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Research Article

Synthesis of Silver Nanoparticles Using *Premna serratifolia* Linn. Leaf Extract as Reducing Agent and Their Antibacterial Activity

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ABSTRACT

Premna serratifolia Linn. leaf extract has been used as a reducing agent in the synthesis of silver nanoparticles (AgNPs). Various synthesis parameters such as reaction time, concentration and the pH of the *Premna serratifolia* Linn. leaf extract, and silver nitrate concentration were investigated. In addition, the stability of synthesized AgNPs and their activity against *Staphylococcus aureus* and *Escherichia coli* have also been investigated. The results of the experiment showed that *Premna serratifolia* Linn. leaf extract reduced silver ions resulting in AgNPs. In addition, the AgNPs colloid showed a gradual change in color from transparent green to yellow. At the same time, its ultraviolet (UV)-Visible spectra exhibited the typical surface plasmon resonance peak at around 400-415 nm. The optimum reaction conditions in the formation of AgNPs are 40 minutes of reaction time using silver nitrate $1.5x10^{-4}$ M and *Premna serratifolia* Linn leaf extract 80 ppm at pH 10. The particle size of synthesized AgNPs distributes from 48.3-157 nm with an average size of 58.7±14.4 nm and is stable at least for 1-month storage under ambient conditions. The antibacterial test shows that synthesized AgNPs are effective against both *Escherichia coli* and *Staphylococcus aureus.*

INTRODUCTION

With the emergence of biofilm community bacterial infections and their resistance, the medical world needs a new antibacterial class. In this era, the use of alternative antibacterial agents such as silver finds momentum. Silver nanoparticles (AgNPs) research provides good knowledge about its efficacy against bacterial infections. As an antibacterial agent, AgNPs have attracted more attention from epidemiology researchers. Many publications have reported the studies of the antibacterial activity of AgNPs (Das *et al*., 2020). The chemical reduction process can be used to produce AgNPs. As the precursor, silver ions are reduced by a reducing agent and then stabilized by a capping agent. In some cases, a reducing agent plays a role as a capping agent simultaneously (Gusrizal *et al*., 2018). The limitation of the chemical reduction process is that the reducing agents are not environmentally friendly. On the other hand, green synthesis using plant material extracts plays a role in minimizing the toxic waste or byproducts. Green synthesis using plant extracts is eco-friendly, rapid, low in cost, and produces nontoxic waste, and provides protection to human health (Ahmad *et al*., 2019; Srikar *et al*., 2016).

The use of plant material extract in the synthesis of AgNPs has been recently reported (Ahmad *et al*., 2019; Chandra *et al*., 2020; Das *et al*., 2020). The extracts of leaves, roots, stems, bark, flowers, and fruit act as a reducing and capping agent. Phenolic and flavonoid content in the plant material extract can be utilized for reducing silver ions and stabilizing the synthesized AgNPs (Jadhav *et al*., 2018).

Premna serratifolia Linn. or *buas-buas* (local name) is widely distributed and well-known as a food component in "*bubur pedas*", a West Kalimantan traditional food. *Premna serratifolia* Linn ethanolic extract contains tannins, flavonoids, saponins, and phenolic compounds. It is also reported that the ethanolic extract of *Premna serratifolia* Linn has an antioxidant activity due to the phenolic and flavonoid content (Isnindar and Luliana, 2020; Purwanti *et al*., 2018).

In this paper, we report the use of *Premna serratifolia* Linn. leaf extract as a reducing agent in the synthesis of AgNPs. Furthermore, the bioactivity of synthesized AgNPs as an antibacterial agent is also presented. The data presented in this paper are taken from the Master's thesis submitted to the Department of Chemistry Universitas Tanjungpura by the first author (Octavianus, 2021).

METHODS

Materials

Dried *Premna serratifolia* Linn. leaves were collected from Sintang, West Kalimantan. The chemicals such as silver nitrate (Merck), sodium hydroxide (Merck), ethanol (J.T. Baker) were used without further purification.

Instrumentations

Ultraviolet (UV)-Visible spectrophotometer (UV-1800 Shimadzu) was used for collecting the surface plasmon resonance (SPR) spectra of AgNPs. Fourier transform infrared (FTIR) (Thermo Scientific Nicolet iS10) was used to identify the functional group of the *Premna serratifolia* Linn leaf extract, and a particle size analyzer (Backman Coulter Delsa Nano) was used to measure the size of synthesized AgNPs.

Preparation of *Premna serratifolia* Linn leaf extract

Premna serratifolia Linn leaf was collected, air-dried, and then powdered using a chopper. The extract of *Premna serratifolia* Linn was prepared by adding 200 g of powdered *Premna serratifolia* Linn. leaf into 1,200 ml of ethanol. After three days, the extract was filtered and concentrated with a rotary evaporator. The extract was stored in an amber bottle for further experiments.

Synthesis of AgNPs

In the synthesis of AgNPs, we modified the previously published procedure (Gusrizal *et al*., 2018). AgNPs were synthesized by reduction of silver nitrate with *Premna serratifolia* Linn. leaf extract. Several synthesis parameters such as reaction time, concentration and the pH of the extract, and silver nitrate concentration were investigated. The color change of the reaction mixture from transparent green to yellow and the peak of SPR at 400-450 nm indicate the formation of AgNPs (Sharma *et al*., 2009).

Effect of time of reaction

The first step in the synthesis of AgNPs is to adjust the pH of *Premna serratifolia* Linn. leaf extract to 11 by adding sodium hydroxide solution into the extract (100 ppm in water). In a test tube, 5 ml of the pH 11 adjusted extract was mixed with 5 ml silver nitrate $1x10^{-4}$ M. The mixture was then heated in a boiling water bath. The formation of AgNPs was monitored at various times of reaction from 10-60 min using a UV-Visible spectrophotometer.

Effect of the pH of Premna serratifolia Linn. leaf extract

In studying the effect of pH of the extract in the synthesis of AgNPs, the pH of extract (100 ppm) was adjusted to 8, 10, and 12 by adding sodium hydroxide solution. In a test tube, 5 ml of the pH adjusted extract was mixed with 5 ml silver nitrate $1x10^{-4}$ M. The mixture was then heated in a boiling water bath for 40 min. The formation of AgNPs was monitored using a UV-Visible spectrophotometer.

Effect of silver nitrate concentrations

In a test tube, 5 ml of the pH 10 adjusted extract (100 ppm) was mixed with 5 ml silver nitrate. The silver nitrate concentration was varied from $5x10^{-5}$-$2x10^{-4}$ M. The mixture was then heated in a boiling water bath for 40 min. The formation of AgNPs was monitored using a UV-Visible spectrophotometer.

Effect of Premna serratifolia Linn. leaf extract concentrations

In a test tube, 5 ml of the pH 10 adjusted extract was mixed with 5 ml silver nitrate $2x10^{-4}$ M. The concentration of the extract was varied from 60-400 ppm. The mixture was then heated in a boiling water bath for 40 min. The formation of AgNPs was monitored using a UV-Visible spectrophotometer.

Characterizations of silver nanoparticles

AgNPs colloid was centrifugated at 10,000 rpm for 30 minutes and dried in an oven at 65^0C. Dried AgNPs were analyzed by KBr plate method on 400-4,000 cm^{-1} scanning range with Thermo Scientific Nicolet iS10 instrument. Backman

Coulter Delsa Nano was used to measure the distribution of particle size.

Stability of silver nanoparticles

The stability of synthesized AgNPs was monitored during one month of storage by observing the change of SPR spectra, including the position of maximum peak (λ_{max}), intensity, and full width half maximum value (FWHM) of the absorption peak.

Antibacterial activity test

In studying the antibacterial activity of synthesized AgNPs, *Staphylococcus aureus* and *Escherichia coli* were used. Ciprofloxacin and chloramphenicol were used as a positive control for gram-negative bacteria and gram-positive bacteria, respectively. Disc diffusion method was used by observing the clear area around the paper disc after two days of incubation.

RESULTS AND DISCUSSION

Effect of time reaction

The formation of AgNPs is indicated by the appearance of yellow color from the mixture of reaction and absorption peak at the visible region. The reaction of silver nitrate and the *Premna serratifolia* Linn. leaf extract was performed at various times from 10-60 min. All of the reactions showed the formation of AgNPs (Figure 1) indicated by the yellow color of the mixture of reaction and maximum absorption peak at around 400-415 nm (Figure 2).

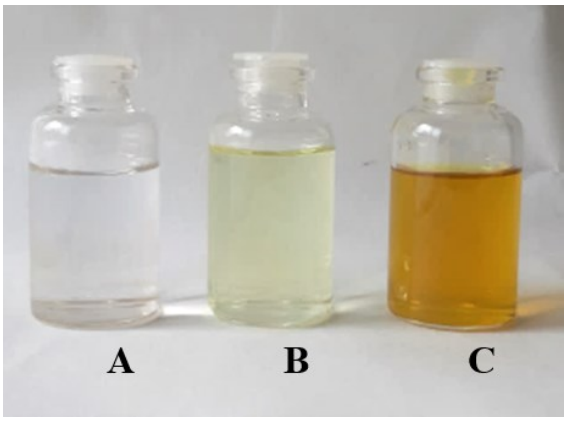

Figure 1. The appearance of AgNO3 solution (A), Premna serratifolia Linn. leaf extract(B), synthesized AgNPs (C).

The observed yellow color is characteristic of AgNPs due to the surface plasmon resonance and is spectrally observed as an absorption peak in the visible region. The position of the maximum absorption peak depends on the size of particles that could be controlled by the reducing agent used in the reduction of silver ions (Agnihotri *et al*., 2014; Annadhasan *et al.*, 2014). The AgNPs synthesized using an aqueous extract of *Teucrium polium* L. and *Cucumis prophetarum* show a peak centered around 434-440 and 420 nm, respectively (Hashemi *et al*., 2020; Hemlata *et al*., 2020).

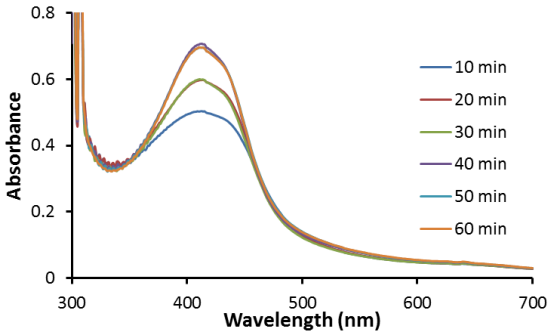

Figure 2. SPR spectra of AgNPs synthesized in various reaction times.

The intensity of SPR spectra correlates with the amount of synthesized AgNPs (Gusrizal *et al*., 2017). Figure 2 shows the intensity of the SPR spectra of AgNPs synthesized at different reaction times. As the reaction time increase, the intensity of spectra gradually increases indicates that AgNPs are continuously formed during the reaction. The maximum intensity was reached if the reaction was carried out for 40 min. The extract of *Cucumis prophetarum* reduced silver ions to AgNPs after 3 h of reaction (Hemlata *et al*., 2020). The chemical composition of extract may determine its reducing power in the synthesis of AgNPs.

Effect of pH

The phytochemistry screening showed that the *Premna serratifolia* Linn. leaf extract contained phenolic compounds. The phenolic compound may release the electron by breaking of O-H bond, and the released electron is then donated to silver ions in the formation of AgNPs (Ravichandran *et al*., 2019). Our initial experiment revealed that AgNPs formation could be achieved

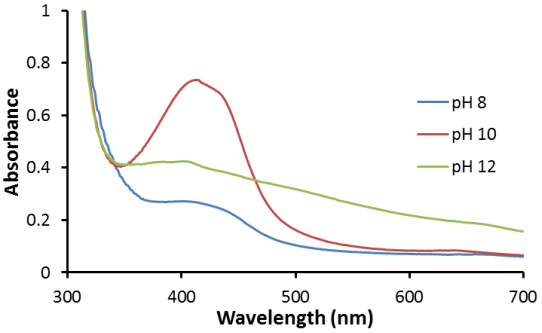

Figure 3. SPR spectra of AgNPs synthesized with different pH of the extract.

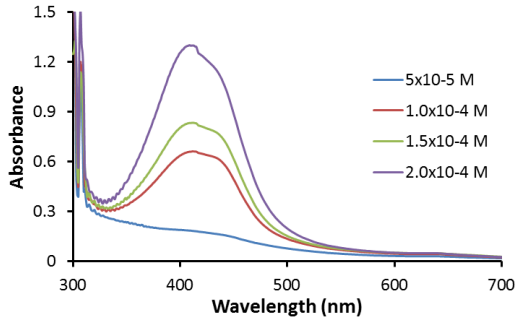

Figure 4. SPR spectra of AgNPs synthesized in the various initial concentrations of silver nitrate.

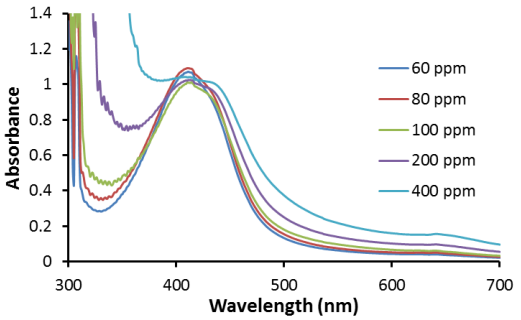

Figure 5. SPR spectra of AgNPs synthesized with different concentrations of extract.

when the pH of *Premna serratifolia* Linn. leaf extract was basic. The reduction of silver ions is initiated by the deprotonation of the phenolic group of the extract with the addition of sodium hydroxide solution. To determine the optimum pH of *Premna serratifolia* Linn. leaf extract for the formation of AgNPs, the reactions were performed using pH 8, 10, and 12 extracts. Figure 3 shows that the optimum pH of extract in reduction of silver ions to form AgNPs is 10.

Effect of silver nitrate concentrations

SPR spectra of synthesized AgNPs show that absorption intensity increases gradually along with the initial concentration of silver nitrate (Figure 4). The results indicate that the concentration of the extract used in the reaction is still available to reduce silver ions in the solution even though the initial concentration of silver nitrate is increased.

Effect of *Premna serratifolia* Linn. leaf extract concentrations

To determine the ratio of silver nitrate and extract for the synthesis of AgNPs, silver nitrate $1.5x10^{-4}$ M was added to *Premna serratifolia* Linn. leaf extract in different concentrations. Figure 5 shows the SPR spectra of the synthesized AgNPs. The highest intensity of SPR spectra was achieved when the reaction used an extract with a concentration of 80 ppm.

Characterizations of silver nanoparticles

Figure 6 shows the FTIR spectra of *Premna serratifolia* Linn leaf extract**.** The spectra show a prominent band at 3406 cm^{-1} which indicated –OH stretching accounts for secondary alcohols and phenols. Medium peaks at 2853 cm^{-1} and 2925 cm^{-1} indicated –CH stretching, and peak 1068 cm^{-1} correspond to –CO stretching. Lower signals at 1605 cm^{-1}, 1654 cm^{-1}, and 1701 cm^{-1} may be due to C=C aromatic stretching (Dachriyanus, 2004). Vibration bands corresponding to the bonds such as –OH, -CH, -CO, and C=C are derived from the phenolic compounds contained in the extract.

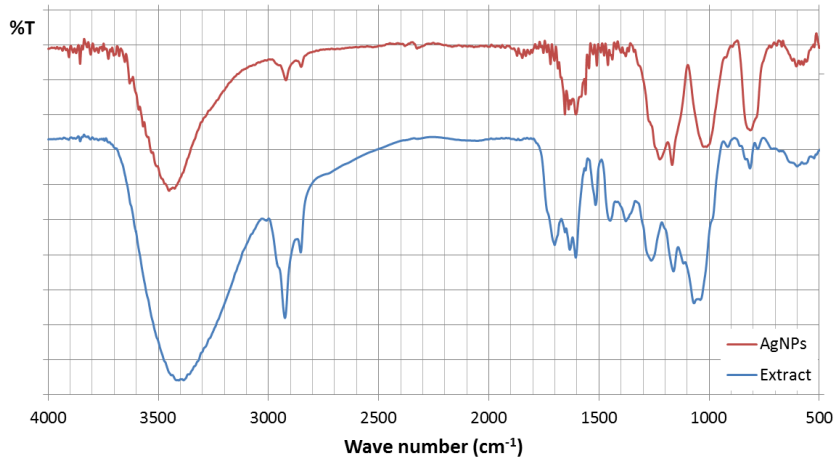

Figure 6. FTIR spectra of *Premna serratifolia* Linn leaf extract and AgNPs

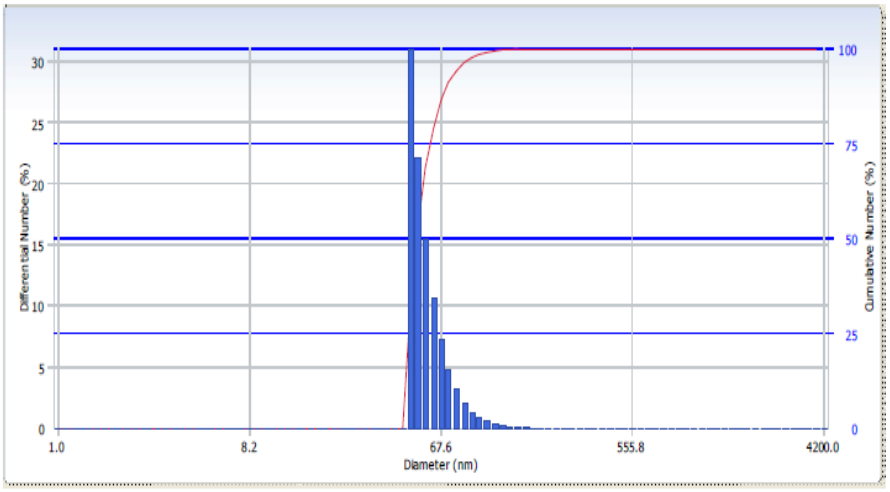

Figure 7. Size distributions of synthesized AgNPs.

Table 1. Stability Test of Synthesized AgNPs

Time of Storages	λmax (nm)	Absorption Intensity	FWHM
Fresh	414	0.941±0.003	93±1.4
1 day	413	0.925±0.006	93±0
2 days	413	1.009±0.001	106.5±0.7
3 days	413	0.906±0.017	94±1.4
1 week	414	1.012±0.004	105.5±2.1
2 week	414	1.010±0.004	104±0
3 week	413	0.903±0.005	92.5±0.7
1 month	413	0.904±0.012	92±0

Table 2. Antibacterial Activity Test of Synthesized AgNPs

Group I Gram-Positive Bacteria (*Staphylococcus aureus*)		Group II Gram-Negative Bacteria (*Escherichia coli*)	
Sample	Clear zone diameter (mm)	Sample	Clear zone diameter (mm)
$AgNO_3$ solution	10.6	$AgNO_3$ solution	12.77
Extract	0	Extract	0
AgNPs	11.1	AgNPs	12.03
Control (+)	13.9	Control (+)	30.1
Control (-)	0	Control (-)	0

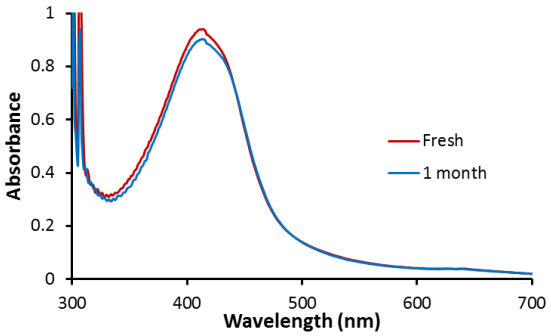

Figure 8. SPR spectra of synthesized AgNPs after one-month storage.

These data are confirmed by the result of the phytochemistry screening of the extract, indicating that the extract has phenolic compounds. This group of compounds is involved in the reduction of silver ions.

FTIR spectra of AgNPs (Figure 6) are similar to the spectra of extracts which indicate the presence of chemical constituents of the *Premna serratifolia* Linn. leaf extract that act as the capping agent. The band of AgNPs around 1,700 cm^{-1} disappeared compared with the spectra of the *Premna serratifolia* Linn. leaf extract. It indicates that the carbonyl group contained in the *Premna serratifolia* Linn. leaf extract participates in forming the capping agent layer on the surface of AgNPs. The action of the capping agent can avoid the agglomeration of AgNPs.

Data from the particle size analyzer in Figure 7 reveal the size of AgNPs distributes

between 48.3-157 nm and has an average size of 58.7±14.4 nm. The polydispersity index of synthesized AgNPs is 0.382 and is considered moderate (Bhattacharjee, 2016).

Stability of silver nanoparticles

Data from the stability test figure out the ability of silver nanoparticles to prevent agglomeration. Table 1 shows the change of λ_{max} position, absorption intensity, and FWHM for the synthesized AgNPs after storage at ambient conditions. After one month of storage, there is a lowering absorption intensity (4%), the redshift of the λ_{max} (1 nm), and FWHM (1 nm). The changes of SPR spectra after one month of storage are shown in Figure 8. Based on the data in Table 1, it can be said that the synthesized AgNPs are stable at least for one month of storage. Formation of capping on the surface of AgNPs by the chemical constituents containing in the *Premna serratifolia* Linn. leaf extract prevents the agglomeration of AgNPs. It indicates that the *Premna serratifolia* Linn. leaf extract acts as a capping agent as well.

Antibacterial activity test

The antibacterial activity of silver nitrate, *Premna serratifolia* Linn. leaf extract, and synthesized AgNPs was checked by disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*, as shown in Table 2. Silver nitrate and synthesized AgNPs can prevent bacterial growth after two days of incubation. On the other hand, the *Premna serratifolia* Linn. leaf extract does not show antibacterial activity. It can be seen that silver ions and AgNPs contribute to antibacterial activity. Gram-negative and gram-positive bacterial strain become sensitive to the synthesized AgNPs.

The mechanism of the antibacterial activity of AgNPs is not well-known or fully understood. However, it has been suggested that AgNPs may act as a reservoir of silver ions in their activity as an antibacterial agent (Le Ouay and Stellacci, 2015). Silver ions released from AgNPs by oxidative dissolution play a significant role in the antibacterial activity of AgNPs (Marambio-Jones and Hoek, 2010).

It has been proposed there may be several candidates for the antibacterial mechanisms of AgNPs (Xu *et al.*, 2021). Silver ions interact with membrane protein and are attached to the surface of bacteria and then penetrate the membrane and bacterial wall. The actions such as destroying the respiratory chain, generating oxidative stress, dephosphorylating the protein, and binding to bases to make DNA lose its replication ability by silver ions disturb the normal metabolism of bacteria. Finally, the membrane and bacterial wall are broken resulting in cytoplasm leaking and leading to apoptosis. Membrane proteins, DNA, enzymes, or intracellular cofactor are the binding target of silver ions. The interaction of silver ions with these biomolecules can inactivate the biological system which causes the death of bacteria.

CONCLUSIONS

Premna serratifolia Linn. leaf extract can be used in the synthesis of AgNPs. The extract acts as a reducing agent and capping agent simultaneously. The dispersity of synthesized AgNPs is considered to be moderate, with an average size of 58.7±14.4 nm. The synthesized AgNPs are stable for one month of storage and active against *Staphylococcus aureus* and *Escherichia coli.* The findings of this present study suggest that *Premna serratifolia* Linn could be the potential source of natural reducing agents for the synthesis of AgNPs as an alternative to being an antibacterial agent.

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REFERENCES

Agnihotri, S., Mukherji, S., Mukherji, S., 2014. Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. *RSC Advances*, 4(8), 3974–3983.

Ahmad, S., Munir, S., Zeb, N., Ullah, A., Ali, J., Bilal, M., Omer, M., Alamzeb, M., Salman, S.M., Ali, S., 2019. Green nanotechnology: a review on green synthesis of silver nanoparticles — An ecofriendly approach. *International Journal of Nanomedicine*, 14, 5087–5107.

Annadhasan, M., Muthukumarasamyvel, T., Sankar Babu, V.R., Rajendiran, N., 2014. Green synthesized silver and gold nanoparticles for colorimetric detection of Hg2+, Pb2+, and Mn2+ in aqueous medium. *ACS Sustainable Chemistry and Engineering*, 2(4), 887–896.

Bhattacharjee, S., 2016. DLS and Zeta potential: what they are and what they are not? *Journal of Controlled Release*, 235, 337–351.

Chandra, H., Kumari, P., Bontempi, E., Yadav, S., 2020. Medicinal plants: treasure trove for green synthesis of metallic nanoparticles and their biomedical applications.

Biocatalysis and Agricultural Biotechnology, 24(101518), 1–11.

Dachriyanus, 2004. Analisis struktur senyawa organik secara spektroskopi. Lembaga Pengembangan Teknologi Informasi dan Komunikasi Universitas Andalas, Padang.

Das, C.G.A., Kumar, V.G., Dhas, T.S., Karthick, V., Govindaraju, K., Joselin, J.M., Baalamurugan, J., 2020. Antibacterial activity of silver nanoparticles (biosynthesis): a short review on recent advances. *Biocatalysis and Agricultural Biotechnology*, 27(101593).

Gusrizal, G., Santosa, S.J., Kunarti, E.S., Rusdiarso, B., 2018. Two highly stable silver nanoparticles: surface plasmon resonance spectra study of silver nanoparticles capped with m-Hydroxybenzoic acid and p-hydroxybenzoic acid. *Molekul,* 13(1), 30–37.

Gusrizal, G., Santosa, S.J., Kunarti, E.S., Rusdiarso, B., 2017. Synthesis of silver nanoparticles by reduction of silver ion with m-Hydroxybenzoic acid. *Asian Journal of Chemistry*, 29(7), 1417–1422.

Hashemi, S.F., Tasharrofi, N., Saber, M.M., 2020. Green synthesis of silver nanoparticles using *Teucrium polium* leaf extract and assessment of their antitumor effects against MNK45 human gastric cancer cell line. *Journal of Molecular Structure*, 1208, 127889.

Hemlata, Meena, P.R., Singh, A.P., Tejavath, K.K., 2020. Biosynthesis of silver nanoparticles using *Cucumis prophetarum* aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines. *ACS Omega*, 5, 5520–5528.

Isnindar, I., Luliana, S., 2020. Synergism of antioxidant activity combination of Buas-Buas (*Premnaserratifolia* Linn.), Meniran (*Phyllanthusniruri* L.), Secang (*Caesalpiniasappan*) and Roselle (*Hibiscus sabdarifa*) extracts. *Traditional Medicine Journal*, 25(3), 138–143.

Jadhav, K., Deore, S., Dhamecha, D., Hr, R., Jagwani, S., Jalalpure, S., Bohara, R., 2018. Phytosynthesis of silver nanoparticles: characterization, biocompatibility studies, and anticancer activity. *ACS Biomaterials Science and Engineering*, 4(3), 892–899.

Le Ouay, B., Stellacci, F., 2015. Antibacterial activity of silver nanoparticles: a surface science insight. *Nano Today*, 10(3), 339–354.

Marambio-Jones, C., Hoek, E.M.V., 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research*, 12(5), 1531–1551.

Octavianus, C., 2021. Sintesis nanopartikel perak dengan reduktor ekstrak daun Buas-Buas (*Premna serratifolia* Linn.) dan uji aktivitasnya sebagai antibakteri. Universitas Tanjungpura.

Purwanti, N.U., Wahdiyanti, R., Susanti, R., 2018. Effect of variation of solvent concentration to antioxidant activity of ethanolic extract of Buas-Buas stem (*Premna serratifolia* L.) using DPPH (2, 2-diphenyl-1 picrylhidrazyl) scavenging method, in: Ningrum, V.D.A. (Ed.), International Conference on Pharmaceutical Research and Practice. Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, 126–132.

Ravichandran, V., Vasanthi, S., Shalini, S., Shah, S.A.A., Tripathy, M., Paliwal, N., 2019. Green synthesis, characterization, antibacterial, antioxidant and photocatalytic activity of *Parkia speciosa* leaves extract mediated silver nanoparticles. *Results in Physics*, 15, 102565.

Sharma, V.K., Yngard, R.A., Lin, Y., 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, 145(1–2), 83–96.

Srikar, S.K., Giri, D.D., Pal, D.B., Mishra, P.K., Upadhyay, S.N., 2016. Green synthesis of silver nanoparticles: a review. *Green and Sustainable Chemistry*, 6, 34–56.

Xu, Z., Zhang, C., Wang, X., Liu, D., 2021. Release strategies of silver ions from materials for bacterial killing. *ACS Applied Bio Materials*, 4(5), 3985–3999.