B12

by Dhony Hermanto

Submission date: 30-May-2023 08:48PM (UTC-0500)
Submission ID: 2105644072
File name: B12.pdf (1.47M)
Word count: 6211
Character count: 34312

Karbala International Journal of Modern Science

Volume 9 | Issue 1 Article 8

Electrochemically Synthesized Biogenic Silver Nanoparticles Using Green Tea Extract as a Promising Antioxidant

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This research paper is available in Karbala International Journal of Modern Science: https://kijoms.uokerbala.edu.iq/home/vol9/iss1/8

RESEARCH PAPER

Electrochemically Synthesized Biogenic Silver Nanoparticles Using Green Tea Extract as a Promising Antioxidant

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Abstract

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Keywords: Biogenic AgNPs, Electrochemical, Green tea extract, Antioxidant

1. Introduction

Silver nanoparticles are one of the nanomaterials that have attracted wide attention because they possess the highest level of commercialization. It is associated with their potential applications, including sensors, biomedical imaging, antistatic, cryogenic superconducting, photocatalytic, pharmaceuticals, antioxidants, and antibacterial [1,2,3,4]. The derivative products also include disinfection agents, health products, cleaning agents, food storage, textile coatings, bactericides, and medical devices. The chemical and physical synthesis of AgNPs involves the reduction of Ag^{+} ions in a solution or a high-temperature gaseous environment [5]. However, the reducing reagents often used, such as ethylene glycol and sodium borohydride pyridine, can produce some toxic compounds during the process [6]. The high-temperature requirements also increase the operational cost. The development of green preparation methods has attracted much interest in producing AgNPs due to their simplicity, low cost, use of environmentally friendly materials, and effectiveness [7,8]. This approach uses natural biological agents, including DNA, enzymes, proteins as biomolecules, and plant extracts in place of chemical compounds, which serve as reducing agents [9,10,11]. These agents can

Received 22 August 2022; revised 23 November 2022; accepted 27 November 2022.
Available online 16 January 2023

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https://doi.org/10.33640/2405-609X.3276

reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}) and also stabilize AgNPs formed during green synthesis and storage [12,13].

The products that are stabilized by extracts of plant parts, such as roots, stems, seeds, fruits, rhizomes, and leaves, are called biogenic AgNPs. Green tea extracts have been widely used because they are rich sources of polyphenolic compounds, carboxylic acids, terpenoids, aldehydes, amides, flavones, and ketones [14]. Despite these unique advantages, the traditional method still needs to be improved due to the long duration and difficulty of increasing the production scale. The presence of ethyl acetate as a precursor during the process, and the high-temperature requirement, increase the costs [15]. There are also difficulties in controlling the shape and size of plant extract-based production due to the variety of phytochemicals that serve as the capping agents [16,17]. The electrochemical synthesis was then employed for a more controlled process to solve his problem. The manufacture of AgNPs with the electrochemical method, namely electrolysis, also reduces the cost of synthesizing these products and does not use expensive materials.

Consequently, electrolysis has been widely used to synthesize AgNPs due to its advantages. Electrolysis is when a solution decomposes into positive and negative ions when a direct electric current flows through electrodes [18,19]. In this technique, bulk silver electrode with high purity is the primary pure precursor, making them a promising straightforward method for producing pure AgNPs with good quality, large scale, and low cost [20,21]. The electrode was dissolved anodically to produce Ag^{+} and then reduced to Ag^{0} in the presence of polyphenol-rich biomolecules from the green tea extract in the electrolyte at the cathode [22]. In this system, the solution was used as a reducing and capping agent, while distilled water served as the reaction medium [23].

The combination of the electrochemical method and green tea extract led to faster production by reducing the reaction time from a few hours to a few minutes. Based on previous knowledge, this is the first study to use local green tea from Karanganyar, Indonesia, as a capping agent in the synthesis process. The extract is expected to increase the antioxidant activity of AgNPs capped biomolecules due to its rich content. This study attempts to fill the knowledge gap by investigating the synthesis, reaction condition, stability, and antioxidant activity of biogenic AgNPs. The particles obtained were characterized using Fourier Transform Infrared (FTIR) spectroscopy and Transmission Electron Microscopy (TEM) to observe the product's morphology and particle size distribution. The stability was then evaluated through a time-dependent evaluation. The antiradical or antioxidant activity was determined using the DPPH method.

2. Experimental section

2.1. Chemicals

Two silver wires with Ø = 1.8 mm and 99.9% purity (Antam, Indonesia) were used as electrolytic electrodes, namely anode and cathode. The local green tea leaves were collected from Kemuning Tea Garden, Karanganyar, on Mount Lawu, at an altitude of 1500 masl, Indonesia. Furthermore, Sigma–Aldrich, Germany, provided DPPH (2-diphenyl-1-picrylhydrazyl). All other reagents used were analytical grade, and double-deionized distilled water served as the primary solvent.

2.2. Preparation and characterization of biogenic AgNPs

The proposed method for the production of biogenic AgNPs was the conventional synthesis process using green electrolysis, as described by Hoang et al. [22]. Inexpensive silver metal rods used as electrodes (anode and cathode) were polished, washed, and mounted on the electrolytic reactor cover. The extract solution was a reducing and capping agent for the AgNPs biogenic synthesis. It was prepared by boiling 1 g of green tea leaves in 100 mL of double deionized distilled for 10 min. Subsequently, it was diluted to 500 mL at room temperature, and the extracts were used as final samples for qualitative analysis of phytochemicals, as described by Panchal and Parvez [24]. The phytochemical assay included alkaloids, assessed with Mayer's and Dragendorf's tests, while terpenoids were evaluated with Libermann's. The phenolics/tannins and flavonoids were confirmed with the ferric chloride and Shinoda tests, respectively. Two parallel silver metal wire electrodes were immersed in a 500 mL beaker, filled with the extract solution under magnetic stirring at 2000 rpm for 5 min, and connected to a 10 V DC voltage source. The polarity of the anode and cathode was varied every 1 min through a switch as the controller [20]. The solution colour changed from greenish-yellow to brownish-yellow in 2 min, indicating the formation of AgNPs, and the electrolysis was stopped. The colloidal products obtained were stored inside a dark bottle in a refrigerator chiller at 5 °C. Then, a stability test of AgNPs in a water

medium and 0.1 M phosphate buffer solution (PBS) was carried out. The effect of electrode distance, electrolysis time, and extract concentration on AgNP formation was also observed.

The LSPR absorbance of the synthesized colloidal biogenic AgNPs was then measured using a UV–Visible spectrophotometer (Spectrophotometer 7809, Labo-hub, China). The results showed that the products were stable for up to 15 weeks, as indicated by a slight decrease in LSPR absorbance. The resulting colloidal AgNPs were diluted with double-deionized distilled water to prepare the required concentrations. Microscopic images were recorded using a TEM (Hitachi H9500, Japan) with an accelerating voltage of 120 kV. The diameter of the particles in the TEM image was measured using the ImageJ application version 1.49. The measurement results were further analyzed using the origin version 8.5 application to obtain the size distribution histogram and descriptive statistics, including average size, standard deviation, minimum, maximum, and median values. Colloidal AgNPs were separated by centrifugation (Tomy Centrifuge MDX-310, Japan) at 12,000 rpm, followed by freeze-drying (Freeze dryer Alpha 1-2LDplus with vacuum pump RZ 2.5, Germany) before measurement.

2.3. Antioxidant susceptibility test

The antiradical or antioxidant activity of the synthesized biogenic AgNPs stabilized by green tea extract was carried out using the Khan et al. method [2] with some slight modifications. The different concentrations of biogenic AgNPs, green tea extract, and ascorbic acid (as a reference) were dissolved in methanol and added 0.5 mL DPPH. The reaction system was then incubated for 30 min in a dark environment. The sample was centrifuged to remove suspended AgNPs at 10,000 rpm for 5 min. The optical density of the supernatant was measured using UV–Visible spectroscopy with a maximum wavelength of 517 nm against methanol as a blank solution. The procedure was repeated three times, and the relative standard deviation was calculated. The percentage inhibition was obtained using the following equation.

$$\%\text{DPPH Inhibition} = \frac{\text{Abs (control)} - \text{Abs (test)}}{\text{Abs (control)}}$$

3. Results and discussion

3.1. Biogenic AgNPs formation

Biogenic AgNPs can be electrochemically synthesized using green tea extract, where biomolecules are essential as reducing agents and stabilizers. Stabilization due to the restriction of natural biomolecules, such as flavonoids and terpenoids, creates a monolayer on the AgNP surface in the solution. Phytochemical assays were used to ensure that the green tea leaves could assist in the preparation of biogenic AgNPs. The results of phytochemical tests of fresh leaves and extracts are presented in Table 1.

Table 1 shows that the most dominant secondary metabolites in the green tea extract sample were terpenoids and phenolics groups, which was indicated by extreme levels obtained, namely +4. The phenolics have several derivatives, one of which is flavonoids in moderate levels of +2. The flavonoid and terpenoid components responsible for the biogenic synthesis process were confirmed to be present in the green tea extract with substantial levels. Furthermore, the flavonoids were divided into several components, including catechin, which is helpful as a potent antioxidant [25].

Based on previous studies, the synthesis process of biogenic AgNPs is presented in Fig. 1. They were electrochemically synthesized using green tea extracts under a direct current (DC) voltage source of 10 V. The presence of current causes electron transfer at the anode side. Biomolecules, including the R–OH, are lowered to form R–O• radicals, and the loss of electrons accompanies this. Ag^+ ions released from the anode side also accept electrons and interact with R–O• radicals to form Ag^0 [26,27]. The potential mechanism for forming biogenic AgNPs was described in stages 1–3, with R–OH representing biomolecules in green tea extract. The colour change to brownish yellow indicated the formation of the products.

Stage 1, formation of R–O• radicals and releasing Ag^+ ions from the anode side:

$$\text{R–OH} \rightarrow \text{R–O}^{\bullet} + \text{H}^+ + \text{e}^- \quad (1)$$

$$\text{Ag}^0 - \text{e}^- \rightarrow \text{Ag}^+$$

Stage 2, formation of the intermediate complex

$$\text{R–O}^{\bullet} + \text{Ag}^+ \rightarrow \text{R-O—Ag}^+ \quad (2)$$

Table 1. Qualitative analysis of green tea.

Phytochemicals	Green tea leaves	Green tea extract
Alkaloids (Mayer)	no precipitate (–)	the precipitate (+1)
Alkaloids	no precipitate (–)	the precipitate (+1)
(Dragendorf)	pink rose (+1)	dark red (+4)
Terpenoids	red (+3)	dark red (+4)
Phenolic	pink rose (+1)	red (+2)
Flavonoids		

Table description: (–): no content, (+1): weak content, (+2): moderate content, (+3): strong content, (+4): very strong.

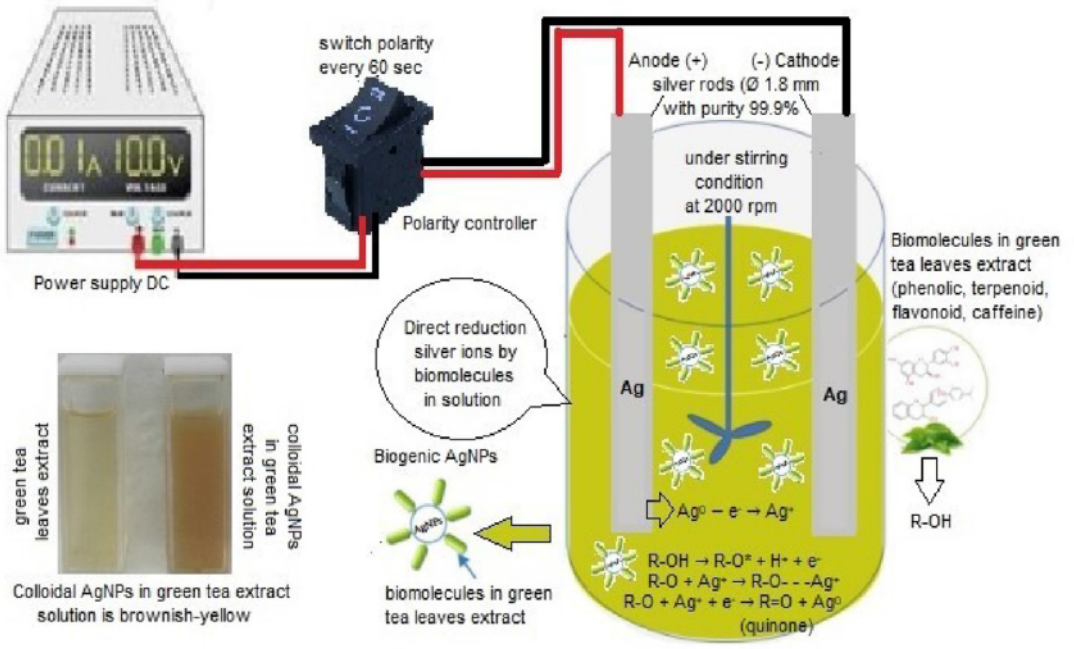

Fig. 1. Schematic formation of biogenic AgNPs electrochemically synthesis using green tea extracts.

Stage 3, formation of Ag^0

$$R{-}O + Ag^+ + e^- \rightarrow R = O\ (quinone) + Ag^0 \quad (3)$$

Ag^0 develops into clusters and then grows to form biogenic AgNPs [22] with biomolecules as the reducing agents and stabilizers. Stabilization was caused by the capping of biomolecules, such as terpenoids and flavonoids, to form a single layer on their surface in the solution. The formation of AgNPs on the cathode side can occur, but this must be prevented due to the production of $R{-}OH{-}{-}Ag^+$ [28] under the stirring conditions of the magnetic stirrer at 2000 rpm. The polarity exchange of positive and negative poles was applied every 1 min to avoid the depletion of Ag at the anode side due to the release of Ag^+ ions [20].

Experimental parameters in AgNP synthesis, including the distance between two electrodes, concentration of green tea extract, and electrolysis time, were found in the supplementary data (S1–S3). The red shift towards a longer wavelength indicates that AgNPs with a larger size were formed [29], while wider peak widths show broad size distribution. A single LSPR band indicates spherical nanoparticles were formed, while anisotropic nanoparticles have two or more LSPRs [30]. Increasing the electrode spacing led to the production of AgNPs with a larger size and broader distribution. Similar results were obtained for variations in electrolysis time. Meanwhile, the lower concentration of tea extract led to broader size distribution. The optimum conditions in this synthesis were 0.5 cm of electrode distance and 2 min of electrolysis with 1% extract concentration.

FTIR measurements were carried out to identify the functional groups of the biomolecules that serve as reducing and stabilizing agents for AgNPs synthesized using green tea extract. They were also used to confirm the schematic of the formation of these products, as previously described by Loo et al. [7]. Three bands in the precise infrared spectra were observed at 3455 cm^{-1}, 1638 $cm^{-1,}$ and 400-800 cm^{-1}, as shown in Fig. 2b. The intense broadband at 3455 cm^{-1} was due to the stretching mode of −OH in the biomolecules of the green tea extract, which was shifted from the previous band of 3400 cm^{-1}, as shown in Fig. 2a. The R−OH was responsible for the reducing agent in the biogenic synthesis of AgNPs. The moderate intensity band at 1638 cm^{-1} emerged from the R = O (quinone) strain mode, indicating the presence of a biomolecule, which functions as a capping agent for AgNPs and enhances the stability of the synthesized nanoparticles. Furthermore, the intense and broad peaks at 400-800 cm^{-1} correspond to Ag^0. The formation of

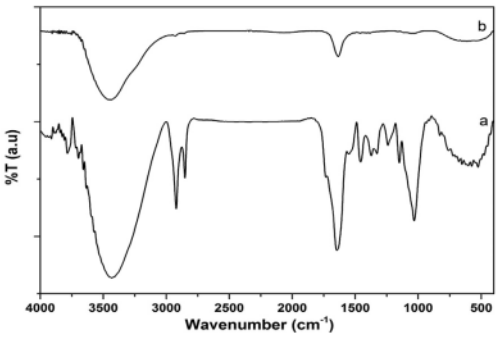

Fig. 2. FTIR spectra of the green tea extract (a) biogenic AgNPs stabilized by biomolecules in this extract (b).

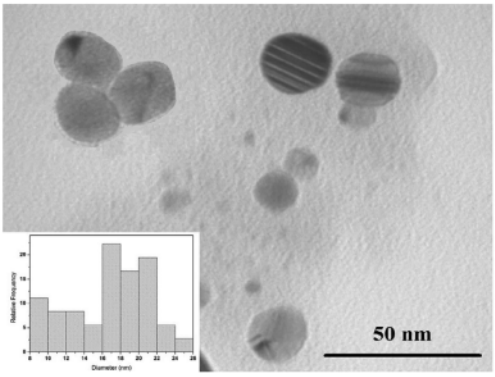

Fig. 4. TEM image of biogenic AgNPs.

biogenic AgNPs was indicated by the characteristics of the LSPR, as shown in Fig. 3.

Biogenic colloidal AgNPs have an LSPR spectrum with an absorption peak at 420 nm, as shown in Fig. 3. The colour of the colloidal AgNPs depends on the shape and size of the particles. Previous studies revealed that colloidal AgNPs with a particle size of 15–50 nm have an LSPR spectrum with an observed absorption peak of 420–438 nm and yellow colouration. Meanwhile, those with a diameter of 1–10 nm and spherical form have LSPR peaks at lower wavelengths. The shape and size of AgNPs can be controlled by solvents and capping and reducing agents [31,32]. It was assumed that prepared AgNPs with the shape of the nanoparticles are spherical, and the average diameter size was 17 nm due to the brownish-yellow colour and peak at 420 nm.

Fig. 4 shows a TEM image of the AgNPs prepared in this current study. As predicted from the LSPR spectrum, the nanoparticles were spherical with quasi-uniform size. The approximate average diameter was 16.82 ± 4.36 nm. Colloidal AgNPs with particle sizes ranging from 8.88 to 24.13 nm have an LSPR absorbance of approximately 420 nm, and their spectra are similar to previously reported data [33,34]. Previous studies reported that smaller Ag nanoparticles have a spherical shape. The TEM images confirmed that the biogenic AgNPs produced using green tea extract were also spherical, and the solution was brownish-yellow. Uniformity of shape and size of AgNPs was achieved in this synthesis. The previous method, which involved conventional heating methods, has a broader size range of 10–60 nm with a longer reaction time of 60 min [9].

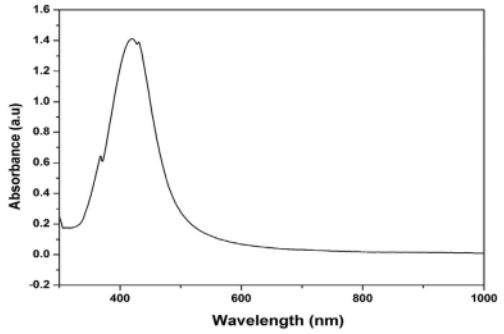

Fig. 3. Spectra LSPR spectra of biogenic AgNPs.

The biomolecular stability of the synthesized AgNPs, which were stabilized by the biomolecules of green tea extract, was measured during the storage period. The measurement of the biogenic stability was carried out within a period of 1 day–15 weeks after the synthesis process using two parameters, namely the position of the maximum wavelength (λ_{max}) and the intensity of the absorption peak, as shown in Fig. 5. A red shift of LSPR was observed during storage from 420 to 431 nm, indicating a change in particle size. Besides the maximum wavelength, absorbance was also observed. There was a decrease of 30% between 1 day and 15 weeks. It indicates that agglomeration occurred during the storage period. Although there was a slight change in the position of λ_{max} and the intensity of the absorption peak, biogenic AgNPs stabilized by biomolecules from green tea extract have a high level of stability within 15 weeks of storage. Previous studies showed that AgNPs synthesized with the addition of the stabilizer Twen-20 can last for two weeks [35], while those produced using *p*-aminobenzoic acid stabilizing agents often have a storage time of 4 weeks [36]. The synthesis method in another study involved the addition of polyvinylpyrrolidone solution into warm silver

nitrate solution under constant vigorous stirring, followed by sodium borohydride. The synthesized products changed at week 6, showing a difference of 11.9% compared to the original profile and a change in absorbance at 18 weeks [37]. The results showed that green tea extract was more effective in producing stable AgNPs.

The stability of AgNPs in PBS media is lower compared to water, as shown in Fig. 5. Furthermore, PBS has a phosphate group (PO_4^{3-}) that is negatively charged, attacks silver [38], and competes with tea extract biomolecules covering the surface of nanoparticles. The release of capping agents on the surface of AgNPs causes susceptibility to agglomeration [39]. Moreover, at low pH, the functional groups of biomolecules can be protonated, reducing their negative charge. Therefore, they are no longer effective as capping agents. This condition cannot provide stability in the PBS system, as indicated by the decreasing intensity and widening of the AgNPs LSPR.

3.2. *Antioxidant activity of biogenic AgNPs*

Antioxidant activity is the ability of a compound or molecule to inhibit or prevent oxidation reactions caused by free radicals [40]. Furthermore, its magnitude is expressed by IC_{50}, which is the concentration of a substance that can inhibit or reduce 50% of free radicals. Testing of antioxidant activity with the DPPH method is based on the ability of a substance to reduce free radicals in DPPH. Samples tested were reacted with DPPH solution, and their absorbance was measured using UV–Visible spectroscopy with a maximum wavelength of 517 nm [41]. A series of solutions were employed for the antioxidant activity determination of biogenic AgNPs, and the results are presented in Fig. 6. As previously reported, the test samples can reduce free radicals through serial dilutions [2,42].

As shown in Fig. 6, ascorbic acid (vitamin C) has a potent antioxidant activity with an IC_{50} value of 7.91 ppm. Meanwhile, green tea extract and biogenic synthesized AgNPs were in the strong category with values of 78.97 and 54.99 ppm, respectively. The results of the sample solution were weaker than the ascorbic acid, which was used as a standard. Green tea extract has lower antioxidant power than biogenic AgNPs capped by biomolecules from the extract. Previous studies revealed that flavonoid compounds are responsible for the antioxidant properties in green tea extracts and silver metal, which showed that the biogenic AgNPs have a better IC_{50} value. Each sample was measured three times, and a relative standard deviation of less than 5% was obtained.

Antioxidant activity occurs through electron donors or hydrogen atoms from antioxidants to free radicals. Elemental silver has one unpaired electron in its configuration, possibly donated to free radical species. Capping agents, namely the biomolecules in tea extract, can also donate hydrogen atoms. It was supported by NMR spectroscopic analysis, which confirmed that aromatic protons played a role in the antioxidant activity of green tea [43]. Fig. 7(1) shows that the one-electron donor of AgNPs on the DPPH radical caused electron pairing and DPPH stability.

Meanwhile, Fig. 7(2) revealed that the hydrogen donation from the capping agent to the DPPH radical led to its stability. One free electron in the radical was paired with the hydrogen atom of the capping agent. These results showed that AgNPs capped biomolecules of tea extracts can be used as an antioxidant to prevent oxidative stress that induces several harmful diseases.

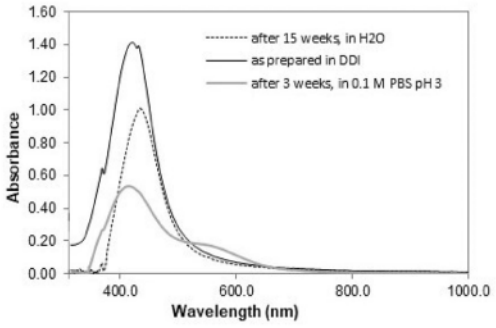

Fig. 5. Stability of biogenic AgNPs in water and PBS.

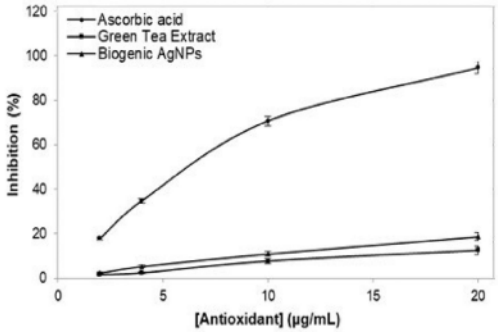

Fig. 6. The results of testing the antioxidant activity of AgNPs synthesized electrochemically using green tea extract by the DPPH method.

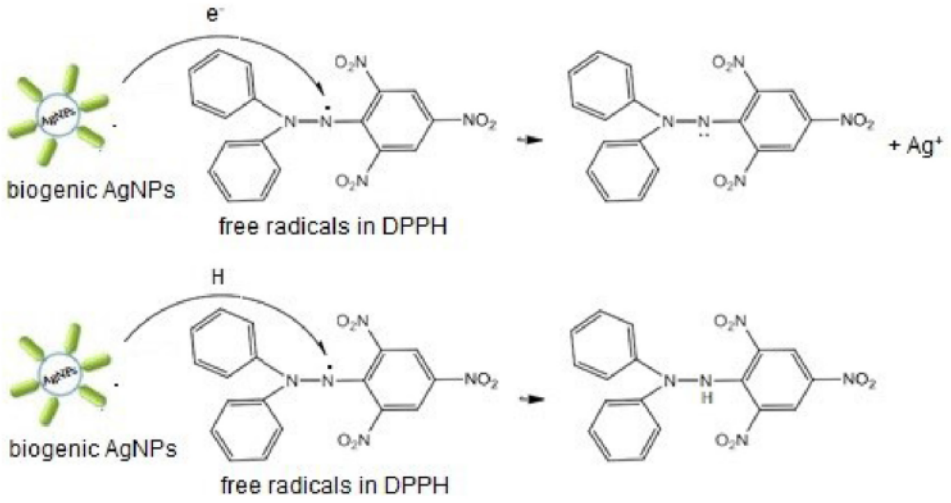

Fig. 7. DPPH radical scavenging reaction mechanism by biogenic AgNPs: (1) AgNPs donate electrons to DPPH radicals; (2) Capping agents of AgNPs donate hydrogen atoms to DPPH radicals.

4. Conclusion

Biogenic AgNPs with excellent characteristics were successfully synthesized using electrolysis and green tea extract as a capping agent within 2 min of the reaction. The AgNPs were spherical, with an average size of 16.82 ± 4.36 nm, ranging from 8.88 to 24.13 nm. The products showed high stability in the aqueous system but were lower in PBS. The tea extract's hydroxyl groups of flavonoids and terpenoids were involved in reducing and stabilizing AgNPs. Biogenic AgNPs also had higher antioxidant activity than green tea extract, with IC_{50} of 54.99 and 78.97 ppm, respectively. The ease of synthesis and excellent properties of AgNPs showed that this method could be used for the large-scale production of antioxidants in the pharmaceutical industry.

Author contributions

D.H. conceptualization, methodology, writing-original draft. N.I. validation, data curation. I.I., I.F., and D.N.C. resources, methodology. U.K.Z. formal analysis, investigation. H.M. visualization, project administration. R.W. software, supervision. All authors contributed to writing, review & editing.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors thank the Kementrian Pendidikan, Kebudayaan, Riset dan Teknologi Republik Indonesia (Kemendikbudristek RI) for the National Competitive Basic Research Program (contract number: 1291/UN18.L1/PP/2022).

Appendix.

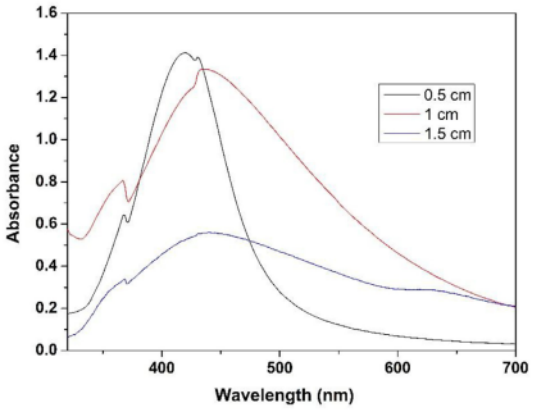

Fig. S1. Effect of distance between two electrodes on AgNP formation.

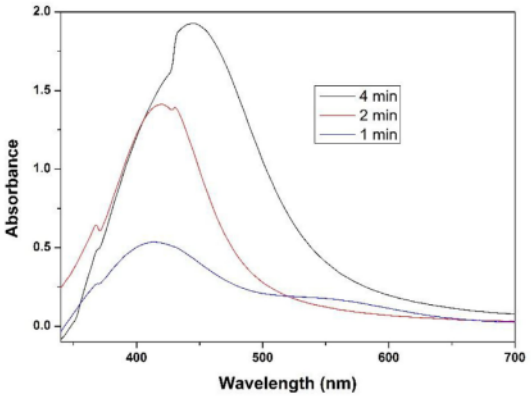

Fig. S2. Effect of electrolysis time on AgNP formation.

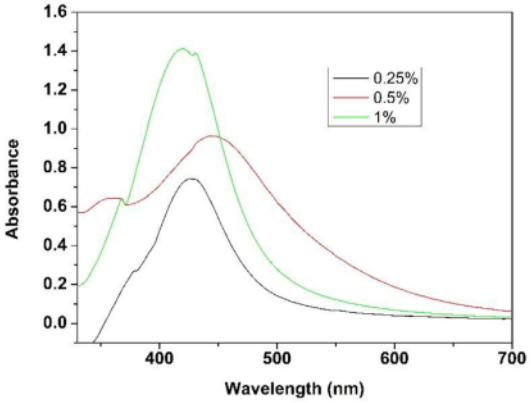

Fig. S3. Effect of concentration of green tea leaf extract on AgNP formation.

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