H. Fujiki, *MPB Research*, 2001, 928, 274.
13. K. Sandeep and S. Nisha, Int.*J.Adv. Res. Bio. Sci.*,2012,1, 348.
14. M. D. Gianeti, D. G. Mercurio and P. M. Maia Campos, *Dermatol. Ther*., 2013, 26, 267.
15. S. Ahmed, M. Ahmad, B. L. Swami and S. Ikram, J. *Adv. Res.*, 2016, 7, 17.
16. D. MubarakAli, N. Thajuddin, K. Jeganathan and M. Gunasekaran, *Colloida surf*., B, 2011, 85, 360.
17. F. Duman, I. Ocsoy and F. O. Kup, *Mater. Sci. Eng.*, C., 2016, 60, 333.
18. A. Demirbas, B. A. Welt and I. Ocsoy, *Mater, Lett.*, 2016, 179, 20.
19. I. Ocsoy, A. Demiras, E. S. McLamore, B. Altinsoy, N. Ildiz and A. Baldemir, J. *Mol.Liq*., 2017, 238, 263-269.
20. I. Ocsoy, M. Temiz, C. Celik, B. Altinsoy, V. Yilmaz abd F. Duman, J. *Mol.Liq*., 2017, 227, 147-152.
21. G.SekerKaratoprak, G. Aydin, B. Altinsoy, C. AltinKaynak, M. Koser and I. Ocsoy, *Enzyme Microb. Technol*., 2017, 97, 21-26.
22. K. Zheng, M. I. Setyawati, D. T. Leong and J. Xie, *ACS Nano*2017, 11, 6904-6910.
23. K. Zheng, M. I. Setyawati, T. P. Lim, D. T. Leong and J. Xie*ACS Nano*, 2016, 10, 7934.
24. Mbata TI. Debiao L and Saikia A. antibacterial Activity of the Crude extract if Chinese Green Tea *(Camelia sinensis)* On *Listeria monocytogenes. The Internet journal of microbiology*. 2006: 2(2).
25. Diane L. McKay and Jeffrey Blumberg B. Roles for Epigallocatechin Gallate in Cardiovascular Disease and Obesity: An Introduction. Journal of the American College of Nutrition. 2007; 26 (4), 362S-365S.

26. N. Duran, A. B. Seabra, Biogenic synthesized Ag/Au nanoparticles: Production, Characterization, and applications. Curr. Nanosci. 14 (2018) 82-94.
27. S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram, A review on plants extract mediated synthesis of silver nanopaarticles for antimicrobial applications: A green expertise, J.Adv. Res. 7(2016) 17-28.
28. J.Y. Song, B. S. Kim, Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng. 32 (2009) 79-84.
29. S. Iravani, H. Korbekandi, S.V. Mirmohammadi, B. Zolfaghari, Synthesis of silver nanoparticles: chemical, physical and biological methods, Res. Pharm. Sci. 9 (2014) 385-406.
30. P. Singh, Y. J. Kim, D. Zhang, D. C. Yang, Biological synthesis of nanoparticles from plants and microorganism, Trends Biotechnol. 34 (2016) 588-599.
31. A. Ebrahiminezhad, A. Zare-Hoseinabadi, A.K. Sarmah, S. Taghizadeh, Y. Ghasemi, A. Berenjian, plant-mediated synthesis and applications of iron nanoparticles, Mol. Biotechnol. 60(2018) 154-168.
32. N. Duran, P. D. Marcato, M. Duran, A. Yadav, A. Gade, M. Raj, Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants, Appl. Microbiol. Biotechnol. 90 (2011) 1609-1024.
33. M. Flores-Gonzalez, M. Talavera-Rojas, E. Soriano-Vargas, V. Rodriguez-Gonzalez, Practical mediated-assembly synthesis of silver nanowires using commercial *Camelia sinensis* extracts and their antibacterial properties, New J. Chem., 42 (2018) 2133-2139.
34. M. C. Moulton, L.K. Braydich-Stolle, M.N. Nadagiuda, S. Kunzelman, S.M. Hussaina, R.S. Varma, Synthesis, Characterization and biocompatibility of " green" synthesized silver nanoparticles using tea polyphenols, Nanoscale 2 (2010) 763-770.
35. A.B. Seabra, N. Manosalva, B.A. Lima, M.T. Pelegrino, M. Brocchi, O. Rubilar, N. Duran, Antibacterial activity of nitro oxide releasing silver naopartilces, J. Phys. Conf. Ser. 838 (2017) 012031.
36. Rani, R., Nagpal, D., Gullaiya, S., Madan, S.,& Agrawal, S. S. (2014). Review article phytochemical, Pharmacological and beneficial effects of green tea. *International*
37. *journal of pharmacognosy and phytochemical Research*, 6, 420-426.
38. Graham HN (1992) Green tea composition, consumption and polyphenol chemistry, prev Med 21:334-350.

40. E.petryayeva and U.J.Krull, *Anal.Chim.Acta*,2011,706,8-24, crossRef, CAS, PubMed.

41. p.Singh, S. Pandit, J. Garnaes, S. Tunjic, V.R. Mokkapati, A. Sultan, A. Thygesen, A. Mackevica, R. V. Mateiu, A.E. Daugaard and A. Baun, *Int. J. nanomed.*, 2018,13,3571, crossRef, CAS, PubMed.

42. S. Tang and J. Zheng, *Adv.Healthcare Mater*.,2018,7, 1701503. crossRef, PubMed.

43. B. Le Ouay and F. Stellacci, *Nano today*, 2015,10,339-354 crossRef, CAS.

44. A.K. Keshari, R. Srivastava, P. Singh, V.B. Yadav and G. Nath, *J. Ayurveda Integr. Med.*, 2020,11,37-44 CrossRef PubMed.

45. H. Gandhi and S.Khan, *J.Nanomed.Nanotechnol.*,2016,7,1000366 Search PubMed.

46. Seyed H, Masoud D (2014) Crystalography, morphology and optical properties of silver nanoparticles synthesized by extract of black pepper as a green method. Optics 3: 19-23.

47. Vilchis-Nestor A, Sanchez-Mendieta V, Camacho-Lopez M (2008) Solvent less synthesis and optical properties of Au and Ag nanoparticles using *Camelia sinensis* extract, Mater Lett 62:3103-3105.

48. Vigneshwaran N, Ashtaputre N, Varadarajan P, Nachane R, Paralikar K (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*, Mater Lett 61:1413-1418.
49. Sharma N, Sahi S, Nath S, Parsons J, Gardea-Torresdey J (2007) Synthesis of plant mediated gold nanoparticles and catalytic role of biomatrix-embedded nanomaterials, Environ Sci Technol 41:5137-5142.
50. Yoon K, Hoon B, Park J, Hwang J (2007) Susceptibility constants o*f Escherichia coli* and *Bacillus Subtilis* to silver nad copper nanoparticles , Sci Total Environ 373: 572-575.
51. Ahmad P, Mukherjee S, Senapati D, Mandal M, Khan R (2002) Extracellular biosynthesis of silver nanoparticles using the Fungus Fuarium oxysporum. Colloids Surf B 28: 313-318.
52. Nestor A, Mendieta V, Lopez M, Espinosa R, Lopez M (2008) Solvent less synthesis and optical properties of Au and Ag nanoparticles using *Camelia sinensis* extract, Mater Lett 62:3103-3105.
53. Kasthuri J, Veerapandian S, Rajendiran N (2009) biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids Surf B Biointerfaces 68:55-60.