ASEAN Engineering Journal

Full Paper

GREEN SYNTHESIZED SILVER NANOPARTICLES USING LAI (*DURIO KUTEJENSIS*) LEAVES INFUSION WATER AND ITS INHIBITION ACTIVITY AGAINST *ESCHERICHIA COLI* FOR WASTEWATER TREATMENT APPLICATION

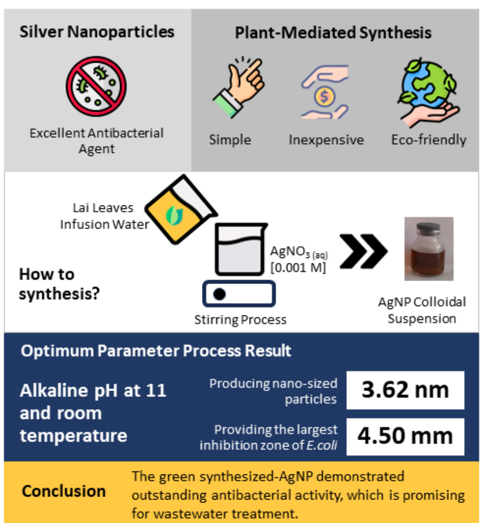
Tajalla, G.U.N.[a*], Prastita, T.P.[a], Anggraeni[a], Lubis, M.P.D.[a], Purwanto, MOCH.[b]

[a]Materials and Metallurgical Engineering Department, Institut Teknologi Kalimantan, Balikpapan 76127, Indonesia
[b]Chemical Engineering Department, Institut Teknologi Kalimantan, Balikpapan 76127, Indonesia

Article history
Received
25 October 2023
Received in revised form
16 June 2024
Accepted
16 July 2024
Published online
28 February 2025

*Corresponding author
gusti.unt@lecturer.itk.ac.id

Graphical abstract



Abstract

Silver nanoparticles (AgNP) have piqued the interest of researchers due to their antibacterial and antimicrobial properties, which are helpful in wastewater treatment applications. Green synthesis was selected because it is simple, quick, inexpensive, low in energy, and environmentally friendly. Many leaves have been studied in research, but the Lai leaves (*Durio kutejensis*), a plant endemic to Kalimantan, has yet to be studied. The leaves contain phenolic compounds, which act as reducing agents and stabilizers during the synthesis of AgNP. The success of AgNP formation is dependent on several process parameters, including pH and temperature. In brief, a 2:1 (v/v) ratio of $AgNO_3$ [0.001M] and Lai leaves infusion water was used in the synthesis. The pH levels were set to 5, 8, and 11, and the temperatures were set to room temperature and 80 °C. UV-Vis and a particle size analyzer (PSA) will be used to characterize the synthesized result. In an alkaline environment, the UV-Vis spectrum shows the peak in all samples. In addition, the highest temperature produced the highest peak at 413-415 nm wavelength, indicating the formation of AgNP. The PSA revealed that all samples produced a homogeneous size because they had a polydispersity index of less than 0.5 with nanoparticle sizes ranging from 1 to 18 nm. In addition, the FTIR analysis of Lai leaves revealed the presence of hydroxyl and carbonyl groups, which play a role in the reduction and stabilization of nanoparticles. Subsequently, the antibacterial properties of AgNP were also investigated using the well-diffusion antibacterial test, which was observed every 24 hours for three days. The optimum zone of inhibition was yielded at alkaline and room temperature due to its nano-sized particles. Therefore, green synthesis using Lai leaves is the potential to produce AgNP with suitable antibacterial activities.

Keywords: Silver Nanoparticles, Green Synthesis, Lai Leaves Infusion Water, pH, Temperature

1.0 INTRODUCTION

Poor water quality causes over 50 different forms of disease, such as diarrhea, skin problems, and cancer [1]. Chlorine is a widely used disinfectant in the water process because it is both simple and effective in killing bacteria. However, employing chlorine to eliminate microorganisms is time consuming. In addition, the use of chemicals will have serious consequence [2]. Therefore, it is still a challenge to develop efficient antimicrobial agents for water purification application.

Currently, nanoparticles are attracting the attention of researchers because of their environmental capabilities. Among metal nanoparticles, silver nanoparticles (AgNP) have good antibacterial activity in the water treatment process [3, 4].

Over the last few decades, various methods production of AgNP have been used, including chemical reduction with $NaBH_4$ or hydrazine. Silver nanoparticles made via chemical reduction yield homogeneous results, but the chemicals are hazardous and environmentally unfriendly [5]. Green synthesis is a safer way to synthesize silver nanoparticles utilizing plants [6]. To date, leaves extracts are most frequently used for the green synthesis of AgNP [7]. Lai (var. *Durio kutejensis*) is an endemic plant of Kalimantan precisely from Kutai Kartanegara, East Kalimantan. Lai leaves contain phenolic compounds which beneficial for reducing Ag^+ into Ag^0 and stabilizing the particles from agglomeration [8]. According to the explanation, it is feasible to use Lai leaves for the green synthesis of AgNP. However, no research has been conducted to examine the use of Lai leaves as a reductor agent in AgNP green synthesis.

The success of green synthesis regarding to its formation and size is dependent on numerous critical parameters, including $AgNO_3$ concentration, extract concentration, pH, and temperature [9]. However, due to the complexity of the plant extract and the different chemical makeup of each type of plant extract, the dependency of activity in each plant extract on operational parameters (e.g., temperature and pH) might provide a different outcome [10]. Therefore, synthesis parameters, likewise pH and temperature, must be carefully controlled to obtain AgNP with lower size and higher homogeneity.

Although some plant extracts have been used in the synthesis of AgNP, until now no research has been reported on the synthesis of AgNP using Lai leaves. Thereby, in this study, an analysis was carried out on green synthesis of silver AgNP using an infusion water of Lai leaves with variations in pH concentration and reaction temperature.

2.0 METHODOLOGY

2.1 Infusion Water Preparation

The fresh of Lai (var. *Durio kutejensis)* leaves were obtained from Balikpapan, East Kalimantan, Indonesia. The Lai leaves were cleaned with distilled water to remove dust and impurities before drying at room temperature for five days. The ground leaves of 0.5 grams were then heated with 500 ml of distilled water until the temperature of the infusion extract solution reached 40℃. After that, the solution filtered using Whatman filter paper No.41. The filtrate was subsequently stored at 4℃ until it was used in next the synthesis process.

2.2 Green Synthesis Of Silver Nanoparticles

A total of 15 ml of extract solution of infusion is prepared. The infusion water's pH was adjusted to 5 with 0.1 M HCl while pH 8 and 11 were set with 0.1 M NaOH. The infusion water was then mixed with 0.001 M $AgNO_3$ as a precursor. The infusion water to precursor ratio was 1:2 (v/v). The synthesis process was carried out under dark conditions at room temperature. The process was repeated for another temperature variation, 80°C. Following the synthesis process, the colloidal sample was observed and further characterized.

2.3 Characterization Methods

The mixture changes color after the synthesis process, indicating the formation of AgNP. To observe those formations, further observations were conducted using a UV-Vis spectrophotometer (Shimadzu UV-1780, Japan). The samples were prepared by diluting six drops of colloidal samples in 5 ml of distilled water and placing them in the cuvette. The measurements were taken at room temperature in the 300-600 nm wavelength range.

Next, dynamic light scattering (DLS, Nano Particle Size Analyzer Biobase BK-802N, China) was used to examine the size distribution of the resulting nanoparticles. The scattering angle was set to 165° and the test was performed at room temperature (25°C). Before characterization, the colloidal samples were diluted in a volume ratio of 1:1 with distilled water and 2 mL of the diluted sample was used for measurement.

The functional groups in Lai leaves infusion extract were confirmed using Fourier Transferred Infrared (FTIR, Bruker Alpha II, Germany). This method identified the essential groups involved in AgNP green synthesis as a reductor and capping agent. As a comparison, dried Lai leaves powder was used. The measurements were taken at room temperature in the 4000-750 /cm range.

2.4 Antibacterial Testing Method

An agar well diffusion assay was employed to evaluate the antibacterial activity of AgNP. The bacterial strain *Escherichia coli* (E. coli) was used as the model. A suspension solution of Escherichia coli bacteria was prepared prior to the test using a turbidity standard of 0.5 McFarland. E. coli bacteria were dissolved in five milliliters of 0.9% NaCl until the turbidity reached 0.5 McFarland. The bacterial suspension solution was then used for antibacterial testing.

On the petri dishes, 100 µL of E. coli bacterial suspension was mixed with 15 mL of Nutrient Agar (NA). The mixture solution had solidified after 10 minutes and was ready to drill five holes with a sterile borer. Each petri dish contained three pH samples (5, 8, and 11), chloramphenicol as a positive control, and Lai leaves infusion water as a negative control of 100 µL. The incubation process then continued for 24 hours at 37 °C. Antibacterial activity was assessed by measuring the diameter of the 'clear zone' formed around the well (in millimeters). This procedure was performed once again for the 80°C samples. The experiment was conducted triplicate and the average values for diameter were later calculated.

3.0 RESULTS AND DISCUSSION

3.1 Observation Visual of Green Synthesized AgNP

The overall synthesis was visually observed, as shown in Figure 1. In comparison, $AgNO_3$(aq) and infusion water have a clear visual, with infusion water having a brownish color (figure 1 (a) and (b)). Ag^+ is reduced to Ag^0 during the synthesis process and then nucleates to form nanoparticles. The color change in the synthesis indicates the formation of silver nanoparticles [11]. There was no change in high color intensity after the synthesis process based on visual observations in samples with acidic conditions (pH 5) either at room temperature or 80°C (Figures 1 (c) and 1. (d)).

Furthermore, alkaline samples exhibit significant color changes. The pH 8 samples at room temperature are lighter brown color than the samples at 80°C (Figures 1(e) and 1(f)). Meanwhile, pH 11 samples at room temperature are dark brown (Figure 1 (g)), whereas samples at 80°C have a blackish brown color and darker than other samples (Figure 1 (h)). The difference in pH causes in different color intensities in each sample.

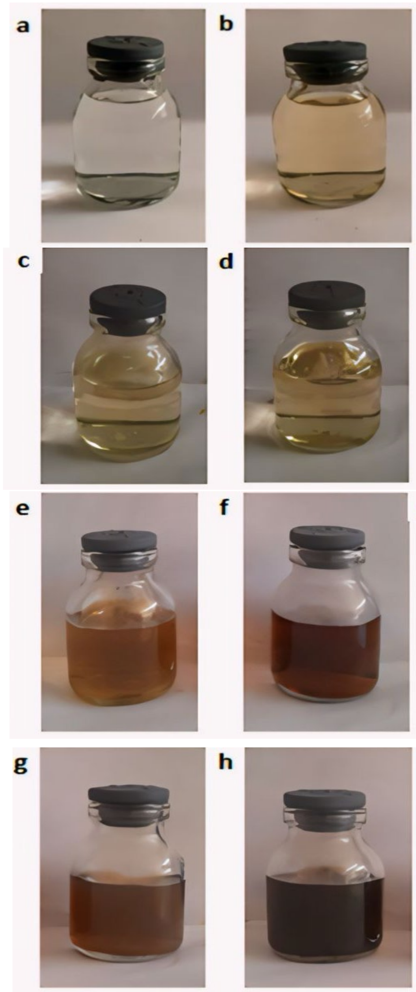


Figure 1 Solution before and after synthesis, (a) 0,001 M $AgNO_{3\ (Aq)}$; (b) Infusion water of Lai leaves;(c) pH 5 at room temperature; (d) pH 5 at temperature 80°C; (e) pH 8 at room temperature; (f) pH 8 at temperature 80°C; (g) pH 11 at room temperature; (h) pH 11 at temperature 80°C

3.2 UV-Vis Spectrophotometer Analysis

The Surface Plasmon Resonance (SPR) band is a unique phenomenon that occurs at the surface of nanoparticles due to collective oscillations between conduction electrons and incoming photons. As a result, UV-Vis analysis became a practical and reliable tool for assessing the synthesis [12].

The presence of peaks in the UV-Vis spectrum at 400-450 nm wavelengths supports previous observations of silver nanoparticle formation in colloidal solutions as SPR bands [13]. Figure 2 shows that peaks in alkaline samples range from 413 to 415 nm. UV-Vis peaks were observed at 414.8 and 415 nm wavelengths in pH 8 samples at room and 80°C, respectively. Furthermore, UV-Vis peaks appear at 413 nm at pH 11 (room temperature and 80°C). SPR effect from nanoparticles causes UV-Vis light absorption. However, there are undetected UV-Vis peaks in pH 5, both of which vary with temperature. At low pH, no SPR bands were detected, as evidenced by absent colour shift in visual observations. This occurence indicated that minimal AgNP formation happened [12].

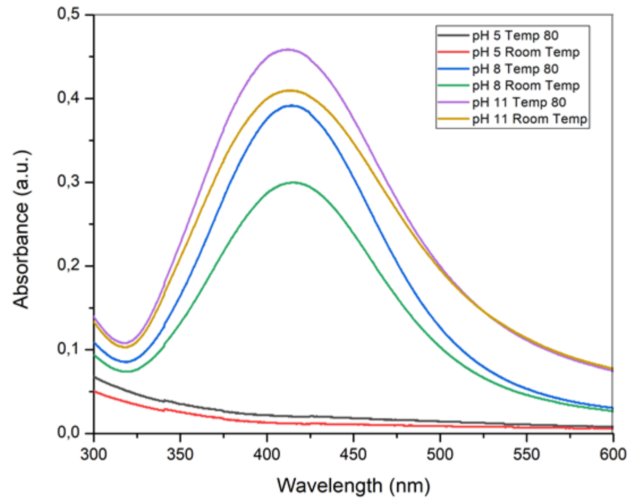


Figure 2 The UV-Vis spectra

The absence of SPR bands in UV-Vis spectra is also regarded an agglomeration. Previous investigations have also found that an acidic pH is unsuitable for green production. An acidic environment produces brighter reaction results. Furthermore, the acidic atmosphere alters the electrical charge of biological molecules, affecting their capping and stabilizing capacity and causing particle size to increase or agglomerate [14].

In addition, peak broadening in the UV-Vis spectrum indicates increased AgNP size [15]. A high absorbance value also indicates that more nanoparticles are being formed. Because of the increased kinetic energy, the spectrum's peak in high-temperature samples is larger and wider than in room-temperature samples [16]. To the findings of this study, green synthesis at high temperatures may result in an increase in the size of nanoparticles, as discussed in the following paragraph.

3.3 Dynamic Light Scattering Analysis

Table 1 depicts the size distribution of AgNP. According to the intensity distribution data, the nanoparticles sizes in all samples ranges from 1 to 18 nm. Furthermore, increased pH resulted in nano-sized particles (Table 2). The average diameter of low pH samples at room temperature tends to agglomerate. This result is consistent with prior UV-Vis observations, that show agglomeration or nil AgNP formation [17].

The polydispersity index (PI) in Table 2 displays the homogeneity of size distribution. A PI value close to 0 indicates a homogeneous or uniform particle distribution, whereas a value greater than 0.5 indicates a high degree of particle heterogeneity [18]. According to the Table 1, all samples have a PI between 0.143 and 0.234. This result demonstrates that all samples have a uniform particle size distribution.

Furthermore, high-temperature samples have a larger average diameter of nanoparticles than room-temperature samples. This is due to the possibility of denaturation of the compounds in Lai leaves extract at high temperatures [15]. The compounds in Lai leaves extract have limited stability at high temperatures. These compounds' structural changes may result in a loss of reducing agent activity. It also gains kinetic energy as the particles collide and merge into larger sizes [19].

Table 1 Silver nanoparticle distribution in all samples

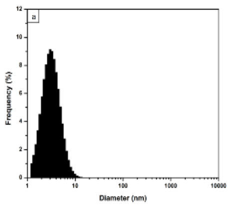
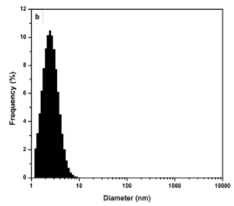
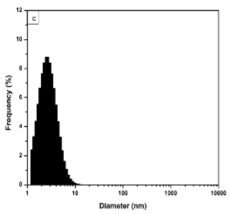
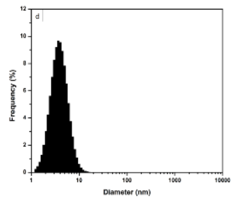
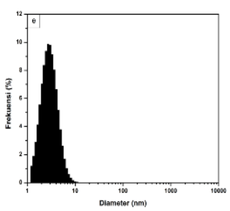
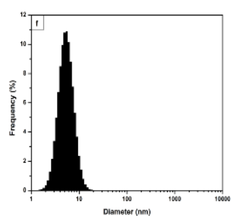
pH	Room Temperature	Temperature 80℃
pH 5		
pH 8		
pH 11		

Table 2 Result of size and homogeneity from silver nanoparticle

pH	Average Diameter (*nm*)		Polydispersity Index, PI	
	Room Temperature	Temperature 80℃	Room Temperature	Temperature 80℃
5	4.11	3.07	0.213	0.162
8	3.57	4.90	0.234	0.186
11	3.62	6.44	0.179	0.143

3.4 Fourier Transform Infrared Spectroscopy Analysis

Bioactive components in Lai leaves contribute to reducing ion Ag into silver nanoparticles. As a result, the FTIR test was performed by comparing the Lai leaves and its filtrate, as shown in Figure 3. The absorption peaks in the origin leaves spectrum were 3337, 2917, 2850, 1731, 1629, 1426, 1373, 1316, 1223, and 1033 cm^{-1}. Those peaks can be attributed to O-H stretching, C-H stretching, C=O stretching, C=C stretching, C-H bending, C-O stretching, and C-O-C stretching.

However, the filtrate after water-based extraction only contained two main peaks, 3284 and 1636 cm^{-1} bands. The absorption peak represents hydroxyl (O-H) and carbonyl (C=C) groups [20]. According to previous studies, the hydroxyl groups aid in reducing ion Ag into AgNP. In Lai leaves extract, the hydroxyl group can be found in phenols [21].

According to earlier research, the hydroxyl and carbonyl groups promote the reduction process. They become the metal-reduction electron donor because they have a more considerable redox potential than other groups. The remaining phenol will be adsorbed as a capping agent and stabilizer on the surface of silver nanoparticles [22, 23]. Thus, Figure 4 illustrates the formation of AgNP.

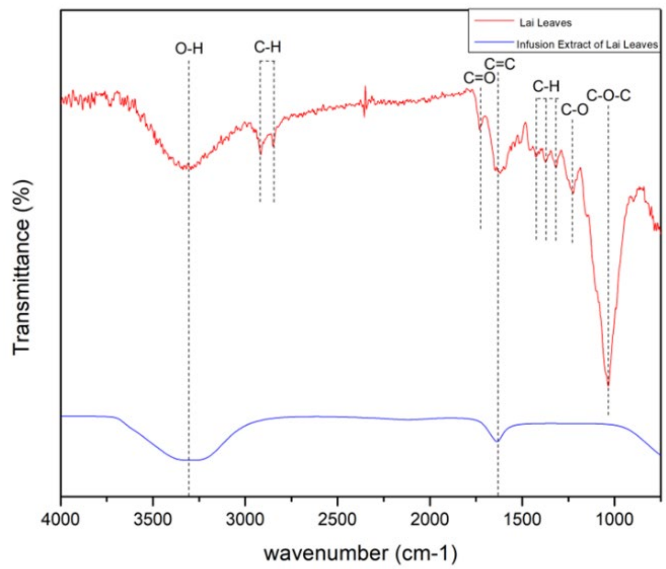


Figure 3 Fourier transform infra-red spectroscopy spectrum of Lai leaves and infusion water of Lai leaves

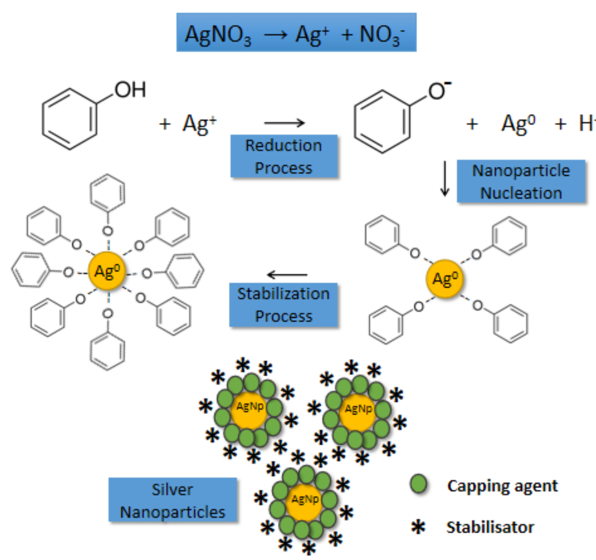


Figure 4 Mechanism of formation of silver nanoparticles

3.5 Antibacterial Activity

The antibacterial assays are limited to samples with alkaline pH because of their UV-vis and DLS evaluations that show AgNP formation and nano-sized particle. The well-diffusion agar method yielded a transparent region surrounding the AgNP, indicating that the bacteria were successfully inhibited (Table 3). The diameter of the zone was measured and calculated to produce the average zone of inhibition (ZOI).

As shown in Tables 4 and 5, ZOI was not detected in infusion water samples (a negative control), indicating that Lai leaves extract lacks antibacterial activity. All green synthesized nanoparticles performed better than chloramphenicol as a positive control, making them potentially used as an antibacterial agent for wastewater treatment. They also achieved better results after three days of incubation.

The currently understood mechanism against bacteria involves four stages. Initially, AgNP firmly adhere to the microbial surface membrane. Then, AgNP penetrates cells and releases reactive oxygen species (ROS), causing biomolecular disruption and intracellular, hence commencing cellular toxicity. Lastly, modulating cell signal transduction pathways [24, 25]. The mechanism is depicted in Figure 5.

Table 3 The result of well-diffusion method

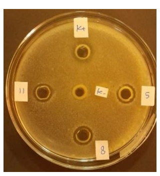
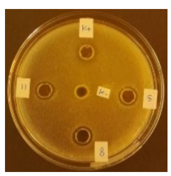
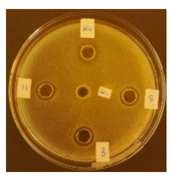
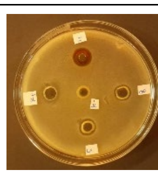
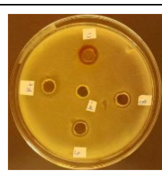
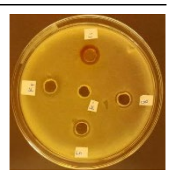
Temperature	24 Hours	48 Hours	72 Hours
Room Temperature			
Temperature 80°C			

Table 4 Zone of inhibition in room temperature conditions

Sample (Room temperature)	24 Hours (*mm*)	48 Hours (*mm*)	72 Hours (*mm*)
Infusion extract of Lai leaves (control -)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Chloramphenicol (control +)	3.75 ± 0.43	3.75 ± 0.43	4.00 ± 0.43
pH 8	3.67 ± 0.76	4.11 ± 0.56	4.33 ± 0.76
pH 11	4.16 ± 1.76	4.42 ± 1.51	4.50 ± 1.39

Table 5 Zone of inhibition in 80 °C condition

Sample (Temperature 80 °C)	24 Hours (*mm*)	48 Hours (*mm*)	72 Hours (*mm*)
Infusion extract of Lai leaves (control -)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Chloramphenicol (control +)	3.83 ± 0.76	3.83 ± 0.76	4.17 ± 0.88
pH 8	3.42 ± 0.95	3.67 ± 1.15	4.10 ± 1.66
pH 11	4.00 ± 0.25	4.35 ± 0.38	4.85 ± 0.26

Previous research has found that nano-sized particles are beneficial for entering bacteria. However, in this study, the bigger size of pH 11 samples demonstrated a wider ZoI than pH 8 samples, which were 4.50 - 4.85 mm and 4.10 - 4.33 mm, respectively. This is due to the fact that AgNP production is increased at high pH [26].

Other than that, the smaller particles at room temperature are effective on day one, whereas the larger particles at 80 °C are effective after a few days. This is possible due to its small size so it moves easily [27]. Nonetheless, more research is required to explain this phenomenon better.

The well-diffusion method is widely used to evaluate antibacterial activity since it is simple and quick. However, agar media may impede AgNP travel, reducing inhibition zone [28]. Furthermore, future studies might employ a sample of raw or synthetic wastewater to provide a more accurate picture of the antibacterial's efficiency in real-world settings.

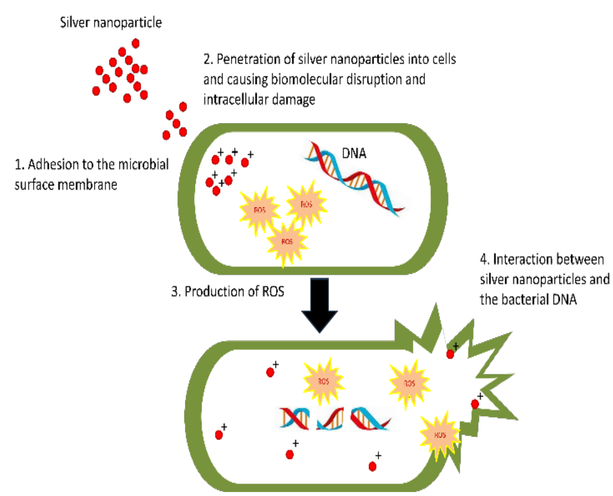


Figure 5 Antibacterial activities of silver nanoparticles

4.0 CONCLUSION

The green synthesis of silver nanoparticles using infusion water from Lai leaves was successful. Two critical parameter processes, pH and temperature, were investigated to produce nano-sized particles with high antibacterial activity. In this study, pH 11 at room temperature has a monodispersed particle size with a diameter of 3.62 nm. These samples also demonstrated excellent antibacterial activity, which is promising for wastewater treatment. This result demonstrates that AgNP with outstanding properties can be produced using a green method.

Acknowledgement

The author would like gratitude to the Ministry of Education, Culture, Research and Technology for providing research grants, 008/E5/PG.02.00.PL/2023. To complete this research, the Institut Teknologi Kalimantan collaborated with the Advanced Membrane Technology Research Center (AMTEC), Universiti Teknologi Malaysia. In addition, the authors would like to thank the Institut Teknologi Kalimantan for providing financial support and facilities for this work.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

References

[1] Lin L, Yang H, Xu X. 2022. Effects of water pollution on human health and disease heterogeneity: a review. *Frontiers In Environmental Science.* 10: 880246. DOI: https://doi.org/10.3389/fenvs.2022.880246

[2] Gelete G, Gokcekus H, Ozsahin DU, Uzun B, Gichamo T. 2020. Evaluating disinfection techniques of water treatment. *Desalination and Water Treatment*. 177: 408-15. DOI: https://doi.org/10.5004/dwt.2020.25070

[3] Huq MA, Ashrafudoulla M, Rahman MM, Balusamy SR, Akter S. 2022. Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: A review. *Polymers*. 14(4):742. DOI: https://doi.org/10.3390/polym14040742

[4] Marimuthu S, Antonisamy AJ, Malayandi S, Rajendran K, Tsai PC, Pugazhendhi A, Ponnusamy VK. 2020. Silver nanoparticles in dye effluent treatment: A review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity. *Journal of Photochemistry and Photobiology B: Biology*. 205:111823. DOI: https://doi.org/10.1016/j.jphotobiol.2020.111823

[5] Vishwanath R, Negi B. Conventional and green methods of synthesis of silver nanoparticles and their antimicrobial properties. 2021. *Current Research in Green and Sustainable Chemistry*. 4: 100205. DOI: https://doi.org/10.1016/j.crgsc.2021.100205

[6] DeAlba-Montero I, Ruiz-Torres CA, Portales-Pérez DP, Martínez-Gutierrez F, Echeverría F, Compeán-Jasso ME, Cataño-Cañizales YG, Ruiz F. 2020. Atmospheric corrosion, antibacterial properties, and toxicity of silver nanoparticles synthesized by two different routes. *Bioinorganic chemistry and applications*. (1): 8891069. DOI: https://doi.org/10.1155/2020/8891069

[7] Vanlalveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B, Rokhum SL. 2021. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC advances*. 11(5): 2804-37. DOI: https://doi.org/10.1039/d0ra09941d

[8] Manurung H, Susanto D, Kusumawati E, Aryani R, Nugroho RA, Kusuma R, Rahmawati Z, Sari RD. 2022. Phytochmical, GC-MS analysis and antioxidant acrivities of leaves methanolic extract of Lai (*Durio kutejensis*), the endemic plant of Kalimantan, Indonesia. *Biodiversitas Journal of Biological Diversity*. 23(11). https://doi.org/10.13057/biodiv/d231104A

[9] Jabeen S, Qureshi R, Munazir M, Maqsood M, Munir M, Shah SS, Rahim BZ. 2021. Application of green synthesized silver nanoparticles in cancer treatment – a critical review. *Material Research Express*. 8(9):092001. https://doi.org/10.1088/2053-1591/ac1de3

[10] Mosaviniya M, Kikhavani T, Tanzifi M, Yaraki MT, Tajbakhsh P, Lajevardi A. 2019. Facile green synthesis of silver nanoparticles using Crocus Haussknechtii Bois bulb extract: Catalytic activity and antibacterial properties. *Colloid and Interface Science Communications*. 33: 100211. DOI: https://doi.org/10.1016/j.colcom.2019.100211

[11] Donga S, Chanda S. 2021. Facile green synthesis of silver nanoparticles using Mangifera indica seed aqueous extract and its antimicrobial, antioxidant and cytotoxic potential (3-in-1 system). *Artificial Cells, Nanomedicine, and Biotechnology*. 49(1): 292-302. https://doi.org/10.1080/21691401.2021.1899193

[12] Miranda A, Akpobolokemi T, Chung E, Ren G, Raimi-Abraham BT. 2022. pH alteration in plant-mediated green synthesis and its resultant impact on antimicrobial properties of silver nanoparticles (AgNPs). *Antibiotics*. 11(11): 1592. DOI: https://doi.org/10.3390/antibiotics11111592

[13] Arshad H, Sami MA, Sadaf S, Hassan U. 2021. Salvadora persica mediated synthesis of silver nanoparticles and their antimicrobial efficacy. *Scientific reports*. 11(1): 5996. DOI: https://doi.org/10.1038/s41598-021-85584-w

[14] Liaqat N, Jahan N, Anwar T, Qureshi H. 2022. Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity, and cytotoxicity study by hemolysis assay. *Frontiers in chemistry*. 10: 952006. DOI: https://doi.org/10.3389/fchem.2022.952006

[15] Dutta T, Chattopadhyay AP, Mandal M, Ghosh NN, Mandal V, Das M. 2020. *Facile Green Synthesis of Silver Bionanocomposite with Size Dependent Antibacterial and Synergistic Effects: A Combined Experimental and Theoretical Studies.* 30: 1839-51. DOI: https://doi.org/10.1007/s10904-019-01332-8

[16] Kaabipour, S. and 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. DOI: https://doi.org/10.3762/bjnano.12.9

[17] Anjum S, Vyas A, Sofi TA, Mirza U, Bera S, Chakraborty S. 2023. Fungal-Based Nanoparticles in Microbial Processes for Synthesizing Nanomaterials. *Singapore: Springer Nature Singapore.* 81-111. DOI: https://doi.org/10.1007/978-981-99-2808-8_4

[18] Gelatik S, Minelli C, Pei Y, Akhtar N. 2020. Evaluation of Particle Size Techniques to Support the Development of Manufacturing Scale Nanoparticles for Application in Pharmaceuticals. *Journal of Pharmaceutical Sciences.* 109(7): 2284-93. DOI: https://doi.org/10.1016/j.xphs.2020.04.001

[19] Hou D, Zong D, Lindberg CS, Kraft M, You X. 2020. On the coagulation efficiency of carbonaceous nanoparticles. *Journal of Aerosol Science*. 140:105478. DOI: https://doi.org/10.1016/j.jaerosci.2019.105478

[20] Palithya S, Gaddam SA, Kotakadi VS, Penchalaneni J Challagundla VN. 2021. Biosynthesis of silver nanoparticles using leaves extract of Decaschistia crotonifolia and its antibacterial, antioxidant, and catalytic applications. *Green Chemistry Letters and Reviews*. 14(1):137-152. DOI: https://doi.org/10.1080/17518253.2021.1876172

[21] Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific reports*. 10(1): 2611. DOI: https://doi.org/10.1038/s41598-020-59451-z

[22] Hamdiani S, Shih YF. 2021. A Green Method for Synthesis of Silver-Nanoparticles-Diatomite (AgNP-D) Composite from Pineapple (*Ananas comosus*) Leaves Extract. *Indonesian Journal of Chemistry*. 21(3): 740-52. DOI: https://doi.org/10.22146/ijc.63573

[23] Rafique M, Sadaf I, Tahir MB, Rafique MS, Nabi G, Iqbal T, Sughra K. 2019. Novel and facile synthesis of silver nanoparticles using Albizia procera leaves extract for dye degradation and antibacterial applications. *Materials Science and Engineering: C*. 99:1313-24. DOI: https://doi.org/10.1016/j.msec.2019.02.059

[24] Jain AS, Pawar PS, Sarkar A, Junnuthula V, Dyawanapelly S. 2021. Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial applications. *International Journal of Molecular Sciences*. 22(21): 11993. DOI: https://doi.org/10.3390/ijms222111993

[25] Salleh A, Naomi R, Utami ND, Mohammad AW, Mahmoudi E, Mustafa N, Fauzi MB. 2020. The potential of silver nanoparticles for antiviral and antibacterial applications: A mechanism of action. *Nanomaterials*. 10(8): 1566. DOI: https://doi.org/10.3390/nano10081566

[26] Guilger-Casagrande M, Lima RD. 2019. Synthesis of silver nanoparticles mediated by fungi: a review. *Frontiers in bioengineering and biotechnology*. 7:287. https://doi.org/10.3389/fbioe.2019.00287

[27] Almatroudi A. 2020. Silver nanoparticles: Synthesis, characterisation and biomedical applications. *Open life sciences*. 15(1):819-39. DOI: https://doi.org/10.1515/biol-2020-0094

[28] Hossain, M. L., Lim, L. Y., Hammer, K., Hettiarachchi, D., & Locher, C. 2022. A Review of Commonly Used Methodologies for Assessing the Antibacterial Activity of Honey and Honey Products. *Antibiotics*. 11(7): 975. DOI: https://doi.org/10.3390/antibiotics11070975