



## Eco-Friendly Ultrasound-Assisted Synthesis of Silver Nanoparticles Mediated by Tea Leaf Extracts and Their Potential Applications

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### Abstract

The green synthesis of silver nanoparticles (AgNP) presents notable benefits, such as environmental sustainability, simplicity, cost-effectiveness, and scalability for industrial applications. This study utilized green tea leaves (*Camellia sinensis*), which are abundant in catechins and polyphenols, as natural reducing and stabilizing agents. Ultrasonic-assisted synthesis was utilized to improve extraction and reduction processes, as acoustic cavitation induced by ultrasonic waves facilitates efficient mass transfer, accelerates nucleation, and enhances nanoparticle dispersion. This method provides an environmentally sustainable and economically viable alternative to conventional AgNP synthesis, enhancing the sustainability of nanoparticle production. The successful synthesis of AgNP was evidenced by the distinct color change of the tea extract from green to brown, accompanied by the formation of a brown colloidal suspension, in accordance with the Surface Plasmon Resonance (SPR) phenomenon. The UV-Vis spectrophotometric analysis confirmed AgNP formation, evidenced by a characteristic absorption peak at approximately 430 nm. The synthesized AgNPs were utilized as a colorimetric sensor for  $\text{Fe}^{3+}$  ions in aqueous solutions, demonstrating distinct color changes and SPR band shifts upon interaction with the metal. This illustrates their capability as environmentally friendly nanomaterials for the swift and precise detection of heavy metals, aiding in sustainable environmental monitoring. This research integrates ultrasonic-assisted synthesis with green tea extract, presenting a viable approach for the large-scale, environmentally sustainable production of nanoparticles.

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## INTRODUCTION

Iron (Fe) is a micronutrient that is essential for many biological systems, but high levels of Fe—especially  $\text{Fe}^{3+}$ —in aquatic environments can cause a decline in water quality, increase the growth of harmful microbes, and pose a risk of oxidative damage to human health. High levels of iron in water—especially concentrations above these limits—can lead to adverse health effects, including gastrointestinal distress, oxidative damage, and in extreme cases, organ dysfunction or death (Suryati & Maksuk, 2024). Therefore, continuous monitoring of iron levels in water is essential to protect public health.

Traditional detection methods such as atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are indeed accurate and sensitive, but they often have drawbacks such as high costs, the need for trained personnel, complex sample preparation, and limited portability. Nanotechnology offers promising alternatives, especially through the development of optical nanosensors. Silver nanoparticles (AgNPs), owing to their localized surface plasmon resonance (LSPR), can produce visible color changes or spectral shifts in



response to the presence of metal ions (e.g.,  $\text{Fe}^{3+}$ ), allowing for rapid, visual, and cost-effective detection. Silver nanoparticles (AgNPs) synthesized using sodium citrate as a reducing agent and formaldehyde cross-linked chitosan as a stabilizer demonstrated effective analytical performance for the detection of  $\text{Fe(III)}$ , achieving a linearity coefficient of  $R^2 = 0.924$  (Sulistiyani et al., 2022). However, the dependence on synthetic reagents for reduction and stabilization complicates the synthesis process and diminishes its environmental sustainability. The synthesis method necessitates elevated temperatures, leading to increased energy consumption and potential impacts on particle stability and size distribution, thereby constraining its feasibility for large-scale or environmentally sustainable applications.

In line with the principles of green chemistry—which emphasizes the reduction of hazardous substances, energy efficiency, and the use of renewable resources—green AgNP synthesis has gained attention. The use of plant extracts as bioreductors and stabilizers avoids many toxic reagents used in conventional nanoparticle synthesis, thereby reducing environmental impact. Green tea extract (*Camellia sinensis*) is particularly attractive because it is rich in polyphenols, flavonoids, and other biomolecules that can reduce silver ions and stabilize the resulting nanoparticles (Widatalla et al., 2022). Moreover, integrating ultrasonic-assisted methods in green synthesis can improve particle uniformity, accelerate nucleation, reduce synthesis time, and produce smaller, less aggregated AgNPs—features that strongly influence sensitivity and selectivity in sensing applications (Manjamadha & Muthukumar, 2016; Jasmin et al., 2025). Although there is increasing interest in green synthesis, limited research has investigated the efficacy of ultrasonic-assisted green tea-based AgNPs for the detection of  $\text{Fe}^{3+}$ . This study investigates the synthesis of green silver nanoparticles (AgNPs) utilizing ultrasound and green tea extract and evaluates their efficacy as sustainable colorimetric sensors for  $\text{Fe}^{3+}$  ions in aquatic environments.

## METHOD

### Materials and Tools

Tea leaves were collected from local suppliers, the chemicals used ethanol 96%, distilled water,  $\text{FeCl}_3$ ,  $\text{AgNO}_3$ , concentrated  $\text{HCl}$ , Folin reagent, Liebermann-Burchard reagent,  $\text{H}_2\text{SO}_4$  2 M, diethyl ether, Mg powder, Dragendorff reagent, Mayer reagent, Wagner reagent, amyl alcohol,  $\text{Na}_2\text{CO}_3$ , beaker glass, analytical balance, spatula, stirring rod, measuring glass, volumetric flask, funnel glass, hotplate, magnetic stirrer, test tube, graduated pipette, bulb, centrifuged tube, centrifuge, burette, Ultrasonic cell crusher noise isolating chamber, cuvette, Spectrophotometer UV-Vis.

### *Preparation of tea leaves extract with ultrasonic method*

Fresh green tea leaves were washed, air-dried, and cut into small pieces to increase the surface area. A 10 g portion of the sample was placed in a glass vessel, followed by the addition of 100 mL of ethanol as the extraction solvent. Ultrasonic-assisted extraction (UAE) was then carried out at 40 °C for 30 minutes using a controlled ultrasonic chamber, where ultrasonic waves facilitated acoustic cavitation to enhance the release of bioactive compounds. The resulting extract was filtered and collected for subsequent nanoparticle synthesis.

### *Biosynthesis of AgNPs*

A stock solution of  $\text{AgNO}_3$  (0.010 M) was prepared and subsequently diluted to obtain a 0.001 M working solution in a 100 mL volumetric flask. For nanoparticle synthesis, 10 mL of green tea leaf extract was transferred into a 100 mL beaker and stirred using a magnetic stirrer. While stirring, 90 mL of the 0.001 M  $\text{AgNO}_3$  solution was added dropwise from a burette to ensure controlled mixing and reduction. The mixture was then ultrasonically treated at 60 °C for 1



hour using an ultrasonic chamber operating at a frequency of 50 Hz to promote the formation of silver nanoparticles (AgNPs). The successful synthesis of AgNPs was confirmed by UV–Vis spectrophotometry, with absorbance spectra recorded in the range of 325–700 nm to identify the characteristic surface plasmon resonance (SPR) peak.

### ***Phytochemical Screening***

#### ***Alkaloid Test***

Three milliliters of extract were mixed with 10 mL of chloroform–ammonia solution (3:1, v/v) and filtered. A few drops of 2 M H<sub>2</sub>SO<sub>4</sub> were added to the filtrate and the mixture was homogenized until two distinct layers were formed: an organic layer and a colorless acidic layer. The acidic layer was divided into three separate test tubes. Mayer's reagent was added to tube 1, Dragendorff's reagent to tube 2, and Wagner's reagent to tube 3. The presence of alkaloids was indicated by a white precipitate (Mayer's), an orange precipitate (Dragendorff's), and a brown precipitate (Wagner's).

#### ***Flavonoid Test***

One gram of tea leaf extract was dissolved in 25 mL of hot distilled water, boiled for 5 minutes, and filtered. Five milliliters of the filtrate were placed in a test tube, followed by the addition of 1 mL concentrated HCl, 0.1 mg Mg powder, and 1 mL amyl alcohol. The mixture was vigorously shaken, and the development of a red or pink coloration indicated the presence of flavonoids.

#### ***Steroid Test***

Three milliliters of extract were dissolved in 25 mL of diethyl ether and mixed thoroughly. The solution separated into two phases, with the diethyl ether layer filtered for steroid testing and the residue reserved for saponin testing. To the diethyl ether layer, Liebermann–Burchard reagent was added. A green to blue coloration indicated the presence of steroids.

#### ***Saponin Test***

The residue obtained from the steroid test was dissolved in 5 mL of distilled water and heated for 5 minutes. After cooling, the solution was shaken vigorously. The formation of a stable foam persisting for at least 15 minutes indicated the presence of saponins.

#### ***Tannin Test***

Three milliliters of extract were dissolved in 1 mL of methanol and filtered. The filtrate was treated with a few drops of 1% FeCl<sub>3</sub> solution. The development of a green, blue, or purple coloration indicated the presence of tannins.

### ***Detection of Fe(III) ion with AgNPs***

A stock solution of FeCl<sub>3</sub> (10 mM) was prepared by dissolving 0.162 g of FeCl<sub>3</sub>·6H<sub>2</sub>O in a 100 mL volumetric flask with distilled water. From this stock, a series of Fe(III) standard solutions were prepared at concentrations of 17.9, 35.8, 71.6, 107.5, 143.3, and 179.1 mM by appropriate dilution in 25 mL volumetric flasks. For the test sample, 7 mL of FeCl<sub>3</sub> solution was transferred into a 25 mL volumetric flask and diluted to the mark with distilled water.

For colorimetric analysis, 1 mL of each Fe(III) standard solution or sample solution was placed into a test tube, followed by the addition of 1 mL of the synthesized AgNPs and 8 mL of distilled water. The mixtures were homogenized using a vortex mixer and filtered to remove particulates. The absorbance spectra were then recorded using a UV–Vis spectrophotometer within the maximum wavelength range of AgNPs surface plasmon resonance (SPR).



## RESULTS AND DISCUSSION

### Biosynthesis of AgNPs Mediated by Tea Leaf Extracts

The green synthesis of silver nanoparticles (AgNPs) offers distinct advantages over conventional chemical and physical approaches, including environmental sustainability, cost-effectiveness, procedural simplicity, and scalability for industrial applications. Green tea leaves are rich in catechins, a class of flavonoid derivatives bearing hydroxyl ( $-OH$ ) groups, which act as natural reducing agents by converting  $Ag^+$  ions into elemental silver nanoparticles ( $Ag^0$ ) (Rahim et al., 2020). Catechins are readily oxidized, and together with polyphenols—present in concentrations of 30–40% in green tea, which is higher than in black tea—they serve as efficient bioreductors by donating protons in the silver reduction process (Windarti et al., 2022). The reduction of  $Ag^+$  to  $Ag^0$  mediated by hydroxyl-containing compounds is visually evidenced by a color change in the tea extract from green to brown, corresponding to the appearance of a characteristic Surface Plasmon Resonance (SPR) band (Handoko et al., 2022).

The extraction of catechins and polyphenols from green tea leaves is strongly influenced by the choice of solvent. In this study, ethanol was selected due to its polarity, which is compatible with phenolic and flavonoid compounds, in accordance with the “like dissolves like” principle. Ethanol has been widely reported as an effective solvent for recovering phenolic compounds from plant matrices, including tea leaves (Putri et al., 2023).

Ultrasonic-assisted extraction (UAE) was employed to enhance the recovery of bioactive compounds. UAE is based on acoustic cavitation, whereby ultrasonic waves generate microbubbles that collapse violently, disrupting plant cell walls and facilitating solvent penetration. This mechanism significantly improves extraction efficiency while minimizing energy use compared to conventional techniques such as maceration and Soxhlet extraction (Kristina et al., 2022); (Haryono et al., 2018). Furthermore, UAE is regarded as an environmentally friendly, rapid, and non-destructive technology that preserves the structural integrity of extracted compounds (Adhiksana, 2017). However, the extraction temperature and duration are critical factors. Elevated temperatures can accelerate antioxidant oxidation, resulting in extract degradation, whereas excessive duration may cause the breakdown of heat-sensitive bioactive compounds such as flavonoids (Ibrahim et al., 2015). Bioactive compounds are particularly unstable above 50 °C, which can lead to structural alterations and a decline in extract quality. Conversely, insufficient heating or short extraction times may yield suboptimal recovery of target phytochemicals (Sekarsari et al., 2019). In this work, UAE was conducted at 40 °C to balance efficiency and compound stability.

Phytochemical screening of the ethanol extract confirmed the presence of several classes of secondary metabolites, including alkaloids, flavonoids, steroids, saponins, and tannins (Rumagit et al., 2015). These metabolites not only serve as markers of extract composition but also contribute to the reduction and stabilization of AgNPs during synthesis. The observed positive reactions to specific reagents provided qualitative evidence of compounds with potential bioreductive activity. The results of the phytochemical analysis of green tea leaf extracts obtained at different extraction conditions are summarized in Table 1.

The ethanol extract of green tea leaves tested positive for the presence of alkaloids, flavonoids, steroids, saponins, and tannins. These findings are consistent with the results reported by (Wulandari et al., 2020), who also confirmed that green tea leaf extracts contain the same classes of secondary metabolites. The thermal stability of these compounds further supports the extraction results obtained in this study. Alkaloids remain stable up to 138 °C, flavonoids are not degraded below 90 °C, saponins are stable up to 70 °C, while tannins begin to decompose within the range of 98.89–101.67 °C. Therefore, the extraction temperature applied



in this study (40 °C) did not adversely affect the integrity of the secondary metabolites obtained from the ethanol extract of green tea leaves.

Table 1. Test results of secondary metabolites of green tea leaves extract through phytochemicals

Phytochemical Parameters	Indicator	Color if the result is positive	Result
Alkaloids	Mayer	White sediment	+
	Dragendorf	Orange	+
	Wagner	Brown	+
Flavonoids	Amyl alcohol	Orange	+
Steroids	Lieberman-Buchard	Green	+
Saponins	-	Foam	+
Tannins	FeCl <sub>3</sub> 1%	Blackish green	+

Secondary metabolites play a critical role in the green synthesis of silver nanoparticles. The hydroxyl (–OH) and carbonyl groups in polyphenolic compounds, including flavonoids and tannins, function as electron donors in the reduction of Ag<sup>+</sup> ions to Ag<sup>0</sup> (Purnamasari, 2021).

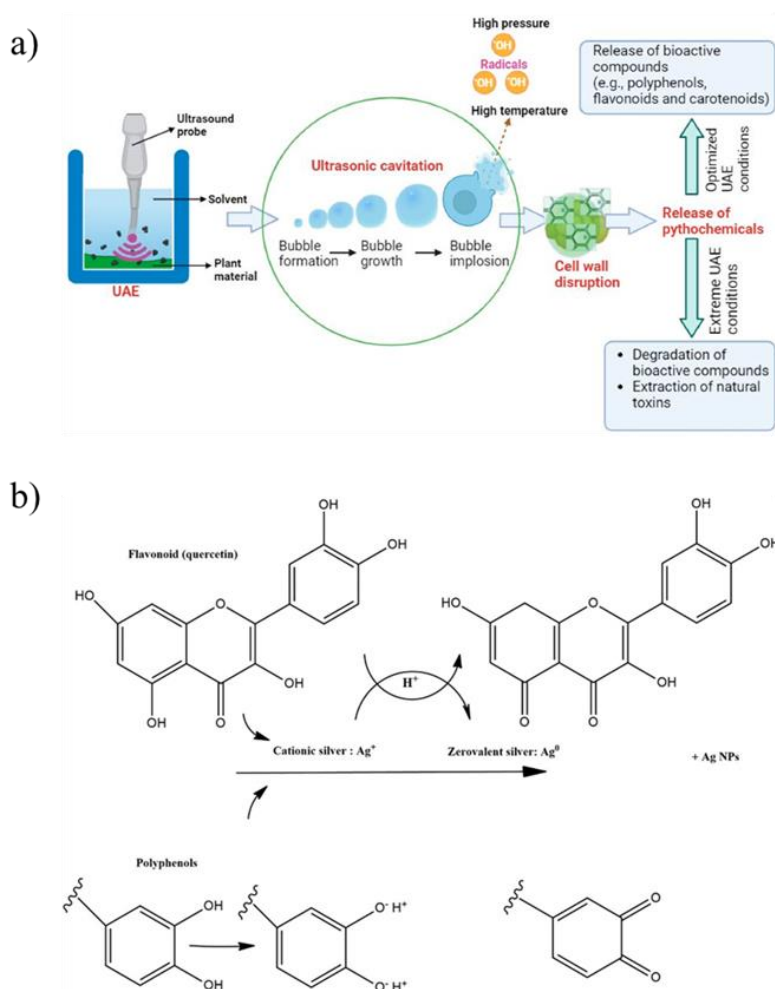


Figure 1. (a) Mechanism of the ultrasound-assisted extraction of bioactive compounds from plant materials (Demesa et al., 2024), (b) Synthesis reaction of silver nanoparticles by polyphenols and flavonoid (Omidi et al., 2018)

Figure 1 demonstrates that polyphenolic compounds experience deprotonation, resulting in the formation of reactive R–O<sup>–</sup> groups that readily interact with Ag<sup>+</sup> ions in solution. This interaction results in the formation of RO–Ag complexes, which then participate in redox



reactions that break the polyphenol chain and release metallic silver ( $\text{Ag}^0$ ) (Lestari, 2019). Besides acting as reducing agents, these biomolecules contribute to stabilization, preventing aggregation and ensuring the colloidal stability of the synthesized AgNPs.

The synthesis of AgNPs using green tea leaf extract was evidenced by a distinct color change of the reaction mixture from green to blackish gray. This observation is consistent with the findings of (Lestari et al., 2019), who reported that the development of a characteristic color is one of the key indicators of AgNPs formation. The intensity of the resulting color correlates with the extent of reduction, where a darker coloration reflects a higher degree of oxidation of organic compounds and, consequently, a greater reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$ . This process enhances the nucleation and growth of nanoparticles, thereby increasing the overall yield and surface area of the synthesized AgNPs (Haryani et al., 2016).

### Characterization of AgNPs

The biosynthesized silver nanoparticles were characterized using UV–Vis spectrophotometry to confirm their formation and optical properties (Sugito et al., 2022). The absorption spectrum of the AgNPs exhibited a distinct Surface Plasmon Resonance (SPR) band with a maximum wavelength at approximately 430 nm, which is consistent with the typical SPR range of silver nanoparticles (400–450 nm) reported in the literature (Shameli et al., 2015); (Ahmed et al., 2016). The presence of this characteristic absorption peak strongly indicates the successful reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  nanoparticles mediated by the phytochemicals present in green tea extract. The observed  $\lambda_{\text{max}}$  at 430 nm suggests the formation of well-dispersed, spherical AgNPs of small size, as variations in the SPR peak position are generally associated with particle size, morphology, and degree of aggregation (Kumar et al., 2019).

The findings of this study align with earlier investigations into the green synthesis of silver nanoparticles (AgNPs) utilizing *Curcuma longa* extract, which indicated a surface plasmon resonance (SPR) peak for AgNPs at around 426–427 nm (Sapkota et al., 2024). The results corroborate the efficacy of ultrasonic-assisted green synthesis in generating stable AgNPs with advantageous optical properties appropriate for metal ion sensing applications (Figure 2). Compared to earlier research that synthesized AgNPs using *Camellia sinensis* (green tea) extracts—resulting in nanoparticles with an average size of approximately 52 nm and significant antibacterial activity (MIC 31.25–62.5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) with distinct SPR bands between 450–500 nm (Ali et al., 2022), as well as other studies producing smaller particles (~26.9 nm) with SPR peaks around 410–420 nm under ambient conditions (Mohamed et al., 2021).

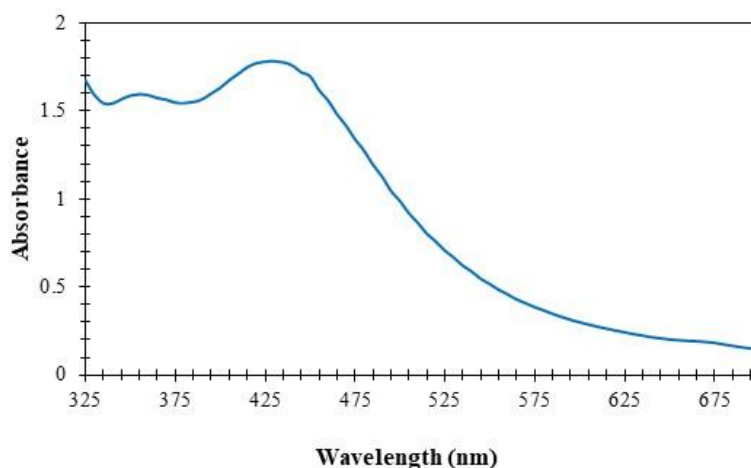


Figure 2. UV-Vis Spectra of AgNPs

The current study exhibits improved synthesis efficiency and enhanced particle uniformity. The use of *Camellia sinensis* extract as a bioreducing and stabilizing agent in ultrasonic-



assisted extraction (UAE) conditions significantly improved mass transfer and expedited nucleation, resulting in the formation of uniformly dispersed AgNPs with a distinct SPR peak around 430 nm. The findings indicate that the UAE-assisted green synthesis method not only maintains the environmentally friendly properties of plant-based reduction but also enhances particle uniformity and reaction kinetics relative to traditional ambient-temperature techniques documented in previous research. The method developed in this study offers a more rapid, energy-efficient, and reproducible approach for the synthesis of AgNPs, while preserving the inherent benefits of phytochemical-mediated reduction.

The particle size analysis (PSA) depicted in Figure 3 indicated that silver nanoparticles (AgNPs) synthesized via tea extract under ultrasonic-assisted extraction (UAE) had a Z-average of  $96.6 \pm 2.1$  nm and a polydispersity index (PDI) of 0.489, suggesting moderately uniform particle dispersion. The small size and acceptable PDI indicate that ultrasound treatment improved the reduction efficiency of phytochemicals in the tea extract, including polyphenols, flavonoids, and catechins, by facilitating rapid nucleation and inhibiting agglomeration during synthesis. Other studies, Ali et al (2022), have reported similar findings, indicating that the application of UAE promotes the formation of smaller and more monodisperse AgNPs. For instance, green syntheses utilizing *Camellia sinensis* or other polyphenol-rich plant extracts have yielded nanoparticles ranging from 52 nm under ultrasonic conditions by using Transmission Electron Microscope (TEM). The results indicate that UAE serves as an effective intensification technique, enhancing both particle size control and distribution uniformity in tea-mediated AgNP synthesis.

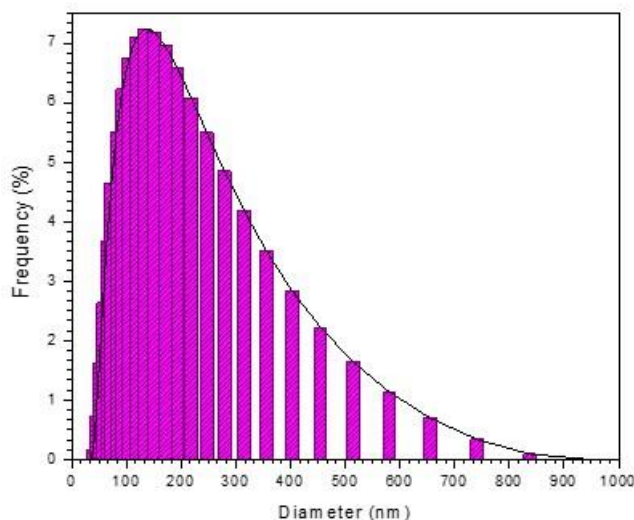


Figure 3. PSA Analysis of AgNPs

### Detection of Fe(III) ion with AgNPs

The performance of the biosynthesized AgNPs from green tea leaf extract in detecting  $\text{Fe}^{3+}$  ions was further evaluated through quantitative analysis. The sensitivity of the system was assessed by constructing a calibration curve, in which the absorbance response of AgNPs to varying  $\text{Fe}^{3+}$  concentrations was measured (Riyanto et al., 2021). The calibration plot exhibited good linearity with a correlation coefficient ( $R^2$ ) of 0.9908, indicating a strong relationship between  $\text{Fe}^{3+}$  concentration and the optical response of AgNPs (Fig 4). Such high linearity demonstrates the reliability of green-synthesized AgNPs as effective colorimetric probes for  $\text{Fe}^{3+}$  detection (Singh et al., 2018). In addition to sensitivity, the accuracy of  $\text{Fe}^{3+}$  quantification was evaluated using recovery studies. Accuracy reflects the degree of conformity between the experimental results and the true or reference values (Sugito et al., 2022).



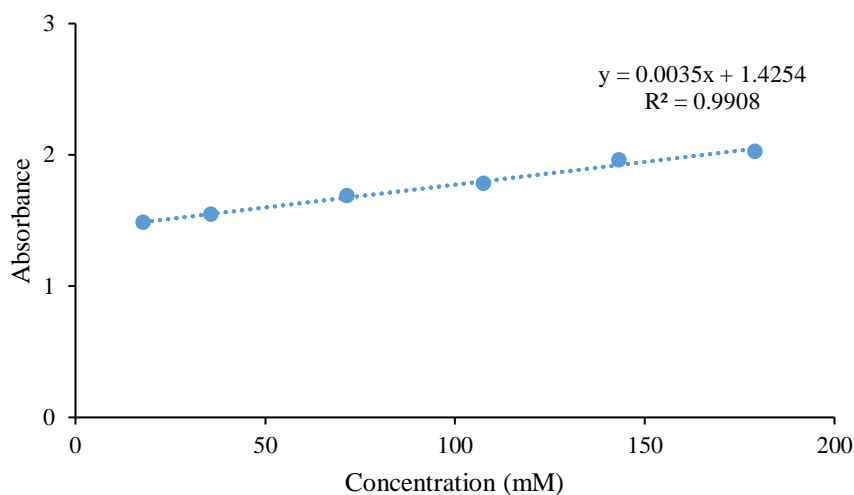


Figure 4. Calibration Curve of  $\text{Fe}^{3+}$  Ion

In this study, the percentage recovery obtained for the test sample was 102%, which falls within the generally accepted range of 95–105% as recommended by (AOAC, 2016). This result indicates that the developed AgNP-based colorimetric method not only provides reliable sensitivity but also ensures accurate determination of  $\text{Fe}^{3+}$  levels in aqueous samples. The combination of high linearity and acceptable recovery highlights the potential of ultrasonic-assisted, green-synthesized AgNPs as eco-friendly and efficient nanoprobes for monitoring  $\text{Fe}^{3+}$  contamination in aquatic environments (Suryani et al., 2022).

Overall, the results demonstrate that silver nanoparticles synthesized via ultrasonic-assisted green synthesis using green tea leaf extract exhibit excellent potential as eco-friendly colorimetric sensors for  $\text{Fe}^{3+}$  ions (Istihara, 2019). The presence of extract played a crucial role as natural reducing and stabilizing agents, enabling the formation of stable AgNPs with distinct optical properties (Muliawati et al., 2021). The AgNPs showed strong sensitivity, high linearity ( $R^2 = 0.9908$ ), and satisfactory accuracy with recovery values within the acceptable range (102%), highlighting their reliability for practical application in water quality monitoring. These findings reinforce the promise of plant-mediated green nanotechnology as a sustainable approach for developing low-cost and effective nanosensors for heavy metal detection in aquatic environments (Kumar et al., 2019); (Riyanto et al., 2021).

## CONCLUSION

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using ethanol extract of green tea leaves assisted by ultrasonic treatment. The polyphenolic compounds, particularly flavonoids and tannins, acted as efficient natural reducing and stabilizing agents, enabling the formation of stable AgNPs, as confirmed by UV–Vis spectrophotometry with a distinct surface plasmon resonance absorption peak at 430 nm. The synthesized AgNPs exhibited excellent performance as colorimetric nano sensors for  $\text{Fe}^{3+}$  ion detection, showing high sensitivity with a strong linear correlation ( $R^2 = 0.9908$ ) and acceptable accuracy (recovery value of 102%).

These findings emphasize the potential of plant-mediated green nanotechnology as an environmentally friendly, simple, and cost-effective approach for developing metal ion sensors, particularly for  $\text{Fe}^{3+}$  monitoring in environmental samples. Future research should concentrate on broadening the application of the synthesized AgNPs for detecting other environmentally significant heavy metal ions, such as  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ , or  $\text{Hg}^{2+}$ , to assess their



selectivity and versatility. Further optimization of the ultrasonic-assisted synthesis process is recommended to enhance yield, improve particle uniformity, and facilitate large-scale production, thereby broadening the practical impact of this sustainable nanomaterial in environmental monitoring and analytical sensing technologies.

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