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Research Article

Antimicrobial Activity Test of Silver Nanoparticle Gel from Green Synthesis using Kelor Leaf (*Moringa oleifera*) as a Bioreductant Against *Cutibacterium acne*

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ABSTRACT

Moringa oleifera leaves contain flavonoid compounds that have the potential to act as bioreducers in the synthesis of silver nanoparticles. The antibacterial activity of silver nanoparticles can reduce topical antibiotic resistance, especially against acne-causing bacteria. This study aims to determine the antimicrobial activity of silver nanoparticles formulated in a gel form at concentrations F1 10%, F2 20%, and F3 30%, using Medi-Klin Gel® as a positive control. Testing was conducted on Nutrient Agar medium using the paper disk method with *Cutibacterium acne* bacteria, incubated for 24 hours at 37°C. Additionally, physical evaluations of the gel, including organoleptic properties, spreading ability, and pH, were performed. The results of the physical properties of the gel were analyzed using one-way ANOVA. Organoleptic evaluation results indicated that F1 had a more transparent gel color, while F2 and F3 had darker gel colors, and all three gels had a characteristic extract aroma. Physical evaluation results for spreading ability (5.5±0.25; 5.7±0.12; 6.27±0.08) and pH values (4.42±0.17; 5.35±0.15; 5.27±0.17) were obtained. Antimicrobial research results showed inhibition zones for F1 10% (7.53±0.20), F2 20% (9.43±0.32), F3 30% (11.16±0.12), and the positive control Medi-Klin Gel® (25.15±0.26). Based on this study, concentration differences influenced the physical evaluation results of the gel formulation. Our finding suggests that the formula with the highest concentration (30%) has physical properties reaching the requirements and exhibited the highest inhibitory effect and potential as an antibacterial agent for acne.

INTRODUCTION

The nanotechnology approach is related to the design, synthesis, and manipulation carried out to obtain particle structures with sizes ranging from 1-1000 nm (Nilavukkarasi *et al*., 2020). One of the widely developed types of nanoparticles is silver nanoparticles. Silver nanoparticles find extensive applications in the skincare industry (Kasmudjiastuti *et al*., 2021), improvement of drinking water quality (Hulu *et al*., 2019), and antibacterial activities (Tri Aksari Dewi *et al*., 2019). Silver nanoparticles have advantages due to their antibacterial activity, and numerous studies have been conducted on their efficacy (Nalawati *et al*., 2021) (Fabiani *et al*., 2019). Silver nanoparticles release soluble Ag+ ions that can interact with sulfur-containing protons in bacterial cell walls and cytoplasmic proteins, providing antibacterial properties (Nalawati *et al*., 2021). The utilization of silver nanoparticles in various fields poses challenges in the synthesis process. Silver nanoparticle synthesis can be achieved using chemical, physical, or biological methods (Nilavukkarasi *et al*., 2020).

The synthesis of silver nanoparticles using biological methods is more economical and poses a lower environmental pollution risk, making the resulting products safer and environmentally friendly (Taba *et al*., 2019) (Rengga *et al*., 2017). The biological synthesis of silver nanoparticles can be achieved by reducing Ag+ ions using plant extracts that involve compounds from plants such as flavonoids, terpenoids, alkaloids, and polysaccharides (Fabiani *et al*., 2019) (Kasmudjiastuti *et al*., n.d.). Moringa, a local plant in the city of Palu, contains flavonoid compounds that can be utilized as a bioreducer in the synthesis of silver nanoparticles (Dewie *et al*., 2022) (Munira *et al*., 2021).

The results of silver nanoparticle synthesis can be applied in pharmaceutical formulations, one of which is gel formulation. Gel formulations are effective in treating various skin conditions, including acne caused by *Cutibacterium acne* bacterial infection (Pariury *et al*., 2021) (Yusu *et al*., 2022). Choosing a gel formulation for silver nanoparticles can enhance drug penetration, maintain skin moisture, and reduce inflammation in acne due to the cooling sensation produced (Ariani Edityaningrum *et al*., 2022). Additionally, gel formulations are easier to clean, preventing pore blockage and the growth of Propionibacterium acnes bacteria that can worsen acne-prone skin conditions (Kusuma *et al*., 2018). Several studies on the application of nanoparticles to inhibit acne have been successfully conducted, such as using the biosynthesis of Chromolaena odornata leaves (Mariani *et al*., 2023), utilizing Citrus sinensis L peel infusion (Ermawati *et al*., 2021), and silver nanoparticles synthesized through chemical methods (Septyarin and Taufikurohmah, 2017). Based on the above description, this research aims to determine the inhibitory effect produced by silver nanoparticles synthesized using moringa extract, formulated into a gel, and tested against *Cutibacterium acne* bacteria.

METHODS

Materials

The materials used in this study were fresh Kelor leaves collected from Guntarano, Palu, $AgNO_3$, aquadest, HPMC, propylen glycol, methyl paraben, propyl paraben, Nutrien agar, *Cutibacterium acne* ATCC 11827, blank disk and Medi-klin Gel (Clindamycin phosphate 1,2%). The tools used in this study were glassware (Pyrex), Centrifugator (Thermo), Spectrophotometer UV-VIS (Thermo), Particle Size Analyzer (Horiba Scientific SZ 100z), Scanning Electron Microscope (Thermo Fisher Scientific), Magnetic Stirrer (Horiba), pH meter, and Viscometer.

Kelor Plant Determination

The determination of the kelor plant (*Moringa oleifera* Lamk) was done at UPT Laboratorium Herbal Materia Medica Batu, Jawa Timur.

Extraction of Kelor Leaf

The extraction process started with weighing 10 grams of herbal powder, which was placed into a 250 mL glass beaker that had been filled with 100 mL of water. It was then heated on a hotplate at a temperature of 90 degrees for 15 minutes, occasionally stirred. Afterward, the mixture was filtered using filter paper and a funnel. The filtrate was transferred to a 100 mL measuring flask.

Preparation of 1 mM $AgNO_3$

1 mM AgNO3 solution was prepared by diluting a 10 mM $AgNO_3$ solution. Silver nitrate ($AgNO_3$) was weighed as much as 0.17 grams and dissolved in 100 mL of aquadest then 20 mL was taken and put into a 200 mL volumetric flask then aquadest was added to the mark (Dwiastuti *et al*., 2022).

Nanosilver Synthesis and Purification

Synthesis of nanosilver use 20 mL of moringa leaf extract was reacted with an 80 mL solution of 1 mM $AgNO_3$. After the addition of the extract for 1.5 hours, the solution was stirred at a temperature of 70º. Subsequently, the solution was centrifuged at a speed of 11,000 rpm to separate the precipitate from the filtrate. The obtained filtrate was then stored in a desiccator and utilized for the characterization of nanosilver (Fabiani *et al*., 2019; Tri Aksari Dewi *et al*., 2019).

Nanosilver characteristics

The compounds obtained from the synthesis of silver nanoparticles using moringa leaf extract were then characterized. The particle size of the silver nanoparticles was tested using a Particle Size Analyzer (PSA)

instrument (Horiba Scientific SZ 100z) at the Integrated Laboratory and Research Center of the University of Indonesia (Nalawati *et al*., 2021). The morphology of the silver nanoparticles was analyzed using a Scanning Electron Microscopy (SEM) instrument (Thermo Fisher Scientific) at the Institute of Biological Engineering and Technology, Airlangga University (Tri Aksari Dewi *et al*., 2019).

Gel Formulation of Silver Nanoparticles and Storage Stability Test

This formulation refers to Edityaningrum, Oktafiani, Widya Wati, and Arimurni (2021), and the selected concentrations are based on the optimal formula. The gel preparation begins by heating approximately 30 mL of distilled water to a temperature of around 80-90 degrees. Then, the pre-weighed Hydroxypropyl Methylcellulose (HPMC) is gradually added to the distilled water, allowed to swell for 15 minutes, and stirred until there are no HPMC lumps. Methylparaben and propylparaben are dissolved in propylene glycol, and then the mixture is added to the silver nanoparticle solution. The blending process is carried out using a mixer at a speed of 200 rpm until homogeneous. The weight of the gel is adjusted by adding the remaining distilled water and stirring until homogeneous. The homogenized gel is stored in plastic containers, and stability tests are conducted (Ariani Edityaningrum *et al*., 2022).

The stability test of the gel was conducted by storing it in a tightly closed container for 21 days at 75% relative humidity and a temperature of 30°C. The evaluation of the gel formulation includes organoleptic assessments (shape, color, and odor), spreading capability tests, and pH measurements.

Table 1. The compositions of the Gel silver nanoparticle

Materials	Concentration of materials in the gel formula (%)		
	F1	F2	F3
Nanosilver Kelor bioreductant	10	20	30
HPMC	15	15	15
Propylene Glycol	15	15	15
Methylparaben	0,075	0,075	0,075
Propylparaben	0,025	0,025	0,025
Distilled Water	100	100	100

Testing the Antibacterial Activity of Silver Nanoparticle Gel

The inhibition zone test for nanosilver was conducted by immersing disc papers into silver nanoparticle gel solutions (at concentrations of 10%, 20%, and 30%), negative control (gel base without active substance), and positive control (Medi-Klin Gel). Subsequently, nutrient agar medium was poured into Petri dishes inoculated with *Cutibacterium acne* bacteria and incubated for 24 hours at 37°C. The formed inhibition zones of the silver nanoparticle gel were then measured (Fabiani *et al*., 2019).

Result Analysis

The evaluation results were analyzed using a one-way ANOVA statistical test using SPSS.

Table 2. Organoleptic, pH, and Spreadability of each gel formula

	Organoleptic	pH	Spreadability
F1	Clear brown and distinctive extract aroma	4,42±0,17	5,5±0,25
F2	Dark brown and distinctive extract aroma	5,35±0,15	5,7±0,12
F3	Dark brown and distinctive extract aroma	5,27±0,17	6,27±0,08

Table 3. The result of the measurement Inhibition zone of Gel silvernanoparticle on the growth of *Cutibacterium acne*

Formulation Test	Inhibition Zone (mm)
Formula 1 (Silver Nanoparticles10%)	7.53±0.20
Formula 2 (Silver Nanoparticles20%)	9.43±0.32
Formula 3 (Silver Nanoparticles30%)	11.16±0.12
Positive control (Medi-Klin® Gel)	25.15±0.26
Negative control (Gel Base)	0.0±0.0

RESULTS AND DISCUSSION

Result of Determination of Kelor Leaves (*Moringa oleifera* Lamk)

The kelor plant was determined at the UPT Laboratorium Herbal Materia Medica Batu, Dinas Kesehatan Provinsi Jawa Timur, Kota Malang, with No. 000.9.3/ 2430/ 102.20/ 2023. The result indicated that plant used in this research corresponds to that the plant in question, namely kelor leaf (*Moringa oleifera* Lamk).

Kelor Leaf Extraction

The infusion method is employed to extract water-soluble compounds, as it utilizes water as the primary solvent (Nur Oktavia *et al*., 2020). Compounds in moringa that can be extracted using this method include flavonoids, tannins, saponins, triterpenoids, and alkaloids. The experimental results show that kelor extract contains flavanoid compounds that useful as bioreductor to reduce Ag^{+} ions into Ag^{0} in nanosilver synthesis (Muna, 2022; Fabiani *et al*.,2019).

Nanosilver synthesis and purification

The biosynthesis result shows that moringa leaf extract is able to produce silver nanoparticles with a characteristic color change to brownish-yellow. The synthesis of silver nanoparticles using the biological method is chosen to minimize the use of environmentally unfriendly chemicals. The synthesis employs moringa leaf extract as a bioreducer due to its secondary metabolite content, capable of reducing Ag+ ions into Ag0. The silver source used originates from a silver nitrate solution ($AgNO_{3}^{-}$), serving as a precursor in silver nanoparticle synthesis (Dwiastuti *et al*., 2022; Fabiani *et al*., 2019).

The synthesis involves reacting a 1 mM silver nitrate ($AgNO_3$) solution with moringa extract. This process occurs through a reduction reaction involving electron transfer. The synthesis is conducted at 70 degrees Celsius, resulting in faster completion and smaller particle size.

The formed silver nanoparticles are then separated from the precipitate by centrifugation, dried in a desiccator, and further subjected to characterization.

Result of Characterization Silver Nanoparticles

In this study, the particle size obtained from testing using a particle size analyzer was 113.3 nm. The resulting size can be influenced by variations in the concentration of reducing agents, namely moringa leaf extract and silver nitrate ($AgNO_3$). The differences in compound content are influenced by the growth location, sunlight exposure, and environmental temperature (Ghasemzadeh *et al*., 2018).

The Scanning Electron Microscope (SEM) is used to observe the morphology of silver nanoparticles. Based on the observations, it is noted that the morphology of the silver nanoparticles is spherical (Figure 1).

Stability Test and Preparation Gel Formulation of Silver Nanoparticles

Silver nanoparticle gel is formulated in three variations with differences in the concentration of moringa leaf extract silver nanoparticles, namely F1 10%, F2 20%, and F3 30%. Stability tests for the three formulations were conducted, assessing organoleptic parameters, pH, and spreading ability on days 0, 7, 14, and 21. The pH and spreading ability test results were analyzed using SPSS one-way ANOVA.

Organoleptic testing on day 0 showed that F1 was clear brown, while F2 and F3 were dark brown. Observations up to day 21 revealed no changes in each formula. The color differences are influenced by the concentration

of nanoparticles used. All three formulations lacked a distinctive aroma, and the texture produced by F1 had a somewhat solid consistency compared to F2 and F3, which had a more liquid consistency than F1. The consistency of each formula remained unchanged from day 0 to day 21. Silver nanoparticle gel is formulated in three variations with differences in the concentration of moringa leaf extract silver nanoparticles, namely F1 10%, F2 20%, and F3 30%. Stability tests for the three formulations were conducted, assessing organoleptic parameters, pH, and spreading ability on days 0, 7, 14, and 21. The results of pH and spreading ability tests were analyzed using SPSS one-way ANOVA. pH testing **(Table 2)** was represented by the mean ± SD from day 0 to day 21 for all three formulations. The analysis was performed using SPSS one-way ANOVA. Organoleptic research findings indicate that there were no significant changes observed in the silver nanoparticle gel formulations during storage. The results of the physical tests for pH and spreadability did not exhibit significant changes, as indicated by ($p<0.05$). Storage did not affect the pH and spreadability test results. However, as the concentration of silver nanoparticles used increases, the resulting spreadability also increases due to the influence of the gel's consistency. All formulations showed good spreadability between 5 and 7 cm during the 21-day storage period (Sulastri and Zamzam, 2018).

Antibacterial Test Results

The formed inhibition zone data are divided into five groups: positive control using Medi-Klin Gel 1% (clindamycin phosphate 1%), negative control using the gel base, F1 10%, F2 20%, and F3 30%. Antimicrobial testing was conducted on Nutrient Agar (NA) medium. Based on Figure 2, it shows the formation of inhibition zones in the antimicrobial activity test. Statistical analysis results indicate that the antibacterial activity data of the silver nanoparticle gel are normally distributed with $P > 0.05$. Statistical testing continued using ANOVA and had a significance value of $p < 0.05$, suggesting a significant difference in each group. The **Table 3** indicates that the formula with the largest inhibitory effect is F3 compared to F1 and F2, with an inhibition zone value of 11.16 ± 0.12 mm, categorized as strong (Sulistyani *et al*., 2022).

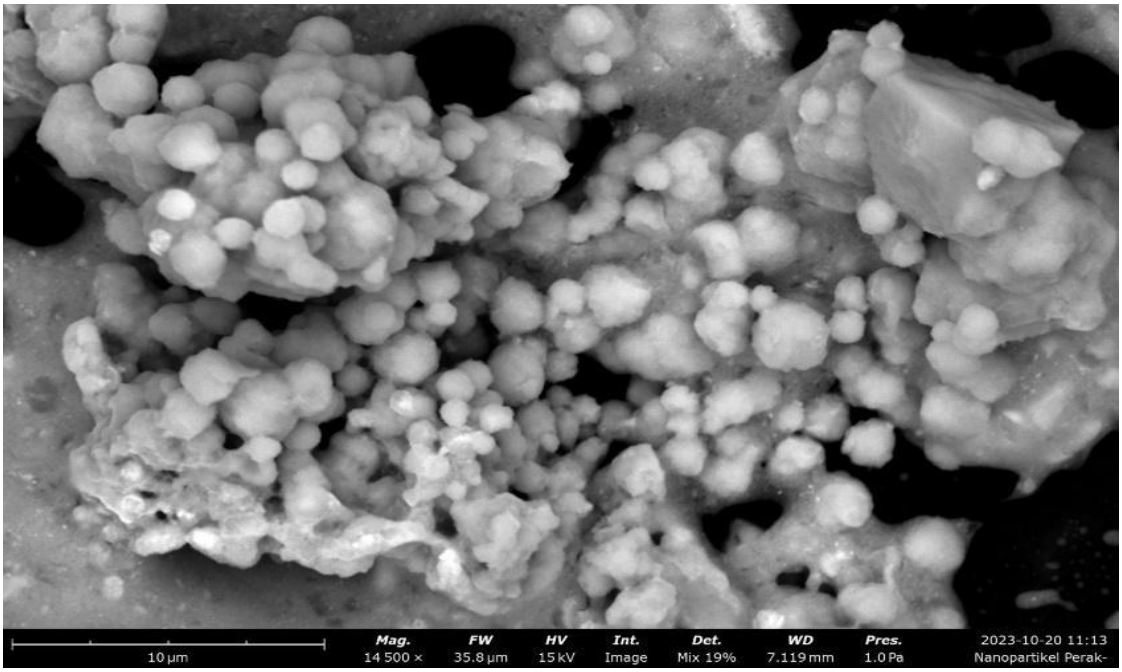

Figure 1. Result of *Scanning Electron Microscopy*

CONCLUSIONS

Based on the research, the physical evaluation results are influenced by the differences in the concentration of silver nanoparticles used. The formula with a concentration of 30% has the highest inhibition zone value, namely 25.15±0.26, and has the potential as an antibacterial agent for acne.

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REFERENCES

Ariani Edityaningrum, C., Oktafiani, A. T., Widiyastuti, L., and Arimurni, D. A., 2022. Formulation and Evaluation of Silver Nanoparticles Gel. In *Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage* (Issue 2).

Dewie, A., Usman, H., and Silfia, N. N., 2022. Pemanfaatan Tanaman Lokal Kelor (*Moringa Oleifera*) Guna Peningkatan Daya Tahan Tubuh Di Era Pandemi Covid-19. *JMM (Jurnal Masyarakat Mandiri)*, 6(5), 4177-4185.

Dwiastuti, R., Irnandari, E., Gani, M. R., Yuliani, S. H., and Nastiti, C. M. R., 2022. Optimization of Nanosilver Synthesis Process with Bioreductor of Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis). *Journal of Pharmaceutical Sciences and Community*, 19(2), 62-70.

Ermawati, D. E., Surya, A. P., and Yugatama, A. 2021. Characterization of Nanosilver Biosynthesis by *Citrus sinensis* (L.) Osbeck and Peel-off Mask Formulation with Variation Polyethylene Glycol 400-Glycerin Concentration. In *Indonesian Journal of Pharmaceutical Science and Technology Journal Homepage*, 1(1), 47-56.

Fabiani, V. A., Putri, M. A., Saputra, M. E., and Indriyani, D. P. 2019. Synthesis of Nano Silver using Bioreductor of *Tristaniopsis merguensis* Leaf Extracts and Its Antibacterial Activity Test. *JKPK (Jurnal Kimia Dan Pendidikan Kimia)*, 4(3), 172-178.

Ghasemzadeh, A., Jaafar, H. Z. E., Bukhori, M. F. M., Rahmat, M. H., and Rahmat, A. 2018. Assessment and comparison of phytochemical constituents and biological activities of bitter bean (*Parkia speciosa Hassk*.) collected from different locations in Malaysia. *Chemistry Central Journal*, 12(1). 1-9.

Hulu, O. P., Sihombing, M., Saputro, R. H., Darmawan, A., and Herbani, Y., 2019. Aplikasi Teknologi Nanopartikel Perak (AgNPs) dalam Air Minum dan Bentuk Kabut terhadap Kadar Amonia Ekskreta Broiler. *Jurnal Ilmu Nutrisi Dan Teknologi Pakan*, 17(2), 26–31.

Kasmudjiastuti, E., Priatni, A., and Rohaeti, E., 2021, Pemanfaatan Nanopartikel Perak Dengan Bioreduktor Daun Sambiloto (*Andrographis peniculata*) Dan Kulit Pisang Kepok (*Musa paradisiaca L*) Sebagai Antibakteri Pada Kulit Domba. *Jurnal Riset Teknologi Industri*, 318-326.

Kusuma, S. A. F., Abdassah, M., and Valas, B. E. 2018. Formulation and evaluation of anti-acne gel containing citrus aurantifolia fruit juice using carbopol as gelling agent. *International Journal of Applied Pharmaceutics*, 10(4), 147-152.

Mariani, H., Mahdi, N., and Umam, K., 2023. Biosintesis Nanopartikel Perak (Agnp) Dengan Ekstrak Daun Kirinyuh (Chromolaena Odorata) Terimpregnasi Zeolit Dalam Menghambat Bakteri Penyebab Jerawat. *Jurnal Biosilampari : Jurnal Biologi*, 5(2), 187–198.

Muna, L. 2022. Aktivitas antioksidan ekstrak air daun kelor (*Moringa oleifera*) dengan metode DPPH serta analisis kualitatif kandungan metabolit sekunder. *Sasambo Journal of Pharmacy*, 3(2), 91–96.

Munira, M., Amalia, D., Khazanah, W., and Nasir, M., 2021. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kelor (*Moringa oleifera* Lamk) Berdasarkan Perbedaan Waktu Panen. In *Indonesian Journal for Health Sciences*, 5(2), 69-76.

Nalawati, A. N., Suyatma, N. E., and Wardhana, D. I. 2021. Sintesis Nanopartikel Perak (Npag) Dengan Bioreduktor Ekstrak Biji Jarak Pagar Dan Kajian Aktivitas

Antibakterinya. *Jurnal Teknologi Dan Industri Pangan*, 32(1), 98–106.

Nilavukkarasi, M., Vijayakumar, S., and Prathip Kumar, S. 2020. Biological synthesis and characterization of silver nanoparticles with Capparis zeylanica L. leaf extract for potent antimicrobial and anti-proliferation efficiency. *Materials Science for Energy Technologies*, 3, 371–376.

Nur Oktavia, S., Wahyuningsih, E., and Deti Andasari, S. 2020. Skrining Fitokimia Dari Infusa Dan Ekstrak Etanol 70% Daun Cincau Hijau (*Cyclea barbata Miers*). In *Jurnal Ilmu Farmasi,* 11(1), 1-6.

Pariury, J. A., Paul Christian Herman, J., Rebecca1, T., Veronica, E., Kamasan, G., and Arijana, N. 2021. Hang Tuah Medical Journal. Potensi Kulit Jeruk Bali (*Citrus Maxima Merr*) Sebagai Antibakteri *Propionibacterium acne* Penyebab Jerawat. In *HTMJ*, 19 (1), 119-131.

Rengga, W. D. P., Yufitasari, A., and Adi, W. 2017. Synthesis Of Silver Nanoparticles from Silver Nitrate Solution Using Green Tea Extract (Camelia Sinensis) As Bioreductor. *Jurnal Bahan Alam Terbarukan*, 6(1), 32–38.

Septyarin, I. P., and Taufikurohmah, D. T. 2017. Uji Aktivitas Antibakteri Nanopartikel Perak (Nanosilver) Terhadap Mutu Sediaan Farmasi Krim Jerawat Silver Nanoparticles (Nanosilver) In *UNESA Journal of Chemistry*, 6(1). 59-63.

Sulastri, L., and Zamzam, M. Y. 2018. The Formulation Gel of Hand Sanitizer of Basil Leaves Ethanol Extract Concentrations of 1,5%, 3%, and 6% with Gelling agent Carbopol 940. 1(1). *Open Journal Systems STF Muhammadiyah Cirebon*, 31-43.

Sulistyani, N., Angelita, L., and Nurkhasanah, N. 2022. Inhibitory Activity of *Parsea americana* Mill. Peels Extract and Fraction Containing Phenolic Compound Against Staphylococcus aureus ATCC 25923. *Journal of Pharmaceutical Sciences and Community*, 19(1), 1–7.

Taba, P., Parmitha, N. Y., and Kasim, S. 2019. Synthesis Of Silver Nanoparticles Using *Syzygium polyanthum* Extract as Bioreductor and the Application as Antioxidant. In *J. Chem. Res.,* 7(1), 51-60.

Tri Aksari Dewi, K., Sukweenadhi, J., and Avanti, C. 2019. Karakter Fisik dan Aktivitas Antibakteri Nanopartikel Perak Hasil *Green Synthesis* Menggunakan Ekstrak Air Daun Sendok (*Plantago major* L.). *Pharmaceutical Sciences and Research*, 6(2), 69–81.

Yusu, A. L., Nugraha, D., Wahlanto, P., Indriastuti, M., Ismail, R., and Himah, F. A. 2022. Formulasi Dan Evaluasi Sediaan Gel Ekstrak Buah Pare (*Momordica Charantia* L.) Dengan Variasi Konsentrasi Carbopol 940, *Pharmacy Genius*, 1(1), 50–61.