Sains Malaysiana 53(9)(2024): 3113-3123
http://doi.org/10.17576/jsm-2024-5309-16

Electrosynthesis of Anisotropic Biogenic Silver Nanoparticles as a Promising Antibacterial Agent using *Stachytarpheta jamaicensis* Leaf Extract

(Elektrosintesis Nanozarah Perak Biogen Anisotropik sebagai Agen Antibakteria yang Berpotensi menggunakan Ekstrak Daun *Stachytarpheta jamaicensis*)

DHONY HERMANTO[1,*], NURUL ISMILLAYLI[1], THIFFANY A. L. WIRANATASARI[1], MUDASIR MUDASIR[2], DWI SISWANTA[2], BAMBANG KUSWANDI[3], SISWOYO SISWOYO[4], SUPRAPTO SUPRAPTO[5] & DIDIK PRASETYOKO[5]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram, Jl. Majapahit 62 Mataram, West Nusa Tenggara 83125, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Gadjah Mada, Sekip Utara BLS 21 Sleman, Yogyakarta 55281, Indonesia*
[3]*Chemo and Biosensor Group, Faculty of Pharmacy, University of Jember, Jl. Kalimantan 37 Jember, East Java 68121, Indonesia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember, Jl. Kalimantan 37 Jember, East Java 68121, Indonesia*
[5]*Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Jl. Teknik Mesin 175 Surabaya, East Java 60111, Indonesia*

Received: 11 May 2024/Accepted: 10 July 2024

ABSTRACT

Antibiotic-resistant bacteria provide a great opportunity to use silver nanoparticles (AgNPs) as potential antibiotic replacements. This work utilized *Stachytarpheta jamaicensis* leaf extract (SJLE)-mediated electrosynthesis of biogenic AgNPs. By using two silver rods and SJLE as the electrolysis medium, biogenic AgNP is produced through electrosynthesis. The properties of the formed AgNPs and SJLE phytochemical composition were examined. The disc diffusion method was utilized to evaluate AgNPs' antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*. The high amount of phenolics, flavonoids, and tannins in SLJE provides biomolecules rich in -OH and carbonyl groups, allowing SLJE to have antimicrobial effects as well as act as a capping agent and bioreductor during electrosynthesis. The presence of functional groups from various phytochemicals leads to the formation of anisotropic AgNP crystals with a size of 38.22 ± 13.06 nm and high purity (95.75 ± 0.43%). Antibacterial activity tests against *E. coli* and *S. aureus* show that anisotropic biogenic AgNPs outperformed spherical AgNPs, probably due to the angular AgNPs' ease of penetration into bacterial cell walls. The characteristics of the AgNPs developed induced outstanding antibacterial efficacy against *E. coli* and *S. aureus*. Thus, SLJE-based electrosynthesis provides a synergistic synthetic design of AgNPs as antibacterial agents with several potential long-term advantages, including high purity, fast synthesis, low cost, absence of hazardous ingredients, and simplicity in scaling up.

Keywords: Anisotropic; antibacterial; biogenic AgNP; electrosynthesis; *Stachytarpheta jamaicensis*

ABSTRAK

Bakteria rintang antibiotik memberikan peluang yang baik untuk penggunaan nanozarah perak (AgNPs) yang berpotensi sebagai pengganti antibiotik. Kajian ini menggunakan ekstrak daun *Stachytarpheta jamaicensis* (SJLE) - pengantara elektrosintesis biogen AgNPs. Dengan menggunakan dua rod perak dan SJLE sebagai medium elektrolisis, AgNP biogen dihasilkan melalui elektrosintesis. Ciri AgNPs dan komposisi fitokimia SJLE yang terbentuk telah dikaji. Kaedah diffusi cakera digunakan untuk menilai keberkesanan antibakteria AgNPs terhadap *Escherichia coli* dan *Staphylococcus aureus*. Jumlah fenol, flavonoid dan tanin yang tinggi dalam SLJE menyediakan biomolekul yang kaya dengan -OH dan kumpulan karbonil, membolehkan SLJE mempunyai kesan antimikrob serta bertindak sebagai agen penghalang dan bioreduktor semasa elektrosintesis. Kehadiran kumpulan berfungsi daripada pelbagai fitokimia membawa kepada pembentukan kristal AgNP anisotropik dengan saiz 38.22 ± 13.06 nm dan ketulenan yang tinggi (95.75 ± 0.43%). Ujian aktiviti antibakteria terhadap *E. coli* dan *S. aureus* menunjukkan bahawa AgNPs biogen anisotropik melebihi AgNP sferik, disebabkan oleh AgNPS mempunyai sudut yang mudah ditembusi ke dalam dinding sel bakteria. Ciri AgNPs yang dibangunkan

menyebabkan keberkesanan antibakteria yang luar biasa terhadap *E. coli* dan *S. aureus*. Oleh itu, elektrosintesis berasaskan SLJE menyediakan reka bentuk sintetik sinergistik AgNPs sebagai agen antibakteria dengan beberapa kelebihan jangka panjang yang berpotensi, termasuk kemurnian yang tinggi, sintesis yang cepat, kos yang rendah, ketiadaan bahan-bahan berbahaya dan kesederhanaan dalam skala.

Kata kunci: AgNP biogenik; anisotropik; antibakteria; elektrosintesis; *Stachytarpheta jamaicensis*

INTRODUCTION

Metal nanoparticles (NPs) have become more prevalent in various areas of human life and provide advantages to the economy due to their wide application in bioscience and biomedicine, pharmaceutical ingredients and cosmetics industry, pollution control, photovoltaic cells, superconducting materials, catalysts, biosensors, and more as antioxidant and antibacterial agents (Hermanto et al. 2023a; Ismillayli et al. 2024a; Khan et al. 2018; Kumari, Barsainya & Singh 2017; Misirli, Sridharan & Abrantes 2021). It is estimated that this nanoparticle product will keep improving, leading to a rise in demand. Silver nanoparticles (AgNPs) are the most rapidly developing metal nanoparticles, which is due to their unique qualities (chemical stability, size, shape, homogeneity, and catalytic activity) (Hermanto et al. 2024a; Ismillayli et al. 2024b; Shah et al. 2015). This development was attempted in a nanotechnology scheme such as designing biogenic AgNP with excellent characteristics and capping agents that can enhance antibacterial action (Dos Santos et al. 2014; Firdhouse & Lalitha 2015).

Research on nanotechnology places significant attention on AgNP preparation in addition to its various applications. Although numerous physical and chemical techniques have been reported for the synthesis of AgNP, most of these techniques are costly or involve hazardous substances that are not preferred due to the approach's adverse environmental impacts. Utilizing several aspects of green chemistry principles offers a straightforward, inexpensive, and efficient synthesis of biogenic AgNPs (Fatimah et al. 2020; Kaabipour & Hemmati 2021). Biogenic AgNPs have been synthesised using green tea leaf extract, showing excellent activity as an antioxidant (Hermanto et al. 2023b) and antibacterial agent (Hermanto et al. 2024b). Biogenic AgNP relies on the systematic use of plant extracts; it serves as a bioreductor of Ag^+ to Ag^0 and a stabiliser of AgNP formation. Innovative improvements in the synthesis of biogenic AgNPs are considered (Hermanto et al. 2024b) to prevent damage to biological resources, such as replacing conventional heating. For this purpose, the electrolysis method was used to prepare biogenic AgNPs, which have the advantages of being simple, quick, inexpensive, non-toxic, and ecologically benign.

In the electrosynthesis technique, the precursor used is a silver electrode, which is promising for producing controlled biogenic AgNP with excellent quality and ease of scale-up (Huang et al. 2015). The primary advantage of electrosynthesis over bioreduction is that it eliminates the risk of $AgNO_3$ toxicity and the influence of metal precursor concentration on the properties of the generated nanoparticles by replacing them with a silver anode. As a result, electrosynthesis provides precise control over shape and size, making the synthesis more reproducible, safe, and highly efficient in its application (Kuntyi et al. 2020). Large-scale manufacturing, high purity, and superior electrochemical and antioxidant properties are all provided by the electrosynthesis of AgNPs (Hermanto et al. 2023b; Hoang et al. 2021). The anodic silver electrode generates Ag^+, which is then reduced to Ag^0 by biomolecules in the electrolyte at the cathode (Hoang et al. 2021). The biomolecules flavonoids, phenols, tannins, alkaloids, and saponins found in SJ plants could be involved in the synthesis of AgNP. This biomolecule contains several electron-rich -OH groups (Tsou & Yang 2020), making it an ideal medium for electrolysis. This ability to be easily oxidized can be utilized to produce AgNPs via Ag^+ ion reduction (Loo et al. 2012; Sun et al. 2014). It has previously been established that the aqueous solution of SJLE, in addition to serving as a bioreductor for the creation of biogenic AgNP, also functions as a stabiliser for the biogenic AgNP generated. In this work, biogenic AgNP was electrosynthesised utilizing SJLE, and the properties of the AgNP biogenic particles generated were investigated. The proposed biogenic AgNP was tested against *S. aureus* and *E. coli* to determine its antibacterial activity.

EXPERIMENTAL SECTION

CHEMICALS

Biogenic AgNP is produced by electrosynthesis employing SJLE as the electrolysis medium; in this instance, SJ was obtained from a nearby yard in Mataram, Lombok, Indonesia. The electrosynthesis of biogenic AgNP is performed similarly to a standard electrolysis procedure, with two electrode rods - the cathode and anode - made of silver rod (Ø 1.8 mm; 99.9%, Antam, Indonesia) dipped in SJLE solution. Test the phytochemical content using several reagents as described in the next stage. Double-distilled water was utilized as the solvent in this investigation, and all other reagents were analytical grade.

TEST THE PHYTOCHEMICAL CONTENT

This phytochemical content test was carried out to determine biomolecules that potentially play a role in the preparation of biogenic AgNP, as described by previous work (Ololade et al. 2017). The Folin-Ciocalteau reagent was utilized to determine the Total Phenolic Content (TPC)

of SJLE, with gallic acid serving as the standard. Each 1 mL of sample and 1000 ppm standard was treated with 1 mL of 10% Folin-Ciocalteau reagent, neutralized with 4 mL of 7.5% Na_2CO_3 and shaken at ambient temperature for 3 h. Quercetin was utilized as a standard to calculate the Total Flavonoid Concentration (TFC) of SJLE using the $AlCl_3$ reagent. Each 1 mL of sample and 1000 ppm standard was added with 1 mL of 10% $AlCl_3$ reagent, followed by 1 mL of 1 M $C_2H_3NaO_2$, 2.8 mL of water, and incubated for 40 min at ambient temperature. The Total Tannin Content (TTC) of SJLE was determined using $FeCl_3$ reagent with tannic acid as a standard. Each 0.1 g sample and standard was mixed with 50 mL of water and heated for 30 min. After filtering, 500 mL of water was added to the filtrate. A 0.5 mL aliquot from the previous stage was transferred to a 10 mL measuring flask, followed by 1 mL of 1% $K_3Fe(CN)_6$ and 1 mL of 1% $FeCl_3$, which were diluted to the limit mark and incubated for 5 min. TFC, TPC, and TTC were determined by measuring absorbance using a UV-visible spectrophotometer at 415, 760, and 720 nm, respectively. Determination based on a calibration curve and three replicated measurements.

ELECTROSYNTHESIS AND CHARACTERIZATION OF BIOGENIC AGNP

SJLE is prepared by air-drying SJ leaves and macerating them with distilled water at a 1:10 ratio overnight. The electrosynthesis method refers to green electrolysis as explained by Hermanto et al. (2023b), with silver rods as electrodes and SJLE as the medium. SJLE functions as an electrolyte (electron transfer medium), a bioreductor for the synthesis of biogenic AgNP, and a stabilising/capping agent for the produced AgNP. The design of the electrolytic reactor is based on previous research. The reactor is a 500 mL beaker filled with SJLE and magnetically stirred at 2000 rpm at room temperature. Two silver rods were put parallel to the reactor at a distance of 0.5 cm, with the electrodes immersed in SJLE. The two electrodes are connected to a 10 V DC power supply, with a switch changing the cathode and anode polarity every 1 min during the electrolysis process. The formation of biogenic AgNPs is characterized by a change in the color of the solution from greenish yellow to brownish yellow, then electrosynthesis is stopped. Biogenic AgNPs were separated from SJLE using a centrifuge (Tomy MDX-310, Japan) at 12,000 rpm for 30 min. The pellets were freeze-dried using a freeze dryer (Alpha 1-2LD plus, Germany), then dispersed in distilled water as needed, and stored in dark bottles in a chiller refrigerator at 5 °C until utilization.

Several instrumentations were used to study the characteristics of biogenic AgNP obtained from the previous stage. The localized surface plasmon resonance (LSPR) absorbance of biogenic AgNP was measured using a UV-visible spectrophotometer (7809, Labo-Hub, China). The functional groups involved in biomolecular interactions resulting in biogenic AgNP synthesis were identified using a Fourier Transform Infra-Red (FTIR) spectrophotometer (Perkin Elmer, USA). Scanning Electron Microscopy (JEOL-JEM, Japan) and Transmission Electron Microscopy (TEM) (Hitachi H9500, Japan) were employed to obtain microscopic images of biogenic AgNP particles' shape and size distribution. Meanwhile, X-ray diffraction (XRD) (Philips X'pert PW3050, Netherlands) was utilized to analyze phase morphology, crystallinity, and crystal size of biogenic AgNP using the Debye-Scherrer equation ($D = k \lambda / \beta \cos \theta$). where D is the crystal size; k is the shape factor (0.94); λ is the X-ray wavelength; β is the full-width at half maximum (FWHM); and θ is the peak Bragg diffraction angle.

ANTIBACTERIAL ACTIVITY OF BIOGENIC AGNP

The disc diffusion method was used to conduct a qualitative antibacterial test to assess the antibacterial activity of biogenic AgNPs. Plates with nutrient agar base media inoculated with bacterial suspension (10^6 CFU, *E. coli*, and *S. aureus* test bacteria) were aseptically injected with 30 µL of each of the following: biogenic AgNP (2 µg/mL), spherical AgNP made using ascorbic acid using the heating method (2 µg/mL), SJLE (0.1%), and Ag^+ ions (2 µg/mL). The observations were made after a 24 h period of incubation at 37 °C. The same bacteria were tested with ciprofloxacin as a positive control and double-deionized distilled water as a negative control. The clear area surrounding the disc indicates the bacteria›s sensitivity to the antibacterial agent employed as the test material. The zone of inhibition is measured by the width of the diameter of the clear zone, which is then classified based on its antibacterial properties (Hermanto et al. 2024b).

RESULTS AND DISCUSSION

PHYTOCHEMICAL CONTENT

Biomolecules play a crucial role in the electrosynthesis of biogenic AgNPs with SJLE. Initial phytochemical analysis of SJLE showed that the plant was quite rich in phenolics, flavonoids, and tannins, as reported by Ruma and Zipagang (2015). TPC, TFC, and TTC were identified as the SJLE biomolecules utilized for this purpose; results are shown in Table 1. SJLE has a TPC of 2.647 µg/mg (Table 1), indicating a significant phenolic concentration, consistent with earlier research (Ololade et al. 2017; Ramadhani & Kurniati 2022). Due to its ability to react with active oxygen radicals through its phenolic level, it has tremendous promise as a therapeutic and antioxidant (Cueva et al. 2017). The flavonoid concentration of SJLE was found to be high, as indicated by its TFC of 28.81 µg/mg (Table 1), which is in line with prior research findings (Ololade et al. 2017). The high amount of flavonoids and phenols may have a significant therapeutic effect (Nguyen et al. 2017). Similar to other studies (Ololade et al. 2017),

SJLE also exhibits high tannin levels, as shown by its TTC value of 29.92 µg/mg (Table 1). Tannins are astringent plant phenolic compounds that can be utilized therapeutically as antibacterial agents (Ashok & Upadhyaya 2012). The phytochemical components of SJLE, including TPC, TFC, and TTC, have shown their potential as antibacterials.

ELECTROSYNTHESIS OF BIOGENIC AGNP

The results of a quantitative analysis of the phytochemical content of SJLE are promising as they show the presence of biomolecules rich in -OH (ROH) groups, including tannins, flavonoids, and phenols, which are abundant in the plant (Hussain & Khan 2014). This suggests that the process of electrosynthesis biogenic AgNP can be executed effectively (Kumar et al. 2015). Figure 1 depicts the manufacturing process of biogenic AgNP, and ROH in an electrolytic cell's aqueous system that serves as an electrolyte bio-media. It will dissolve and become an electrical conductor (Yanilkin et al. 2018). In the initial stage, when a DC voltage of 10 V is applied to the electrolytic reactor through two silver rods that act as electrodes, the silver anode is oxidized to water-soluble Ag^+ ions by releasing electrons. Meanwhile, ROH radicals in the aqueous solution transform into RO· radicals. Commonly, Ag^+ ions migrate to the cathode to undergo reduction, resulting in electrodepositing on the cathode side. However, the presence of RO· radicals prevent electrodepositing on the cathode side by interacting with Ag^+ ions to form an intermediate complex, R-O—Ag^+ intermediate complex (Hermanto et al. 2024b, 2023b; Huang et al. 2015). This complex facilitates the formation of biogenic AgNP in the final step. Changing polarity every 1 min was done to avoid anode depletion due to oxidation to Ag^+ ions (Hermanto et al. 2024b).

The electrosynthesis of biogenic AgNP was facilitated by the presence of a DC 10 V, as seen by the solution's color changing from yellow to brownish yellow. The R-O-Ag^+ intermediate complex forms rapidly when DC 10 V is applied while stirring continuously at 2000 rpm and ambient conditions. The reduction of Ag^+ to Ag^0 leads to the formation of R=O radicals (quinone) (Hoang et al. 2021). Finally, the silver particles cluster and form AgNP, with R=O capping and stabilising them, creating an AgNP template in an environment surrounded by biomolecules, which form a layer on the surface of AgNP. Here, SJLE biomolecules play a principal role as bio-media electrolytes, bioreductors, and cappings of biogenic AgNP (Hermanto et al. 2023b; Yanilkin et al. 2018). Long-term electrolysis in an aqueous system enables water electrolysis to occur, allowing the Ag^+ produced by the anode in the medium to combine with the oxygen released by the cathode to form black silver oxide, which thickens due to electrode deposits on the anode side (Hermanto et al. 2024b). Cheon et al. (2011), described the synthesis of AgNPs utilizing electrolysis with two silver electrodes and confirmed the development of silver oxide on the anode surface after one hour of process. As a result, the current flow was disturbed, causing the electrolysis process to stop. In this work, changing the polarity of the anode and cathode every 1 min prevented water electrolysis. Therefore, the proposed biogenic AgNP electrosynthesis process has several prospective future benefits, such as high purity (obtained from the high-purity silver rod), quick synthesis, cheap cost, no hazardous chemicals, and ease of scaling up.

CHARACTERIZATION OF BIOGENIC AGNP

UV-Vis and FTIR spectroscopy were used to confirm the initial characteristics of biogenic AgNP production. Figure 2(a) depicts the UV-visible spectrum of biogenic AgNP produced electrochemically in an aqueous system. Meanwhile, the types of functional groups on the surface of NPs generated by the electrolysis approach were determined by FTIR, as shown in Figure 2(b). Figure 2(a) depicts the maximum absorption wavelengths for SJLE and biogenic AgNP at 309 and 426 nm, respectively. Certain flavonoids, phenolics, and tannins in SJLE possess carbonyl chromophore, which undergoes n,π* excitation to react from the n,π* excited state, absorbing light in the 300 nm region (Patle et al. 2020). The highest wavelength at 426 nm indicates the biogenic AgNP's surface plasmon resonance (SPR) band (Hermanto et al. 2024c), implying that the biogenic AgNP synthesis was successful. The color change from yellow to brownish-yellow confirmed that biogenic AgNP was formed, marked by the SPR absorption spectrum of biogenic AgNP. The appearance of two shoulders in the absorption spectra of biogenic AgNP indicates the formation of anisotropic biogenic AgNP (Thammawithan et al. 2021), as confirmed by the TEM image.

To reduce measurement errors, the produced biogenic AgNP was centrifuged at 12,000 rpm, and the pellets were redispersed in double distilled water to remove residual organic groups from the remaining SJLE biomolecules. Figure 2(b) illustrates the function of biomolecules in the biogenic AgNP formation as stabilising and capping agents. The FTIR spectra of SJLE showed absorption peaks at ~3415, ~1645, and ~1058 cm^{-1}, corresponding to several oxygen-containing functional groups. The absorption peak at ~3415 cm^{-1} represents the stretching mode of the hydroxyl group (ROH) of the SJLE biomolecule, which is crucial for the synthesis and stability of biogenic AgNP. This peak shifted to ~3406 cm^{-1}, confirming that ROH played a role in the formation of biogenic AgNP. The absorption peak at ~1645 cm^{-1} corresponds to the vibration mode of the carbonyl group (C=O) in the SJLE biomolecule. This peak shifted to ~1639 cm^{-1} and intensified, indicating the presence of a C=O group (R=O, quinone) in the biomolecule. This group acts as a covering agent for

AgNP and stabilizes the formed NPs by interacting with the empty d orbital of silver (Hermanto et al. 2024b; Muthaiah, Bhatia & Kannan 2020). The absorption peak at ~1058 cm^{-1} was confirmed to be the stretching vibration mode of the C-O group of the SJLE biomolecule. This peak shifted to ~1054 cm^{-1}, implying that the molecule participated in the reduction of silver ions. Finally, another weak peak appearing in the range of ~521 cm^{-1} in the FTIR spectrum of electrosynthesised biogenic AgNP confirmed metal NP was formed (Siakavella et al. 2020). As a result, the appearance of these peaks in the FT-IR spectrum of electrosynthesised biogenic AgNPs confirms the dual role of SJLE, both as a bioreductor and a stabiliser, this is in line with the literature (Loo et al. 2012; Wirwis & Sadowski 2023). The involvement of several functional groups from many phytochemical substances in the capping action mechanism promotes anisotropic AgNPs to develop.

Other crucial characteristics of biogenic AgNP produced by electrosynthesis are their size, shape, and crystallinity. Several tests were conducted to confirm this, including PSA, XRD, SEM-EDS, and TEM. To eliminate measurement errors caused by residual organic components in SJLE biomolecules, biogenic AgNP centrifuged and re-dispersed in double-distilled water were utilized. Figure 3 shows the results of biogenic AgNP characterization by PSA, XRD, SEM-EDS, and TEM.

PSA was utilized to characterize the size distribution of NPs, and the particle size distribution of biogenic AgNP was measured in a colloidal system. According to the PSA characterization data (Figure 3(a)), the AgNP distribution size is approximately 65 nm. The nanotechnology method is concerned with the design, production, and manipulation of particle architectures ranging in size from 1 to 100 nm, and the biogenic AgNP generated remains within that range. Meanwhile, the crystallinity of biogenic AgNP was determined by XRD. In Figure 3(b), the biogenic AgNP XRD pattern with SJLE biomolecule stabiliser is shown. This pattern corresponds to the cubic Ag phase with a diffraction point at a 2θ value of 38.11˚; 44.16˚; 64.56˚; 77.56˚; and 81.70˚. It could be indexed to the (111), (200), (220), (311), and (211) planes of the face-centered cubic (FCC) crystal structure (ICCD file: 65-2871), which is consistent with another report (Meva et al. 2019). The XRD pattern's (111) comparatively sharp peak suggests that biogenic AgNP is the crystal phase. The Debye-Scherrer equation can be used to estimate the crystal size of silver particles by calculating the peak width of the Bragg reflection index (111), providing an estimated particle size of 17.94 nm (Figure 3(a)). As a result, the biogenic AgNPs obtained were of similar size to prior research (Meva et al. 2019).

The biogenic AgNP obtained through electrosynthesis was next analyzed using SEM-EDS tools to identify the morphology and constituent elements of the NP material by digitally imaging the NP surface. The majority of the biogenic AgNP created during SJLE electrolysis are formed like rods, as evidenced by the SEM image of biogenic AgNP in Figure 3(c). SEM is a digital image of the material's surface, hence it cannot confirm the real shape of the biogenic AgNP particles; TEM can be employed instead. Meanwhile, the chemical composition of the generated biogenic AgNPs can be determined using EDS equipment. Figure 3(d) depicts EDS analysis of biogenic AgNP obtained via electrolysis using SJLE, providing quantitative information of a prominent silver peak (3 KeV) with a composition of (95.75 ± 0.43)% supported by peaks of other elements such as O, Al, and Si, indicating constituents of the SJLE biomolecule.

TEM analysis projects a digital image of the sample by giving an image of its true morphology. Figure 3(e) depicts a TEM image of biogenic AgNPs generated by SJLE electrolysis, demonstrating that these biogenic AgNPs exist in several forms. Biogenic AgNP exists in an anisotropic form as spherical, nanorod, and nanoprism forms. The variety of biomolecules engaged in the AgNP capping and stabilising process makes the biogenic of AgNP with its non-uniform forms possible (Khodashenas & Ghorbani 2019). Similar to the previous explanation, flavonoids, phenols, and tannins are among the biomolecules from SJLE that are involved in the electrosynthesis process and contribute to the biogenic AgNP's non-uniform morphologies. The UV-visible spectra (Figure 2(a)) show a peak and two shoulders, confirming the mixture shapes of biogenic AgNP. Furthermore, the histogram (Figure 3(f)) shows the size of the biogenic AgNP particles obtained during SJLE electrolysis. The particle size is measured at 38.22 ± 13.06 nm. Many types of biomolecules are responsible for the non-uniform shape and size of the AgNP formed in this synthesis.

ANTIBACTERIAL ACTIVITY OF BIOGENIC AGNP

Infections caused by microorganisms (such as bacteria) are a major cause of chronic infections, and the rise in these instances poses a serious and obvious threat to public health. Due to their proven efficacy and higher perceived cost-effectiveness, antibiotics are frequently used to treat bacterial illnesses. However, the current use of antibiotics has resulted in another harmful phenomenon: the growth of antibacterial-resistant bacteria, which threatens antibiotic effectiveness (Wang, Hu & Shao 2017). In addition to the possibility of resistance, the ability of bacteria to build and generate biofilms is a concern. As a very successful survival strategy for bacteria, biofilms are clusters of microorganisms on a surface that are covered in a slimy matrix (EPS, extracellular polymeric material) that also improves resistance to antibacterial drugs (Nazir, Zaffar & Amin 2019). AgNP is considered to have promising potential as an antibiotic substitute.

AgNP can enter the cell wall surface of microorganisms, damaging the strength of the cell membrane and killing the bacterium. Other methods include protein denaturation in bacteria, which has negatively charged plasma on its surface, whereas AgNP can be positively charged. Furthermore, AgNP can denaturize proteins by attaching to thiol groups, or -SH functional groups, on bacterial proteins (Tang & Zheng 2018). AgNP's ability to serve as antibacterial agents by disrupting the bacterial organisms' respiratory metabolism is another significant mechanism. Thus, the potential for biogenic AgNPs as antibacterial agents was investigated.

Using the disk diffusion method, the antibacterial activity of biogenic AgNP against *E. coli* and *S. aureus* was examined, as shown in Figure 4. This method examines the effect of antibacterial agents on bacterial growth in nutrient agar culture. The substance is effective against bacteria at a specific concentration if colonies do not develop on the media; hence, the agar concentration is higher or equal to the effective concentration. The biogenic AgNP produced from SJLE exhibited antibacterial action against two laboratory pathogenic strains, including *E. coli* and *S. aureus*, according to the results of the antibacterial activity test, which are shown in Figure 4.

TABLE 1. Determination of TFC, TPC, and TTC of SJLE

TPC (µg/mg)	TFC (µg/mg)	TTC (µg/mg)
2.647	28.81	29.92

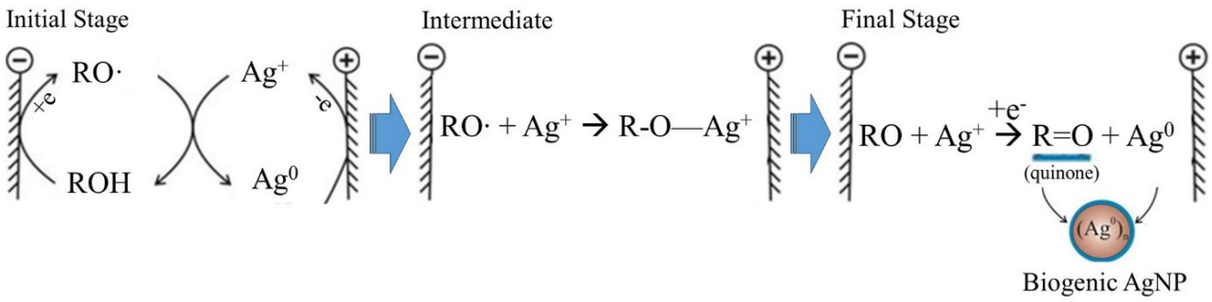
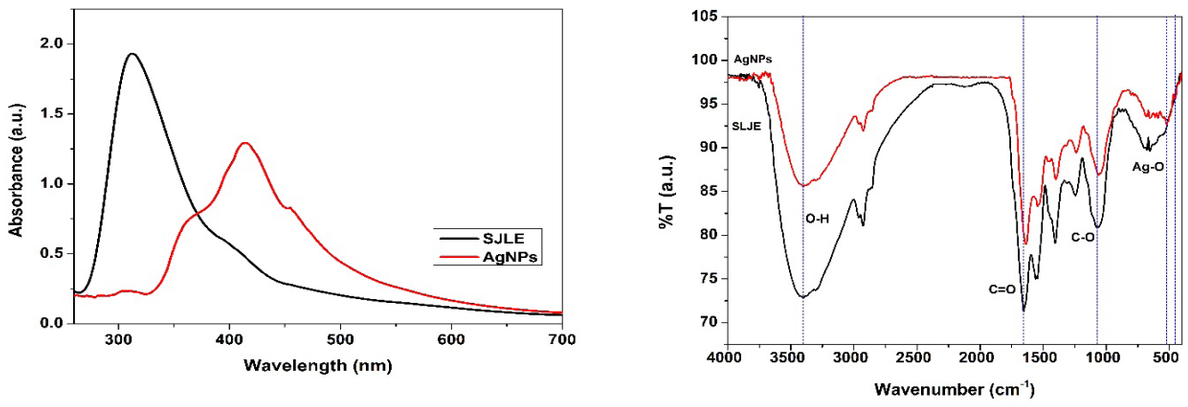
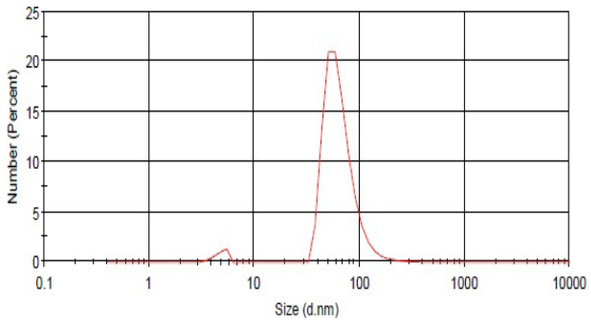


FIGURE 1. Schematic electrosynthesis biogenic AgNP using SJLE

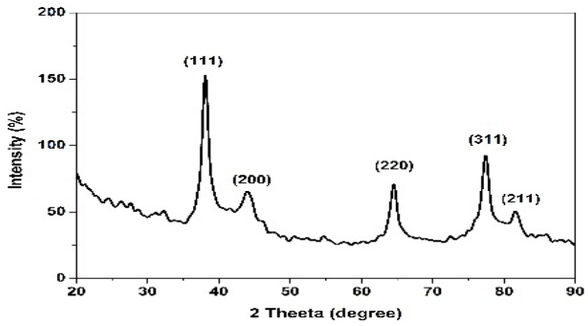


(a) (b)

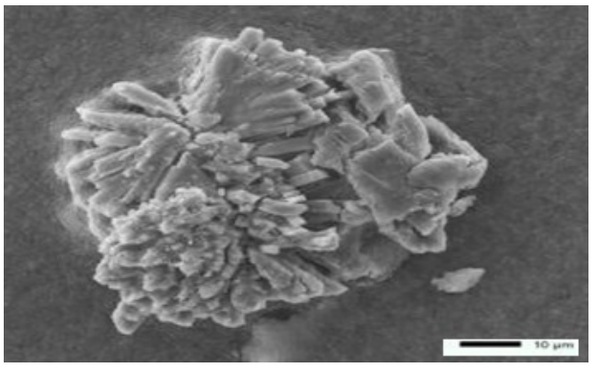
FIGURE 2. UV-Visible spectra biogenic AgNP formed using SJLE (a); FTIR spectra of biogenic AgNP (b)



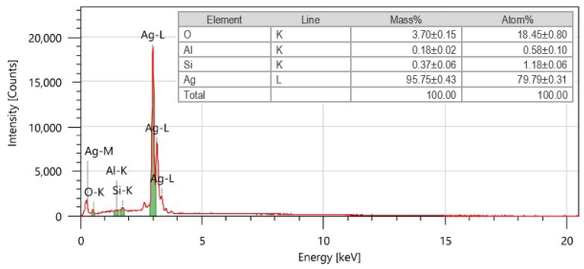
(a)



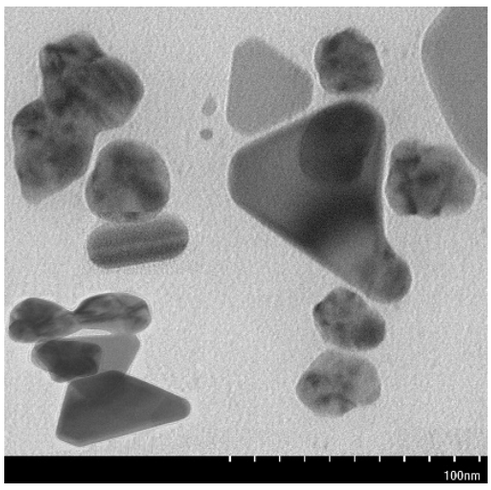
(b)



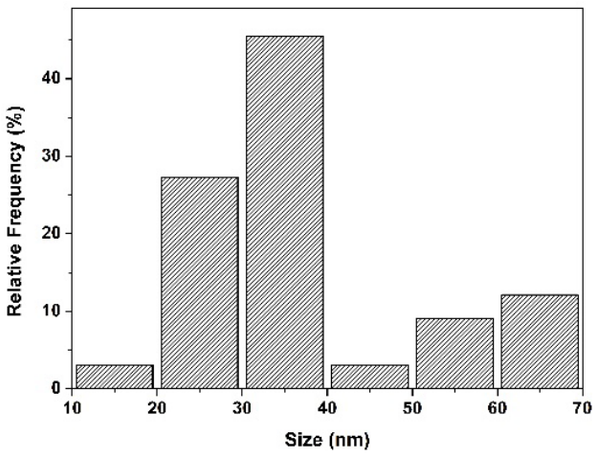
(c)



(d)



(e)



(f)

FIGURE 3. Characteristics of biogenic AgNP (a) PSA; (b) XRD pattern; (c) SEM images; (d) EDS pattern; (e) TEM images; and (f) Particle size distribution

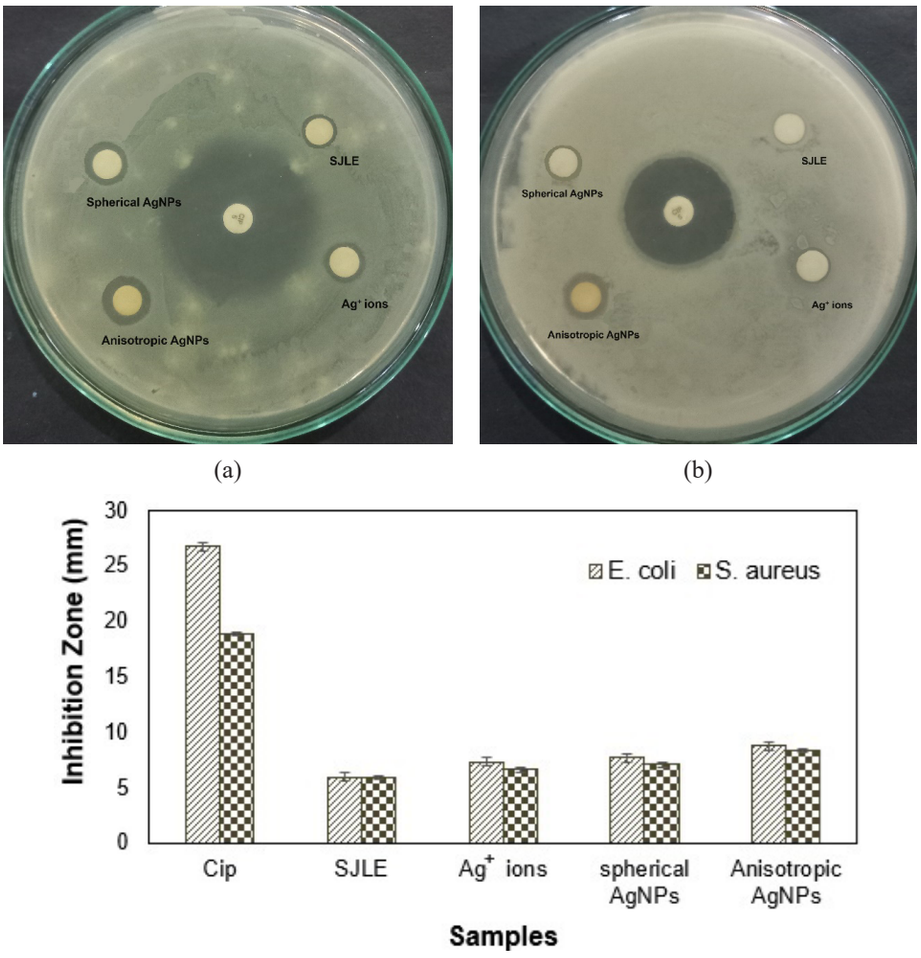


FIGURE 4. Antibacterial activity of biogenic AgNP against (a) *E. coli*; (b) *S. aureus*; (c) bar chart of inhibition zone

The clear zone around the test sample (30 µL) was used to determine antibacterial activity in a 1×10^6 colony forming unit (CFU) bacterial culture. Compared to other test samples, biogenic AgNP (2 µg/mL) showed greater antibacterial efficacy against *S. aureus* and *E. coli* (Figure 4(c)). The inhibition zone in this study was narrower than in earlier investigations (Fatimah et al. 2020; Ghatage et al. 2023) due to the lower concentration of AgNPs applied. The concentration of silver nanoparticles (AgNPs) directly affects the inhibition zone; a higher concentration of AgNPs commonly results in a larger inhibition zone against bacteria (Alowaiesh et al. 2023).

Biogenic AgNP produced by electrosynthesis using SJLE has better antibacterial activity than previous studies (Fatimah 2016; Fatimah & Mutiara 2016), that employed AgNP synthesised with different plant extracts via microwave irradiation. It is suggested that biomolecules derived from SJLE serve as capping and stabilising agents, facilitating these benefits (Rai, Yadav & Gade 2009). The size and form of the generated biogenic AgNP are influenced by SJLE biomolecules, as was previously explained. The small particle size enhances reactivity, electronic effects, and surface-to-volume ratio, allowing NPs to penetrate bacterial cell walls more efficiently. Since they may more readily pass through bacterial barriers, prior studies have shown the high antibacterial activity of nano-prism AgNP (Tanvir et al. 2017). In this study, anisotropic biogenic AgNPs have different morphologies with edges that allow them to penetrate bacterial cell walls. Furthermore, SJLE biomolecules exhibit antibacterial properties (Table 1) due to their flavonoid, phenolic, and tannin content (Ololade et al. 2017). SJLE activity may enhance the intrinsic antibacterial activities of AgNPs. Thus, SJLE-mediated electrosynthesis of biogenic AgNPs is a synergistic synthetic design with the potential to encourage the development of AgNPs as prospective antibacterial agents.

CONCLUSION

Biogenic AgNPs are effectively electrosynthesised using SJLE, with benefits such as high purity, rapid synthesis, low cost, no hazardous components, and ease of scaling up. SJLE's phytochemical composition, including total phenolics (2.647 µg/mg), flavonoids (28.81 µg/mg), and tannins (29.92 µg/mg), suggests the presence of biomolecules rich in -OH and carbonyl groups, which serve as capping agents, electrolyte media, and bioreductors. Biogenic AgNPs are brownish-yellow in color and exhibit a distinct peak at 426 nm. AgNP production involves many functional groups on biomolecules, resulting in anisotropic AgNPs with an average size of 38.22 ± 13.06 nm, fcc crystal structure, and 95.75% purity. This characteristic also affects the antibacterial activity against *E. coli* and *S. aureus*, which is superior to spherical AgNPs. This knowledge, together with SLJE's antibacterial properties as a capping agent, promotes the development of AgNP synthesis as a promising antibacterial agent.

ACKNOWLEDGMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This study is a collaboration between the Faculty of Mathematics and Natural Sciences University of Mataram and the University of Jember (IA contract no. 900/UN18.F7/KS/2024; 625/UN25.1.9/DN/2024).

REFERENCES

Alowaiesh, B.F., Alhaithloul, H.A.S., Saad, A.M. & Hassanin, A.A. 2023. Green biogenic of silver nanoparticles using polyphenolic extract of olive leaf wastes with a focus on their anticancer and antimicrobial activities. *Plants* 12(6): 1410.

Ashok, P.K. & Upadhyaya, K. 2012. Tannins are astringent. *Journal of Pharmacognosy and Phytochemistry* 1(3): 45-50.

Cheon, J.M., Lee, J.H., Song, Y. & Kim, J. 2011. Synthesis of Ag nanoparticles using an electrolysis method and application to inkjet printing. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 389(1-3): 175-179.

Cueva, C., Gil-Sánchez, I., Ayuda-Durán, B., González-Manzano, S., González-Paramás, A.M., Santos-Buelga, C., Bartolomé, B. & Moreno-Arribas, M.V. 2017. An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. *Molecules* 22(1): 99.

Dos Santos, C.A., Seckler, M.M., Ingle, A.P., Gupta, I., Galdiero, S., Galdiero, M., Gade, A. & Rai, M. 2014. Silver nanoparticles: Therapeutical uses, toxicity, and safety issues. *Journal of Pharmaceutical Sciences* 103(7): 1931-1944.

Fatimah, I. 2016. Green synthesis of silver nanoparticles using extract of *Parkia speciosa* Hassk pods assisted by microwave irradiation. *Journal of Advanced Research* 7(6): 961-969.

Fatimah, I. & Mutiara, N.A.L. 2016. Biosynthesis of silver nanoparticles using Putri Malu (*Mimosa pudica*) leaves extract and microwave irradiation method. *Molekul* 11(2): 288-298.

Fatimah, I., Hidayat, H., Nugroho, B.H. & Husein, S. 2020. Ultrasound-assisted biosynthesis of silver and gold nanoparticles using *Clitoria ternatea* flower. *South African Journal of Chemical Engineering* 34(1): 97-106.

Firdhouse, M.J. & Lalitha, P. 2015. Biosynthesis of silver nanoparticles and its applications. *Journal of Nanotechnology* 2015: 29526.

Ghatage, M.M., Mane, P.A., Gambhir, R.P., Parkhe, V.S., Kamble, P.A., Lokhande, C.D. & Tiwari, A.P. 2023. Green synthesis of silver nanoparticles via *Aloe barbadensis* miller leaves: Anticancer, antioxidative, antimicrobial, and photocatalytic properties. *Applied Surface Science Advances* 16: 100426.

Hermanto, D., Ismillayli, N., Hamdiani, S., Kamali, S.R., Wirawan, R., Muliasari, H. & Sanjaya, R.K. 2024a. Inhibitive determination of organophosphate pesticides using acetylcholinesterase and silver nanoparticle as colorimetric probe. *Environmental Engineering Research* 29(4): 230503.

Hermanto, D., Ismillayli, N., Fatwa, D.H., Zuryati, U.K., Muliasari, H., Wirawan, R., Prasetyoko, D. & Suprapto, S. 2024b. Bio-mediated electrochemically synthesis of silver nanoparticles using green tea (*Camellia sinensis*) leaves extract and their antibacterial activity. *South African Journal of Chemical Engineering* 47(1): 136-141.

Hermanto, D., Ismillayli, N., Muliasari, H., Wirawan, R. & Kamali, S.R. 2024c. Silver-based plasmonic nanoparticles for biosensor organophosphate pesticides using a single film containing acetylcholinesterase/choline oxidase. *Global Journal of Environmental Science and Management* 10(1): 39-50.

Hermanto, D., Ismillayli, N., Hamdiani, S., Kamali, S.R., Wirawan, R. & Muliasari, H. 2023a. Paper biosensors utilize silver nanoparticles for onsite pesticide residue detection. *E3S Web of Conferences* 467: 01026.

Hermanto, D., Ismillayli, N., Irmawanti, I., Fathulloh, I., Cahyani, D.N., Zuryati, U.K., Muliasari, H. & Wirawan, R. 2023b. Electrochemically synthesized biogenic silver nanoparticles using green tea extract as a promising antioxidant. *Karbala International Journal of Modern Sciences* 9(1): 80-88.

Hoang, V.T., Dinh, N.X., Pham, T.N., Hoang, T.V., Tuan, P.A., Huy, T.Q. & Le, A.T. 2021. Scalable electrochemical synthesis of novel biogenic silver nanoparticles and its application to high-sensitive detection of 4-nitrophenol in aqueous system. *Advances in Polymer Technology* 2021: 6646219.

Huang, Z., Jiang, H., Liu, P., Sun, J., Guo, D., Shan, J. & Gu, N. 2015. Continuous synthesis of size-tunable silver nanoparticles by a green electrolysis method and multi-electrode design for high yield. *Journal of Materials Chemistry A* 3(5): 1925-1929.

Hussain, S. & Khan, Z. 2014. Epigallocatechin-3-gallate-capped Ag nanoparticles: Preparation and characterization. *Bioprocess and Biosystems Engineering* 37: 1221-1231.

Ismillayli, N., Suprapto, S., Santoso, E., Nugraha, R.E., Holilah, H., Bahruji, H., Jalil, A.A., Hermanto, D. & Prasetyoko, D. 2024a. Microwave-assisted synthesis of silver nanoparticles as a colorimetric sensor for hydrogen peroxide. *RSC Advances* 14(10): 6815-6822.

Ismillayli, N., Suprapto, S., Santoso, E., Nugraha, R.E., Holilah, H., Bahruji, H., Jalil, A.A., Hermanto, D. & Prasetyoko, D. 2024b. The role of pH-induced tautomerism of polyvinylpyrrolidone on the size, stability, and antioxidant and antibacterial activities of silver nanoparticles synthesized using microwave radiation. *RSC Advances* 14(7): 4509-4517.

Kaabipour, S. & Hemmati, S. 2021. A review on the green and sustainable synthesis of silver nanoparticles and one-dimensional silver nanostructures. *Beilstein Journal of Nanotechnology* 12(1): 102-136.

Khan, A.U., Yuan, Q., Khan, Z.U.H., Ahmad, A., Khan, F.U., Tahir, K., Shakeel, M. & Ullah, S. 2018. An eco-benign synthesis of AgNPs using aqueous extract of Longan fruit peel: Antiproliferative response against human breast cancer cell line MCF-7, antioxidant and photocatalytic deprivation of methylene blue. *Journal of Photochemistry and Photobiology B: Biology* 183: 367-373.

Khodashenas, B. & Ghorbani, H.R. 2019. Synthesis of silver nanoparticles with different shapes. *Arabian Journal of Chemistry* 12(8): 1823-1838.

Kumar, B., Smita, K., Cumbal, L., Debut, A., Camacho, J., Hernández-Gallegos, E., Chávez-López, M.D.G., Grijalva, M., Angulo, Y. & Rosero, G. 2015. Pomosynthesis and biological activity of silver nanoparticles using *Passiflora tripartita* fruit extracts. *Advanced Materials Letters* 6(2): 127-132.

Kumari, R., Barsainya, M. & Singh, D.P. 2017. Biogenic synthesis of silver nanoparticle by using secondary metabolites from *Pseudomonas aeruginosa* DM1 and its anti-algal effect on *Chlorella vulgaris* and *Chlorella pyrenoidosa*. *Environmental Science and Pollution Research* 24: 4645-4654.

Kuntyi, O., Mazur, A., Kytsya, A., Karpenko, O., Bazylyak, L., Mertsalo, I., Pokynbroda, T. & Prokopalo, A. 2020. Electrochemical synthesis of silver nanoparticles in solutions of rhamnolipid. *Micro and Nano Letters* 15(12): 802-807.

Loo, Y.Y., Chieng, B.W., Nishibuchi, M. & Radu, S. 2012. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. *International Journal of Nanomedicine* 7: 4263-4267.

Meva, F.E., Mbeng, J.O.A., Ebongue, C.O., Schlüsener, C., Kökçam-Demir, Ü., Ntoumba, A.A., Kedi, P.B.E., Elanga, E., Loudang, E.R.N., Nko'o, M.H.J., Tchoumbi, E., Deli, V., Nanga, C.C., Mpondo, E.A.M. & Janiak, C. 2019. *Stachytarpheta cayennensis* aqueous extract, a new bioreactor towards silver nanoparticles for biomedical applications. *Journal of Biomaterials and Nanobiotechnology* 10(02): 102-119.

Misirli, G.M., Sridharan, K. & Abrantes, S.M.P. 2021. A review on nanostructured silver as a basic ingredient in medicine: Physicochemical parameters and characterization. *Beilstein Journal of Nanotechnology* 12(1): 440-461.

Muthaiah, S., Bhatia, A. & Kannan, M. 2020. Stability of metal complexes. In *Stability and Applications of Coordination Compounds*. Intechopen.

Nazir, R., Zaffar, M.R. & Amin, I. 2019. Bacterial biofilms: The remarkable heterogeneous biological communities and nitrogen fixing microorganisms in lakes. In *Freshwater Microbiology*, edited by Bandh, S.A., Shafi, S. & Shameem, N. Massachusetts: Academic Press. pp. 307-340.

Nguyen, C., Savouret, J.F., Widerak, M., Corvol, M.T. & Rannou, F. 2017. Resveratrol, potential therapeutic interest in joint disorders: A critical narrative review. *Nutrients* 9(1): 45.

Ololade, Z.S., Ogunmola, O.O., Kuyooro, S.E. & Abiona, O.O. 2017. *Stachytarpheta jamaicensis* leaf extract: Chemical composition, antioxidant, anti-arthritic, anti-inflammatory, and bactericidal potentials. *Journal of Scientific and Innovative Research* 6(4): 119-125.

Patle, T.K., Shrivas, K., Kurrey, R., Upadhyay, S., Jangde, R. & Chauhan, R. 2020. Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV–vis and FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 242: 118717.

Rai, M., Yadav, A. & Gade, A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances* 27(1): 76-83.

Ramadhani, P.N. & Kurniati, I.D. 2022. Antimicrobial activity of *Pecut Kuda* leaf extract (*Stachytarpheta jamaicensis* (L. Vahl) against *Mycobacterium smegmatis*. *Mutiara Medika* 23(1): 21-27.

Ruma, O.C. & Zipagang, T.B. 2015. Determination of secondary metabolites and antibacterial property of extract from the leaves of *Stachytarpheta jamaicensis* (L.) Vahl. *Journal of Medicinal Plants and Studies* 3(4): 79-81.

Shah, M., Fawcett, D., Sharma, S., Tripathy, S.K. & Poinern, G.E.J. 2015. Green synthesis of metallic nanoparticles via biological entities. *Materials* 8(11): 7278-7308.

Siakavella, I.K., Lamari, F., Papoulis, D., Orkoula, M., Gkolfi, P., Lykouras, M., Avgoustakis, K. & Hatziantoniou, S. 2020. Effect of plant extracts on the characteristics of silver nanoparticles for topical application. *Pharmaceutics* 12(12): 1244.

Sun, Q., Cai, X., Li, J., Zheng, M., Chen, Z. & Yu, C.P. 2014. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 444: 226-231.

Tang, S. & Zheng, J. 2018. Antibacterial activity of silver nanoparticles: Structural effects. *Advanced Healthcare Materials* 7(13): e1701503.

Tanvir, F., Yaqub, A., Tanvir, S. & Anderson, W.A. 2017. Poly-L-arginine coated silver nanoprisms and their anti-bacterial properties. *Nanomaterials* 7(10): 296.

Thammawithan, S., Siritongsuk, P., Nasompag, S., Daduang, S., Klaynongsruang, S., Prapasarakul, N. & Patramanon, R. 2021. A biological study of anisotropic silver nanoparticles and their antimicrobial application for topical use. *Veterinary Sciences* 8(9): 177.

Tsou, C. & Yang, R. 2020. Low-cost green synthesis of silver nanoparticles using *Taiwan oolong tea* extract. In *Importance & Applications of Nanotechnology.* MedDocs e-Books. pp. 1-5.

Wang, L., Hu, C. & Shao, L. 2017. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine* 12: 1227-1249.

Wirwis, A. & Sadowski, Z. 2023. Green synthesis of silver nanoparticles: Optimizing green tea leaf extraction for enhanced physicochemical properties. *ACS Omega* 8(33): 30532-30549.

Yanilkin, V.V., Fazleeva, R.R., Nasretdinova, G.R., Nastapova, N.V. & Osin, Y.N. 2018. Methylviologen mediated electrosynthesis of silver nanoparticles in a water medium. Effect of chain length and concentration of poly (N-vinylpyrrolidone) on particle size. *New Materials, Compounds and Applications* 2(1): 28-41.

*Corresponding author; email: dhony.hermanto@unram.ac.id