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RESEARCH ARTICLE

Biosynthesis and Characterization of Silver Nanoparticles using Single Garlic Callus Extract (*Allium sativum* L.)

Abstract Nanotechnology is a relatively new and innovative field with huge potential for application in the food and drug industries. Due to their excellent physicochemical and biological properties, silver nanoparticles (AgNPs) are often utilized in various applications and have been the subject of substantial research. AgNP synthesis using plant extracts has recently gained popularity due to its environmental friendliness, affordability, and potent function in several applications. The present study aimed to evaluate the biosynthesis, stability, and characteristics of AgNPs using single garlic callus extract (Allium sativum L.) The biosynthesis of AgNPs using single garlic callus extract (AgNPs-As) was performed by adding 1 mM AgNO3 to ethanolic extracts of single garlic callus. Spectrophotometry (absorption at 200–800 nm), SEM, EDX, PSA, FTIR, and XRD were used for their characterization. The present study showed a colloidal color change to brown, indicating the formation of AgNPs-As. The characterization of AgNPs-As using SEM, EDX, PSA, and XRD revealed a spherical morphology with an average size of 201.9 nm. Several active compounds were also identified using FTIR.

Keywords: Silver Nanoparticle, Biosynthesis, Stability, Garlic, Callus

Introduction

Nanotechnology is the field of science that deals with the synthesis, engineering, and manipulation of matter. Materials that have a size of less than 100 nm are classified as nanoparticles [1]. The application of nanoparticles is growing rapidly in various disciplines because of the novel properties and increased activity related to their size, distribution, and morphology, and they are widely used in health, energy, environment, and several industrial fields [2]. Research has been conducted on nanoparticles from various types of metals including magnesium [3], titanium [4], gold [5], and silver [6].

Silver nanoparticles (AgNPs) are among the most effective and efficient metal nanoparticles as electronic components and antibacterial, antifungal, antiviral [7], and anti-inflammatory compounds [8] and are used in various industrial fields because they are safe for human health [9]. The synthesis of AgNPs can be carried out by various methods, namely physical, chemical, and biological methods. The biosynthesis of AgNPs using plant extracts is an alternative method for the manufacture of AgNPs because it is environmentally friendly, economical, and easy to perform on an industrial scale without the need for large amounts of energy and hazardous chemicals [10].

The plant extract used in the biosynthesis of AgNPs acts as a reductant capable of reducing silver salts (Ag^{+}) to metallic silver (Ag^{0}). The phytochemicals present in plant extracts also act as capping agents for nanoparticles, providing the advantages of preventing the agglomeration of nanoparticles, reducing toxicity, and increasing antimicrobial activity [11]. One of the plants with a high phytochemical content is a single garlic plant (*Allium sativum* L.), which contains organosulfur, the main bioactive compounds (including alliin, allicin, allyl sulfide, ajoene, and dithiane) [12], and phenolic compounds that act as antioxidants [13].

The production and harvesting of secondary metabolites from plants in vivo require a long time, and limited land availability is a consideration for the production of secondary metabolites through tissue culture techniques on an industrial scale. Cell biomass is produced in large quantities and in a short time by stimulating cells through cell growth media [14]. According to Nurchayati*et al.,* [15], the crumb callus structure can be used in the production of secondary metabolites by cell suspension culture.

Previous studies successfully used the callus extract of *Cinnamomum camphora* [9], *Solanum incanum* [16], and *Celastrus paniculatus* [17] as bioreductors for AgNP biosynthesis. Moreover, Hasanin*et al.*, [18] studied the biosynthesis of zinc and selenium oxide nanoparticles, which have potential antimicrobial properties against Gram-negative and -positive bacteria, in combination using the callus extract of *Ziziphus spina-christi*.

Tissue culture to produce callus involves growing cells, organs, tissues, or the entire plant in vitro under aseptic and regulated environmental and nutritional conditions [19]. Plant tissue culture techniques have recently been used as an important tool for large-scale plant propagation, disease management, and the improvement of plant secondary metabolite synthesis. In a continuing process, a small portion of the tissue (explant) is employed to reproduce plants [20]. The use of plant tissue culture avoids exposing the plant to biotic and abiotic stressors that impact secondary metabolite synthesis [21]. Furthermore, the advantages of using callus for the biosynthesis of AgNPs include obtaining a higher yield value at a low cost, reducing the use of plants in nature in high quantities, and preventing contamination; additionally, the callus acts as a source of phytochemicals that can be propagated in the laboratory [22].

Although various studies regarding the use of plant extracts and callus for AgNP biosynthesis have been performed, the biosynthesis of AgNPs using a single garlic callus (AgNPs-As) has not been done thus far. Therefore, the present study aimed to biosynthesize AgNPs-As using various concentrations of $AgNO_3$ and evaluate their characteristics using scanning electron microscopy (SEM), energy dispersive X-ray (EDX), particle size analyzer (PSA), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD).

Materials and Methods

Sterilization and callus induction

Single-clove garlic was collected from Bandung, West Java, Indonesia. Fresh garlic was sterilized using sterile water, fungicides, and bactericides for 20 min followed by Clorox (10%) for 1 min and then rinsed with distilled water. Thereafter, garlic embryos were inoculated in Murashige and Skoog supplemented with 90 $g.L^{-1}$ sucrose and 5 ppm picloram. After the callus cells had formed, the callus was sub-cultured in fresh medium after 3 weeks.

Preparation of ethanolic callus extract

The callus was macerated using 95% ethanol for 48 h until the maceration solution was clear; then, the extract mixture was filtered using Whatman paper no. 2. After filtration, the filtrate was concentrated using a rotary evaporator (Buchi, Switzerland) at a temperature of 50–55 °C to eliminate the solvent. Then, 2 g of the thick extract was weighed, dissolved in 20 mL of deionized water, and used for the biosynthesis of nanoparticles [23].

Biosynthesis of AgNPs-As

The biosynthesis of AgNPs-As was carried out using 1 mM of $AgNO_3$ as a precursor. AgNPs-As biosynthesis was performed by mixing 1 mL of single garlic callus extract with 9 mL of 1 mM $AgNO_3$ solution (1:9) and incubating at 35 °C for 48 h, followed by observation for colloidal color change. process. Based on the docking result, the binding energy of the protein-ligand complexes was evaluated.

Characterization of AgNPs-As

The stable nanoparticle colloid was centrifuged at 10,000 rpm for 15 min and then dried using a freeze dryer (Alpha 1-2 LO, Germany). The AgNPs were characterized using UV-visible spectroscopy (UV-VIS; SP-UN52N, Beijing, China), SEM (SU 3500, Hitachi, Europe), FTIR (Alpha II, Bruker, USA), PSA (SZ-100, Horiba, Kyoto, Japan), and XRD (Bruker D8, Blue Scientific, USA).

Results and Discussion

Color change and UV-VIS analysis of AgNPs-As

The combination of a 10% solution of yellow garlic callus ethanol extract and a clear solution of 1mM $AgNO_3$ solution resulted in a color change from light yellow to brown (Figure 1), indicating the biosynthesis of AgNPs-As. Such a color change was previously observed by Botcha and Prattipati [24] in the aqueous extract of *Hyptis suaveolens* callus, which changed from light yellow to dark brown. The color change during the formation of AgNPs-As is mediated through the reduction of Ag^+ to Ag^0 by a reducing agent contained in the extract. Furthermore, phenolic compounds in the extract may play a role in the reduction of Ag^+ to Ag^0, while the presence of protein promotes the stability of AgNPs [24]. Other phytochemical compounds that are important for Ag^+ reduction include terpenoids, flavonoids, ketones,

aldehydes, amides, and alkanoic acids [25].

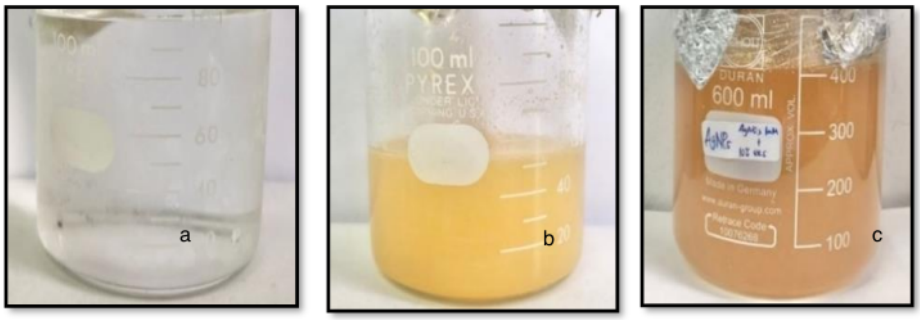

Figure 1. Color change during the biosynthesis of AgNPs-As. a) AgNO3 solution; b) Garlic callus ethanol extract; c) Resulting AgNP-As after 24 h.

The secondary metabolites in the callus extract that function as reducing, stabilizing, and capping agents during the formation of AgNPs-As caused the reaction mixture to change color as they converted silver ions (Ag^+) into elemental silver (Ag^0) [26, 27]. In addition, differences in the color formed depend on the compound contained in the callus extract [28].

Furthermore, the wavelengths of the AgNPs-As colloidal suspension were examined between 200 and 800 nm, with the typical UV absorption spectra being found at 400 nm (Figure 2). The color shift of the solution caused by Surface plasmon resonance (SPR) stimulation during the green synthesis of AgNP-As has been shown in several previous studies [29-31]. Along with the obvious color shift, a peak was also observed in the UV-VIS absorption spectra around 400 nm in this study in accordance with previous work [32].

Characterization of AgNPs-As

SEM of the AgNPs-As revealed that particles were evenly distributed and spherical in shape (Figure 3). The results of the characterization using SEM with EDX analysis revealed strong signals from C and K (49.57%), O and K (6.15%), Cl and K (10.07%), and Ag (34.21%; Figure 4). Because of their SPR, these AgNPs-As exhibited a strong peak at 3 keV, confirming the existence of silver ions as well as other elements, carbon, oxygen, and chlorine, as reducing and capping agents (Figure 5). The lack of nitrogen guaranteed the full reduction of $AgNO_3$ to silver ions, and no additional trace ions were found in the nanoparticles (Table 1).

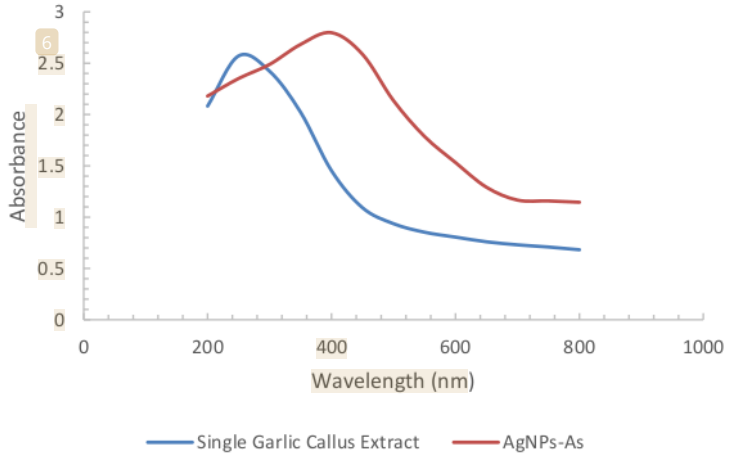

Figure 2. UV-VIS spectra of the biosynthesis of AgNPs using the ethanolic extract of single garlic callus and 1mM of $AgNO_3$ (AgNPs-

As).

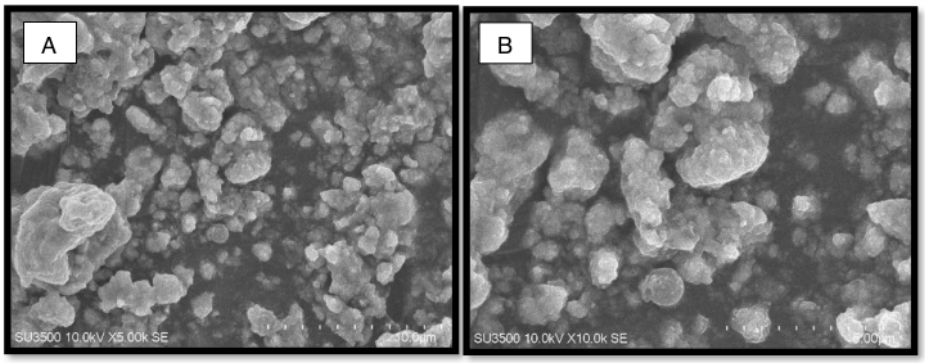

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Figure 3. Scanning electron micrographs of AgNPs synthesized from single garlic callus *(Allium sativum)*. a) Mag 5.000; b) Mag 10.000.

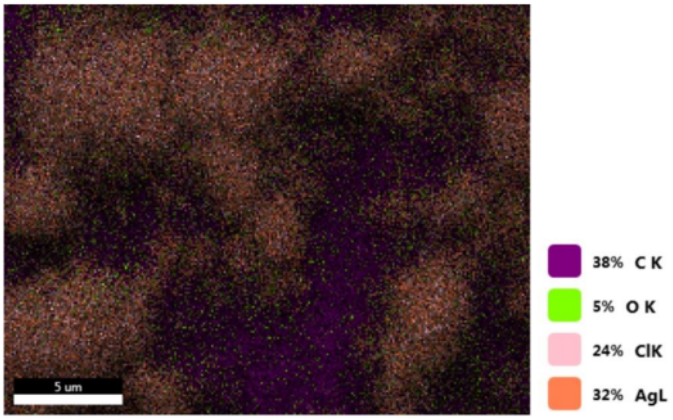

Figure 4. The EDX element overlay map of AgNPs-As synthesized using single garlic callus extract.

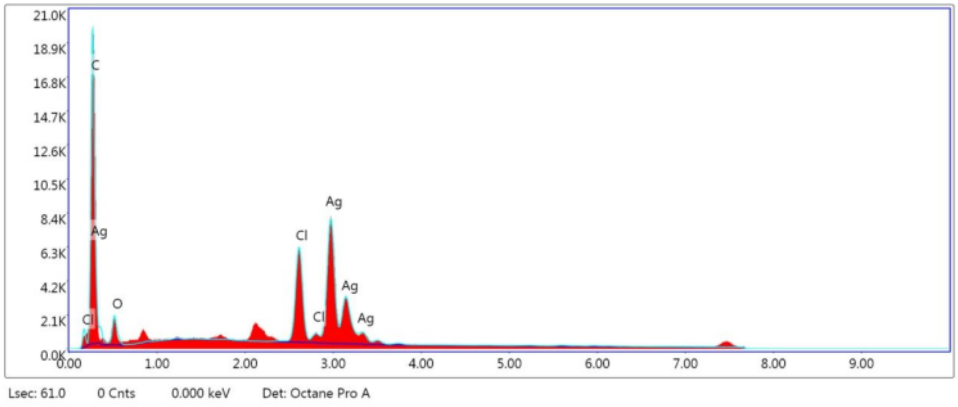

Figure 5. The EDX spectrum of AgNP-As depicts an Ag peak between 2 and 3 keV.

Table 1. The EDX spectrum analysis of AgNPs-As synthesized using single garlic callus extract.

Element	Weight (%)	Atomic (%)	Net. Int.
C K	49.57	80.73	2866.60
O K	6.15	7.51	276.20
CIK	10.07	5.56	1833.60
AgL	34.21	6.20	2635.10

The AgNPs-As size was analyzed using a PSA, revealing an average size of 201.9 nm (Figure 6). The biosynthesis of AgNPs using callus extracts of *Hyptis suaveolens* [24], *Solanum incanum* L [16], and *Taxus yunnanensis* [22] produced AgNPs with a spherical shape. The particle size is strongly influenced by the concentration of plant extracts containing phenols, polyphenols, polysaccharides, anthocyanins, and tannins, which play a role in the reduction of Ag^+ in the formation of stable nanoparticles [33]. Particles with a diameter of 100–2500 nm are referred to as fine nanoparticles [34].

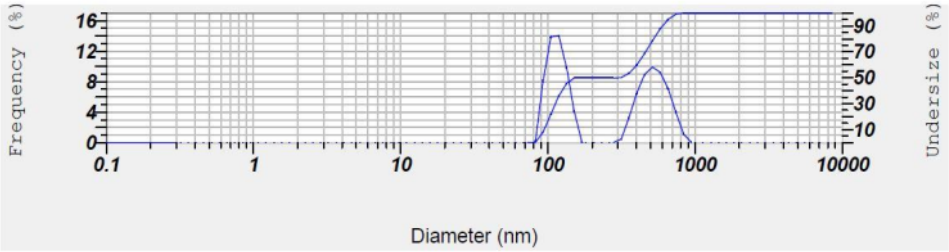

Figure 6. Particles size distribution of AgNPs-As synthesized using single garlic callus extract.

The FTIR spectrum recorded from 4000 to 600 cm^{-1} revealed different peaks indicating the presence of several types of biological functional groups that may play a pivotal role in reduction and stabilization during the biosynthesis of AgNPs-As. The results of the FTIR test on the ethanolic extract of single garlic callus (Figure 7a) revealed absorption peaks (%) of 3265.86, 2926.98, 1634.81, 1514.69, 1407.93, 1348.99, 1264.13, 1032.32, and 923.33. The high-intensity peak 3265.86 belonged to an O-H bond, which is an alcohol compound with hydrogen or phenol bonds. Meanwhile, the medium absorption peak 2926.98 corresponded to a C-C bond, which is an alkane compound. At the absorption peaks 1634.81 and 1514.69 belonging to a C=C bond were alkenes and benzene compounds. The intensity peaks 1407.93 and 1348.99 belonged to a C-H bond, which is an alkane compound. The peak at 1264.13 belonged to a C-N bond, which is an amine or amide compound. Meanwhile, the weak absorption peaks 1032.32 and 923.33 belonged to a C-O bond, which is an alcohol, ester, carboxylic acid, or ether compound.

Furthermore, the results of the FTIR test on colloidal AgNPs-As (Figure 7b) showed that the strong absorption peaks formed were 3313.94, 2118.94, and 1636.28. The peak 3313.94 belonged to an O-H bond, which is an alcohol compound with hydrogen or phenol bonds. Furthermore, the absorption peak 2118.94 belonged to a C≡C bond, which is an alkyne compound. Meanwhile, the absorption peak 1636.28 belonged to a C=C bond, which is an alkene compound.

The FTIR spectrum data from previous studies revealed that AgNPs were surrounded by plant secondary metabolites such as alkaloids, terpenoids, carbonyl groups, esters, halides, and alcohol, which function as active binding sites for AgNPs to provide stability [35, 36].

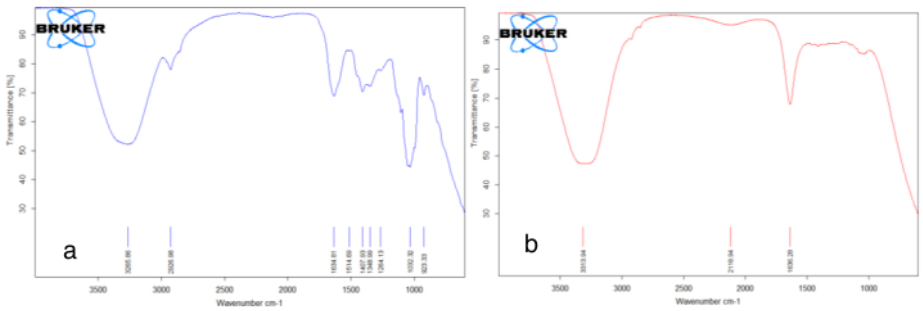

Figure 7. FTIR. a) Ethanolic single garlic extract (*Allium sativum* L.); b) AgNPs synthesized using single garlic callus extract.

XRD determination

The phase formation, crystallinity, and purity of the produced AgNPs-As were all evaluated by XRD analysis. The XRD patterns of AgNPs-As produced using single garlic callus extract are shown in Figure 8. The biosynthesis of AgNPs-As showed diffraction peaks at 21.832°, 24.136°, 28.198°, 32.617°, 46.58°, 55.128°, 57.786°, 67.729°, 74.755°, 77.013°, and 85.936°. The crystallite size of AgNPs-As was calculated to be 201.9 nm using Debye–Scherrer's equation.

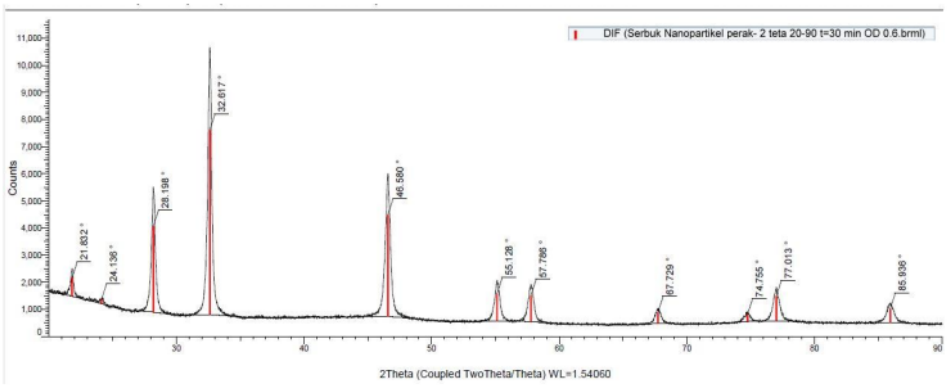

Figure 8. The XRD spectra of AgNPs synthesized using single garlic callus extract and 1 mM AgNO3.

Conclusions

During the biosynthesis of AgNPs-As from garlic callus ethanol extract, a color change indicated the formation of AgNPs-As. A wave peak at a wavelength of 400 nm is the typical optimum UV absorption spectra for AgNPs As. The characterization of AgNPs are performed using SEM, EDX, PSA, FTIR, and XRD, showing that the morphology of the nanoparticles was spherical, with an average size of 201.9 nm and contains several active compound on AgNPs-AS such as alkaloids, terpenoids, carbonyl groups, esters, halides, and alcohol.

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