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Green Synthesis of Silver Nanoparticles using *Coffea canephora* L. Leaves Aqueous Extract with its Antioxidant Activity and H_2O_2 Sensor Ability

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ABSTRACT

Green synthesis provides a sustainable approach for nanoparticle production by minimizing toxic reagents and environmental impact. Given the increasing demand for eco-friendly nanomaterials with biomedical and environmental relevance, this study aimed to synthesize and characterize silver nanoparticles (AgNPs) using aqueous extracts of *Coffea canephora* L. leaves, which are rich in antioxidant compounds serving as natural reducing agents. The biosynthesized AgNPs exhibited a localized surface plasmon resonance (LSPR) peak at 438 nm, while SEM analysis revealed irregular morphologies with an average particle size of ~70 nm. Dynamic light scattering confirmed a Z-average of 70.16 nm and a low polydispersity index (0.244), indicating high dispersion stability. Compared to the crude leaf extract, the AgNPs showed significantly enhanced antioxidant potential, with stronger DPPH radical scavenging activity (IC_{50} = 51.94 ± 0.59 mg/L vs. 85.29 ± 3.67 mg/L). Moreover, the AgNPs demonstrated excellent sensing capability toward H_2O_2, achieving detection limits as low as 0.0068 mM and 0.0575 mM. These results suggest that *Coffea canephora* L. mediated AgNPs not only provide an eco-friendly route for nanoparticle synthesis but also hold strong potential for biomedical, biosensing, and active food packaging applications.

***Keywords*:** Silver Nanoparticles, Agnps, *Coffee canephora* l. Leaf, Green Synthesis, Antioxidant Activity; Hydrogen Peroxide Sensor.

Introduction

Nanoparticles are fine particles with diameters ranging from 1 to 100 nanometers.[1] The shape of nanoparticles is usually spherical, but they can also have various geometric or irregular forms.[2] The factor that makes nanoparticles more attractive is their nanoscale size, which makes them suitable biomedical applications such as targeted therapy to increase the therapeutic effect of a drug, cellular repair, gene-based therapy,[3] biosensor applications,[4] cosmetics,[5] diagnostic purposes,[6] and drug delivery systems.[7] Silver nanoparticles (AgNps) are more widely studied than other metal nanoparticles because they have distinctive physicochemical properties and low toxicity levels on the skin.[8,9,10,11] Nanoparticles can be synthesized using several methods, namely physical, chemical, and "green synthesis" methods. Among these, the green synthesis method is currently being developed due to its advantages, such as being simpler, more cost-effective, reliable, and environmentally friendly. Biological reducing agents in green synthesis include plant extracts, microbial cell biomass, and biopolymers. Plant extracts can serve as reagents to synthesize nanoparticles quickly, and stably and can reduce the level of toxicity of chemical compounds or strong reducing agents.[12] One of the plants that has the potential to be a reductant is the *Coffea canephora* L. plant. Phytochemical aspects of *Coffea canephora* L. leaves include secondary metabolites such as phenolics, alkaloids, terpenoids, carotenoids, enzymes, inorganic substances, and vitamins.

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Secondary metabolites, proteins, and chlorophyll in plant extracts can be used as capping agents to synthesize AgNPs.[13] AgNPs synthesized via bioreduction have demonstrated potential as antioxidant agents because one free valence electron can be donated to free radicals. Previous research showed that AgNPs synthesized with plant extract showed better antioxidant activity. Wang et al., (2018), reported that the antioxidant activity of AgNPs by bioreducing guava leaf extract (Psidium guajava L.) has high antioxidant capacity and greater efficiency.[14] The antioxidant activity of AgNPs from Pongamia pinnata leaf extract shows a strong natural antioxidant because it has a lower IC_{50} value than leaf extract.[15] The strong natural antioxidant activity of AgNPs, obtained from Solanum melongena L. peel extract, is confirmed by their IC_{50} value being lower than that of the peel extract.[16] In addition, phenolic compounds and flavonoids on the surface of AgNPs with bioreductants can act as free radical reducing agents.[17] AgNPs are promising materials for the recognition of highly sensitive chemical and biological molecules. AgNPs have been successfully applied to detect several chemical and biological substances such as glucose, folic acid, H_2O_2, and nitroaromatic compounds.[13] Researchers have conducted several studies on plant-based silver nanoparticles produced by green synthesis with H_2O_2 sensor activity. Research on AgNPs of Acacia nilotica leaf extract showed a high and selective H_2O_2 sensor capability by producing a color change from brown to a colorless solution.[18] Kumar (2021) used Capsicum baccatum L., the results showed that AgNPs had moderate colorimetric sensor activity for H_2O_2 decomposition (> 50%, 15 min) at a concentration of 100 mM.[19] . The H_2O_2 AgNPs sensor of sugarcane leaf extract has a low detection limit of 30 mM and research on cellulose AgNPs can detect H_2O_2 exhibits high sensitivity and strong selectivity toward H2O2 in real sample analysis or with the addition of other interfering substances.[20,21,22] Furthermore, AgNPs synthesized from Solanum melongena L. peel extract also exhibited a low detection limit for H_2O_2 sensing.[16] From these studies, it is proven that AgNPs synthesized with plant bioreduction have H_2O_2 sensor activity.

In the present study, *Coffea canephora* L. leaves were used as both stabilizing and reducing agents for the synthesis of AgNPs (Ag^+ to Ag^0), and their antioxidant activity was evaluated using the DPPH method. Further, the sensing property of *Coffea canephora* L. leaves aqueous extract stabilized AgNPs towards H_2O_2 was investigated.

Materials and Methods

Plant collection and identification

The *Coffea canephora* L. leaves for this study were sourced from a garden in Blita, East Java, Indonesia, at GPS coordinates 8.0955° S, 112.1609° E, on September 20th, 2024 and authenticated by the Materia Medika Herbal Laboratory, Batu, Indonesia. A voucher specimen, No. 074/514/102.7-A/2024, was deposited. The leaves were washed with tap water five times. Then the leaves were washed five times with tap water, shade-dried and ground into a fine powder.

Coffea canephora L. Leaf Extract Preparation

Coffea canephora L. leaf extract was prepared by weighing 1 g of *Coffea canephora* L. leaf powder, which was then dissolved in 100 mL of deionized water (DI water). The mixture was heated on a hot plate at 60 °C for 30 minutes. The resulting extract was cooled to room temperature and then centrifuged for 5 minutes at a speed of 3,000 rpm. Finally, the solution was filtered using a double layer of filter paper.

Precursor ($AgNO_3$ 5.17 x 10^{-3} mol L^{-1}) Preparation

Silver nanoparticles were synthesized using a green synthesis method, 0.0394 g of $AgNO_3$ powder was weighed and then dissolved in a 50 mL volumetric flask using distilled water as the solvent to obtain a concentration of 5.17 x 10^{-3} mol L^{-1} $AgNO_3$.[16]

Green Synthesis Method

AgNPs were produced through a green synthesis approach, in which Ag^+ ions were reduced to nanoparticles by the gradual dropwise addition of 250 µL of a 1% *Coffea canephora* L. solution. Aqueous leaf extract to 500 µL a 5.17 x 10^{-3} mol L^{-1} $AgNO_3$ and 2.5 mL DI water, followed by heating at 45°C for 20 minutes. The formation of silver nanoparticles was visually confirmed by a color change from yellow to dark brown. [16]

Silver Nanoparticle Characterization

UV-Vis Spectroscopy

The absorption characteristics of the synthesized silver nanoparticles in the ultraviolet and visible range were determined using a Shimadzu UV-1280 spectrophotometer (Japan). This method also verified the material's quality. Spectral analysis confirmed the formation of the leaf extract-mediated silver nanoparticles. UV-Vis spectra were obtained by continuously scanning the samples from 200 to 800 nm, with DI water used as a baseline reference.

Scanning Electron Microscopy (SEM)

Morphological analysis, including shape and size determination, was performed using a Hitachi TM 3000 Scanning Electron Microscope (Japan).[16]

Dynamic Light Scattering (DLS)

The nanoparticles' size and particle size distribution were examined using Dynamic Light Scattering (DLS) on a Zetasizer nano series instrument from Malvern Instrument Ltd (England).[16]

Antioxidant Activity Test

Antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and it is expressed as the IC_{50} value. This involved preparing a 0.1 mM DPPH solution in methanol and adding 1.2 mL of it to 0.3 mL of *Coffea canephora* L. aqueous leaf extract (20-100 ug/mL) and AgNPs (20-100 ug/mL15). After incubation for 10 and 20-minute, absorbance was read at 517 nm with a Shimadzu UV-Vis-1280 spectrophotometer.[16] The percentage scavenging activity was calculated using following equation (equation 1)[23]:

$$\% \text{ Scavenging Activity} = \frac{(A0-A1)}{A0} x100 \quad \text{(equation 1)}$$

A0: Absorbance of control; A1: Absorbance of sample. Vitamin C was used as a positive control.

The determination of the IC_{50} value was performed by plotting the sample concentrations against their respective percent inhibitions on the x-axis and y-axis of a linear regression equation. The IC_{50} value is expressed as the x-value obtained when the y-value is 50. The IC_{50} value represents the concentration of the sample solution required to reduce DPPH free radicals by 50%.

H_2O_2 Sensor Ability

Direct detection of H_2O_2 was achieved using the AgNPs. Hydrogen peroxide solutions, ranging from 0.001 to 1 mM in DI water, were reacted with the AgNP solution (1:4 ratio). Monitoring the 437 nm band over an optimized incubation time (30 minutes) allowed the construction of two linear calibration curves: 0.001–0.08 and 0.1–1 mM. The sensor's selectivity and real-world applicability were evaluated through interference studies with various cations and organic compounds (e.g., glucose), as well as by analyzing tap water, river water, and commercial milk samples. The interference and real sample solutions were prepared using DI water and treated under identical ratios and incubation periods.

Data Analysis

The IC_{50}, defined as the concentration inhibiting 50% of DPPH activity, was determined via linear regression from a plot of inhibition percentage against concentration, using the equation $y = bx + a$ (where $y=50$ and $X=IC_{50}$). Subsequently, the IC_{50} values from the three samples were tested for data normality, followed by one-way ANOVA and LSD post-hoc test with a 95% confidence level ($p < 0.05$), performed using IBM SPSS version 23.

Result and Discussions

AgNPs Characterization

The successful synthesis of AgNPs ~~(silver nanoparticles)~~ was evidenced by a distinct visual color shift of the solution from yellow to dark brown. This change is attributed to the surface plasmon resonance (SPR) effect, confirming nanoparticle formation. Furthermore, UV-Vis spectroscopy revealed characteristic absorption within the range of 320–520 nm, typical of monodisperse silver nanoparticles. The *Coffea canephora* L. aqueous leaf extract showed an absorption peak between 200 and 278 nm. Following the formation of silver nanoparticles during incubation for 20 minutes, a shift occurred in the spectrum, as shown in (Figure 1). The UV-Vis spectrum obtained displayed a distinct absorption peak at around 438 nm, corresponding to the characteristic band of Ag^+, due to the activation of longitudinal plasmon resonance of AgNPs present in the medium.

The results of the particle size distribution measurement of the sample using the DLS technique are shown in (Figure 2). Based on these results, the hydrodynamic diameter (Z-average) of the reduced Ag particles with *Coffea canephora* L. aqueous leaf extract is 74.25 nm, and the Polydispersity Index (PDI) value was 0.237. The resulting AgNPs have a size that aligns with the theory, where a particle can be categorized as a nanoparticle if its diameter is between 1and a1000 nm.[16] The PDI value of 0.237 for the AgNPs indicates that these AgNPs are in moderately polydisperse because PDI values between 0.08–0.7 fall within the medium polydispersity category.[24] These findings suggest that *Coffea canephora* L. aqueous leaf extract has the ability to reduce Ag^+ and form Ag^0.

Scanning Electron Microscopy (SEM) can provide details about the morphology of materials at the submicron level and information about their elemental composition at the micron level. Scanning Electron Microscopy (SEM), which provides higher-resolution imaging compared to Transmission Electron Microscopy (TEM), was employed to analyze the morphology and size of the synthesized nanoparticles. The resulting images clearly showed that the silver nanoparticles were distinct and spherical in shape, with an average particle size of 76.79 ± 2.28 nm as depicted in (Figure 3). This relatively narrow size distribution indicates a uniform synthesis process, supporting the UV-Vis spectroscopy results that suggested the formation of monodisperse AgNPs.

Antioxidant Activity Test

The antioxidant activity results are summarized in (Table 1). The antioxidant capacity of the compounds was determined using DPPH,~~a~~

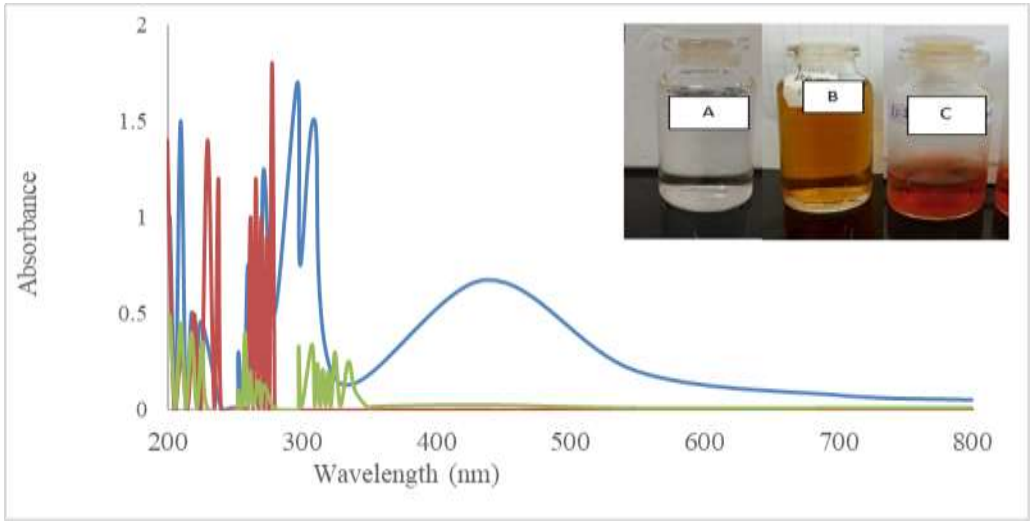

Figure 1: Spectra UV-Vis of (A) 5.17 x 10^{-3} mol L^{-1} $AgNO_3$, (B) 1% *Coffea canephora* L. aqueous leaf extract, (C) AgNPs

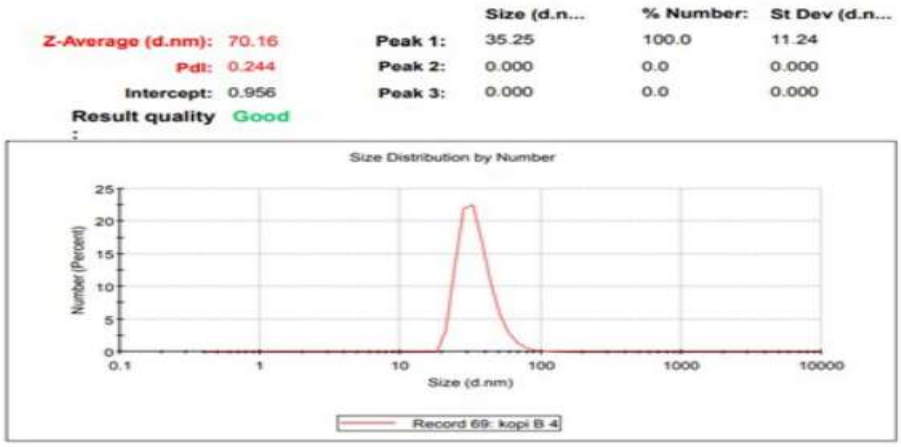

Figure 2: Particle size distribution of AgNPs using Dynamic Light Scattering (DLS)

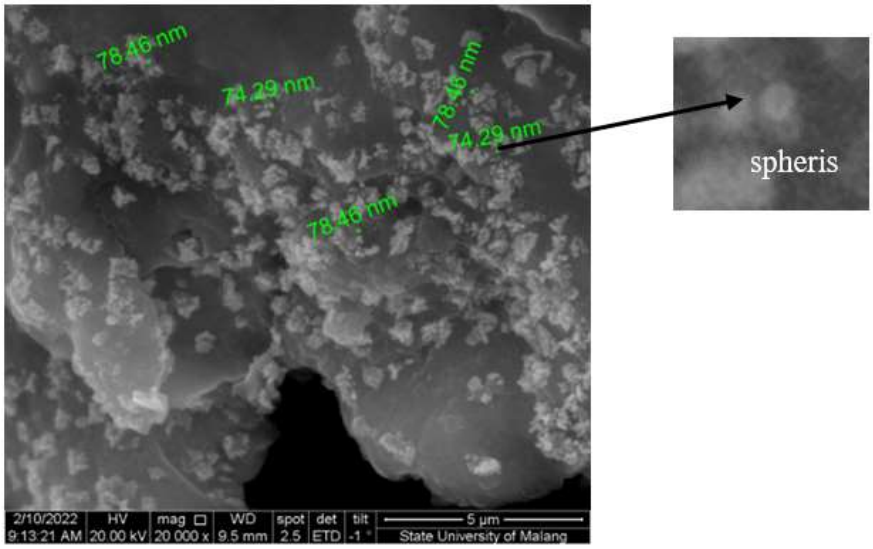

Figure 3: Morphological observations of AgNPs using SEM

Table 1: Antioxidant activity of the Vitamin C, *Coffea canephora* L. aqueous leaf extract, and AgNPs

Sample	IC_{50} (µg/ml ± SD*)
Vitamin C	3.660 ± 0.200
Coffea canephora L. aqueous leaf extract	85.288 ± 3.669
AgNPs	51.935 ± 0.587

* 3 replication

a widely recognized and stable artificial radical. DPPH is reduced by accepting a hydrogen atom or an electron, a process that causes a color change from purple to yellow, which was quantified using spectrophotometry to determine the DPPH radical-quenching activity of AgNPs. AgNPs displayed higher antioxidant activity than plant extract in the DPPH scavenging assay.

The antioxidant capacity of silver nanoparticles produced through green synthesis and the plant aerial extract showed an enhanced capacity to scavenge free radicals. This suggests that AgNPs could be valuable in developing new antioxidants, among others. Furthermore, the plant-mediated AgNPs displayed superior antioxidant capacity relative to the

crude plant material, indicating their potential application in the treatment of numerous diseases resulting from oxidative stress.

Data analysis

A one-way ANOVA performed on the data (Table 2) demonstrated that the IC_{50} values of vitamin C, AgNPs, and *Coffea canephora* L. aqueous leaf extract were significantly different from one another ($p < 0.05$). Subsequent analysis using the Least Significant Difference (LSD) test (Table 3) confirmed these distinctions, showing significant differences ($p < 0.05$) in IC_{50} values when comparing vitamin C to the extract, vitamin C to AgNPs, and the extract to AgNPs.

Table 2: Summary of ANOVA Results

IC_{50}	Sum of Squares	df	Mean Square	F	Sig.
Between	10105.885	2	5052.9	1079	.000
Groups			43	.565	
Within	28.083	6	4.681		
Groups					
Total	10133.968	8			

Table 3: The LSD-test

Sampel (I)	Sampel (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Vitamin C	*Coffea canephora* L. aqueous leaf extract	-81.627333*	1.766453	.000	-85.94969	-77.30498
	AgNPs	-48.275333*	1.766453	.000	-52.59769	-43.95298
Coffea canephora L. aqueous leaf extract	Vitamin C	81.627333*	1.766453	.000	77.30498	85.94969
	AgNPs	33.352000*	1.766453	.000	29.02964	37.67436
AgNPs	Vitamin C	48.275333*	1.766453	.000	43.95298	52.59769
	Coffea canephora L. aqueous leaf extract	-33.352000*	1.766453	.000	-37.67436	-29.02964

*. The mean difference is significant at the 0.05 level.

The colorimetric assay of hydrogen peroxide

The sensitivity of the colorimetric assay was determined by examining hydrogen peroxide solutions ranging from 0.001 to 1 mM. Absorbance measurements at 438 nm were used to assess the system's color and quantify hydrogen peroxide. The appearance of a yellow hue with strong absorbance at this wavelength corresponded to the observation of evenly distributed AgNPs, whereas low absorbance indicated AgNPs degradation. Based on the UV–Vis absorption spectra, higher H_2O_2 concentrations led to a reduction in absorbance at 438 nm, with the minimum observed at 1 mM (Figure 4~~a~~), demonstrating the gradual degradation of AgNPs. ~~This was visually confirmed by a color change from yellow to colorless in the as-prepared AgNPs (Figure 4b) with increasing H_2O_2 concentration,~~ suggesting that the degradation of AgNPs is dependent on the H_2O_2 concentration.

The detection limit (D.L.) was calculated using the equation D.L. = 3.3σ/S, where σ denotes the standard deviation of absorbance at 438 nm (based on five replicate measurements) and S corresponds to the slope of the calibration curve (Figure 5). Using this approach, the colorimetric assay yielded detection limits of 0.0068 mM within the 0.001–0.08 mM range and 0.0575 mM within the 0.1–1.0 mM range. A comparative evaluation with previously reported H_2O_2 detection methods is summarized in Table 4. The findings suggest that the silver nanoparticle-based sensor, obtained via a facile synthesis route, is highly suitable for the colorimetric detection of H_2O_2 in practical applications.

The orange color observed in the suspension arises from the uniform distribution of AgNPs. After these simple preparatory steps, the sensor becomes ready for application. When exposed to a strong oxidizing agent such as H_2O_2, the reaction proceeds in the opposite direction, leading to the oxidation of AgNPs into silver oxide (Ag_2O), and the decomposition of H_2O_2 into water and oxygen.[27].

This oxidation-reduction reaction can be represented as follows:

$$H_2O_2\ (l) + 2\ Ag(s) \rightarrow Ag_2O(s) + H_2O(l)$$

$$H_2O_{2(l)} + 2Ag_{(s)} \rightarrow Ag_2O_{(s)} + H_2O_{(l)}$$

As a result, the solution gradually fades in color, with the reduction in absorbance directly corresponding to the level of the target compound (H_2O_2). This decolorization phenomenon is attributed to the decrease in AgNPs size and the production of Ag_2O, which shows no detectable absorbance in this region of the UV spectrum.[24]The selectivity of the developed colorimetric assay for H_2O_2 was further investigated by introducing potential interferents, including Cu^{2+}, Fe^{3+}, Zn^{2+}, Cd^{2+}, glucose, and a blank sample. As illustrated in Figure 5, only H_2O_2 (at a concentration of 10 mM) induced a distinct color change in the

AgNPs suspension, confirming the high specificity of the assay toward the target analyte.
These results demonstrate that the brown-to-colorless transition is specifically triggered by the presence of H_2O_2, highlighting the assay's selectivity. The observed color variation is associated with a marked reduction in the absorbance at 438 nm indicative of AgNPs

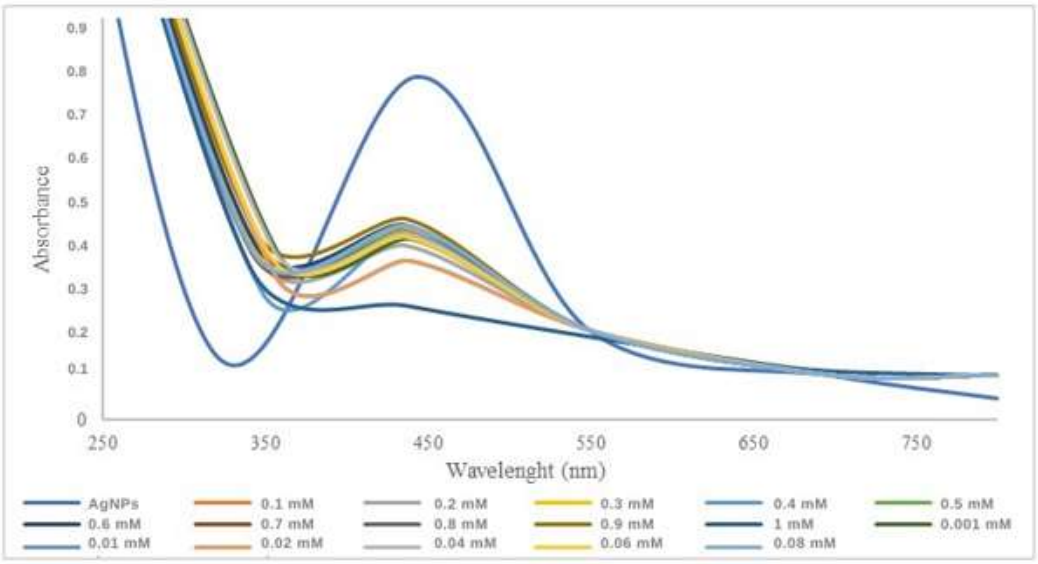

Figure 4: UV–Vis absorption spectra in the presence of different concentrations of H_2O_2 (0.001 mM–1 mM)

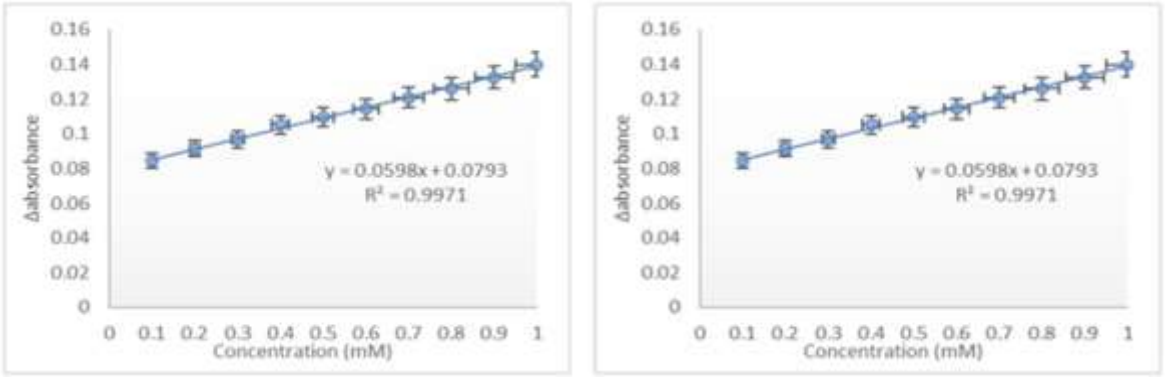

Figure 5: Linear correlation between the absorbance response of the colorimetric assay and different H_2O_2 concentrations

Table 4: H_2O_2 sensor performance with various bioreductive agents

Method	**LOD (mM)**	**inearity nge (mM)**	**Reference**
Colorimetric sensor with AgNPs acacia lignin	9.10^{-4} - 10^{-3}	10^{-3}-100	Aadil et al. (2016)[25]
Colorimetric detection based on CNW: AgNPs	14.10^{-6} 0.112	10^{-7}- 3.10^{-2} 6.10^{-2}- 6.10^{-1}	Teodoro et al. (2019)[21]
Electrochemical sensor with AgNPs and AuNPs Rumex roseus extract	1.1. 10^{-3}	0.035 –1.95.10^{-3}	Chelly et al. (2021)[26]

Colorimetric sensor with AgNPs *Solanum melongena* L. peel	0.0066 0.0432	0.001–0.08 0.1-1	Retnaningtyas et al. (2025)[16]
Colorimetric sensor based on CcL.f -AgNPs	6.8. 10^{-3} 0.0575	1.10^{-3}- 0.08 0.100-1	This research

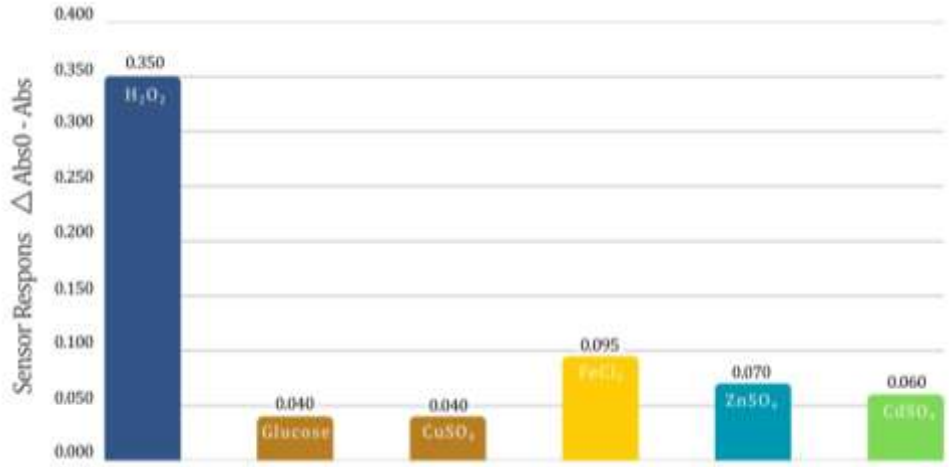

Figure 6: Selectivity test of the AgNPs-based sensor toward H_2O_2 over common interfering species.

Table 5: % Recovery of sample addition

Sample	%Recovery* ± RSD
Mineral water	87.935 ± 2.664%
River water	93.899 ± 1.133%
Milk	87.083 ± 1.222%

*3 replication

decomposition. In contrast, the presence of other tested substances, including cations and the organic compound, produced no significant effect on the suspension (Figure 6). This provides evidence of the suitability of the AgNPs -based system for colorimetric detection of H_2O_2. To confirm the real-world feasibility of the developed colorimetric method, H_2O_2 determination was conducted in commercial water, river water, and milk matrices through the spiking technique.[21] The river water samples were collected from the Bedadung River (Sumbersari, Jember, Indonesia) and filtered using a paper filter. Analyses involved adding 160, 320, and 480 µM of H_2O_2, and the percentage yield was determined, as shown in Table 5, with values ranging from 87.083% to 93.899%, thereby demonstrating the reliability and accuracy of the proposed method for real-sample analysis.

Conclusion

This work reports the first comprehensive evaluation of antioxidant activity, hydrogen peroxide sensing capability, and examination of AgNPs obtained through green synthesis using aqueous leaf extract of *Coffea canephora* L. The results verify that *Coffea canephora* L. aqueous leaf extract is a suitable candidate for the eco-friendly synthesis of silver nanoparticles. Stability was confirmed through UV-Vis, SEM, and DLS characterization, and the nanoparticles demonstrated strong antioxidant activity, underscoring their potential as therapeutic agents.

The AgNPs were employed for the colorimetric sensing of H_2O_2. The developed H_2O_2 sensor demonstrated low detection limits within two concentration ranges: 0.001-0.08 mM and 0.1 mM to 1.0 mM. In addition, the sensor demonstrated excellent sensitivity and selectivity for H_2O_2 determination in real samples, even in the presence of interfering species. Thus, the proposed system offers a promising strategy for sensitive and selective monitoring of H_2O_2.

The present study has highlighted the potential of a sustainable, eco-friendly, and low-cost biological reducer. The extract exhibits promise as a potent green reductant for synthesizing AgNPs. Furthermore, the aqueous leaf extract of *Coffea canephora L.* and the AgNPs derived from it could be beneficial for applications in combating free radical-induced disorders and functioning as a colorimetric probe.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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