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Biosynthesis of silver nanoparticles (AgNPs) from coconut leaf extract and their antifungal activity against *Ganoderma boninense* mycelia

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Abstract

Basal stem rot disease caused by *Ganoderma* is a major problem in palm cultivation in Indonesia, so an appropriate solution is needed to overcome this problem. One of the solutions that can be applied is through silver nanoparticles. Synthesis of silver nanoparticles can use coconut leaves as a source of flavonoids. Flavonoids are one of the phenolic compounds that are widely used in the process of synthesizing silver nanoparticles. Flavonoid compounds have a hydroxyl group (OH), which can be reduced by donating electrons to the Ag^+ ion from $AgNO_3$ to Ag^0. This research aims to synthesize silver nanoparticles (AgNPs) using coconut leaf water extract and its assessment as an antifungal for *Ganoderma boninense*. Silver nanoparticles were synthesized by mixing coconut leaf extract in 1 mM of $AgNO_3$ solution with a ratio of 1:9 (v/v). The concentrations of coconut leaf water extract were 1% and 3% with a heating temperature of 60°C. Silver nanoparticles were characterized using a UV-Vis spectrophotometer, PSA, FTIR, TEM, and SEM-EDS. The AgNPs had a maximum wavelength of 430 nm, with morphologies like a ball, triangle, and square, a mass percentage of Ag of 51.77%, smallest particle size of 63.29 nm, PdI value of 0.3361, and a zeta potential of -16.98 mV. The FTIR spectra show that the functional group that plays a role in the reduction process is the –OH group. The antifungal activity assay produced the highest percentage of colony growth inhibition (68.37%) at a concentration of 4.9 ppm in the 10-day incubation.

[Keywords: antifungal, Bali, characterization, coconut leaf waste]

Introduction

Bali has a culture of arranging offerings in the form of flowers, leaves, and fruit at almost every traditional ceremony, which causes waste after the ceremony (Yadav et al., 2015). The dominant waste is organic waste, mainly coconut leaves, as the primary material for making canang. A recent study from the Bali Partnership shows that the accumulation of waste in Bali reaches 4,281 tons per day. Sixty percent of the total waste is organic, 20% is plastic, and the remainder is paper, metal, and glass (Wijaya & Putra, 2021). One of the advantages of coconut leaves is that they can be used in synthesizing silver nanoparticles (AgNPs). The use of coconut leaves as a bioreductor for making AgNPs is due to the phenolic, flavonoid, and condensed tannin content in coconut leaves (Katja & Suryanto, 2008; Hamzah et al., 2012).

Basal stem rot disease caused by the fungus *Ganoderma boninense* is a major problem in palm cultivation in Southeast Asia. This fungus attacks palm plants at all ages with relatively slow disease development, and symptoms appear in the final stages of the attack, with a tree death rate of 3.7% per year (Barcelos et al., 2015). *Ganoderma boninense* can attack palms at the mature and seedling stages with typical symptoms, namely rot at the basal of the stem, causing necrosis on the inside of the leaves (Widiastuti et al., 2017). To overcome this problem, it is necessary to control stem rot disease using appropriate techniques, one of which is environmentally friendly pest control using silver nanoparticles.

Nanoparticles is a term that was first adapted in Greek works with the meaning "nano" which means small (Jamkhande et al., 2019). Silver nanoparticles have a size of 1–100 nm, and silver nanoparticles are most widely studied because of their proven benefits as antibacterial, antifungal, antiviral, and antioxidant (Sivakumar et al., 2021). In general, silver nanoparticles can be synthesized by several methods, including chemistry, physics, and biology. Physical and chemical methods have the advantage of high purity. However, these two methods are not environmentally friendly because they use dangerous reagents. Biological methods with a

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green synthesis approach are widely used because they are environmentally friendly, cheap, and simple (Siddiqi & Husen, 2016). The biosynthesis method uses media from biological materials, either microorganisms or extracts from plants. Plants contain secondary metabolite compounds (flavonoid compounds, alkaloids, tannins, saponins, and phenolic acids), which can reduce metal ions such as Ag^+ ions to Ag^0 atoms (Bayani, 2016).

According to Pulit et al. (2013), silver nanoparticles can inhibit the growth of fungi by attaching to all parts of the fungus from sporangiophores, conidia, and spores. Silver nanoparticles attached to fungal spores cause the fungus to be unable to develop and the growth of the fungus is hampered. Research by Faramitha et al. (2022) stated that silver nanoparticles made from $NaBH_4$ and L-cysteine showed an inhibition percentage of *Ganoderma boninense* mycelia of 65.17%. Until now, the effect of long exposure to silver nanoparticles as an antifungal or nanopesticide on plants has not been studied further. However, several studies have said that nanopesticides from nanoparticles using the green synthesis method are very safe for the environment, have a high level of efficacy, and have a low risk of pest resistance, can reduce toxic residues from the environment (Ariningsih, 2016). The amount of Ag material used to form silver nanoparticles is very small, namely one mM, so it is cost-effective in making silver nanoparticles on a large scale.

In this research, AgNPs were synthesized by bioreduction using coconut leaf. Silver nanoparticles characterization was carried out using a UV-Vis spectrophotometer, scanning electron microscope-energy dispersive X-ray spectroscopy, transmission electron microscope, particle size analyzers, and Fourier transform infrared spectroscopy, while the activities of AgNPs to control *Ganoderma boninense* was asses in vitro.

Material and Methods

Materials

The tools used in this research were hot plate (IKA C-MAG HS 7), magnetic stirrer (IKA), oven (B-ONE), blender (Philips), analytical balance (OHAUS), UV-Vis spectrophotometer (Thermo), Particle Size Analyzer (Malvern), Centrifuge (Top Centrifuge PLC 03), Scanning Electrom Microscope-Energy Dispersive American), spirit lamp, volume pipette, glassware (Herma), Fourier-Transform Infrared Spectrometer (Spectrum Two System L160000A). The materials used in this research were $AgNO_3$ crystals (PT Brataco), distilled water (PT Brataco), aqua demineralisata, 96% ethanol (MERCK), *Ganoderma boninense* isolate (Simalungun Sumatera Utara (SSU) code) (IOPRI), Potato Dextrose Agar media (MERCK), concentrated HCl (PT. Brataco), Liebermann-Buchard reagent (PT Brataco), anhydrous acetic acid (PT Brataco), concentrated sulfuric acid (MERCK), chloroform (MERCK), NH_4OH (PT Brataco), 2M H_2SO_4, reagent Meyer (PT Brataco), Wagner reagent (PT Brataco), Dragendorf reagent (PT Brataco), and $FeCl_3$ (PT Brataco). The bioreductor sample used was coconut leaves, which were waste from the environment around the Kediri area, Tabanan, Bali.

Preparation of coconut leaf powder

The coconut leaves were collected and sorted to separate the samples from impurities or unwanted parts. Then, the samples were washed with clean water to remove other impurities attached to the leaves. After that, the samples were dried under indirect sunlight covered with black cloth, then ground using a blender to powder (simplicia). The powder obtained was stored in a clean and closed container to prevent damage.

Water coconut leaf extraction

A total of 20 g of coconut leaf powder *(Cocos nucifera* L.) was heated using 100 mL of demineralized aqua for 15 minutes at a temperature of 80°C to obtain a concentration of 20% (w/v). After that, it was removed and filtered while hot (Ni'mah, 2022). The extract was then diluted to make concentrations of 1% and 3% to be used in the biosynthesis of silver nanoparticles.

*Phytochemical qualitative analysis of coconut (*Cocos nucifera *L.) leaf water extract*

a. Flavonoid content

A 0.1 g extract was added to 10 mL of hot water and filtered. A 10 mL filtrate was mixed with 0.5 g of Mg powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol. The mixture was shaken vigorously. A positive test for flavonoids is indicated by the appearance of a red-orange color (Harborne, 1987).

b. Terpenoid and steroid content

This test used the Liebermann-Buchard reagent. The filtrate was put into a test tube, then 2-3 drops of anhydrous acetic acid were added, then stirred slowly for a while until dry, then 1-2 drops of concentrated sulfuric acid were added, and the color that appeared was observed. Red, brown, or violet rings indicate triterpenoids, while the formation of a bluish-green color indicates steroid positivity (Harborne, 1987).

c. Alkaloid content

A total of 0.1 g of simplicia was dissolved in 10 mL of $CHCl_3$ (chloroform) and 4 drops of NH_4OH then filtered and the filtrate was put into a closed test tube. The $CHCl_3$ extract in a test tube was then shaken with 10 drops of 2 M H_2SO_4, until 2 layers were formed. The acid layer above was separated into another test tube and added with Meyer's reagent, the positive result produced a white precipitate, while the addition of Dragendorff's reagent produced a positive result, a red-orange precipitate, and a yellow-brown precipitate with Wagner's reagent (Harborne, 1987).

d. Saponin content

A total of 0.1 g of extract powder was put into a beaker, then 10 mL of hot water was added and boiled for 5 minutes. After that, it was filtered and the filtrate was used as a test solution, put into a closed test tube and then shaken vigorously for 30 sec. The formation of steady foam for no less than 10 seconds with a height of 1 cm to 10 cm indicates the presence of saponin (Harborne, 1987).

e. Tannin content

The tannin test was carried out by adding 2-3 drops of 5% $FeCl_3$ solution to the filtrate. Positive results are indicated if a blackish-blue, brownish-green, or greenish-black color is formed, indicating tannins' presence (Harborne, 1987).

Biosynthesis of silver nanoparticles

Synthesis of silver nanoparticles was carried out by mixing 1 mM $AgNO_3$ solution with water extract of coconut leaves (*Cocos nucifera* L.). The treatment carried out was by using a ratio of coconut leaf water extract to 1 mM $AgNO_3$ solution, namely 1:9 (v/v). The mixture was heated to a temperature of 60°C. A visual indicator of the formation of silver nanoparticles is a change in the color of the solution from clear yellow to brownish red.

Characterization of silver nanoparticles

a. Characterization with a UV-Vis spectrophotometer

In this research, the formation of silver nanoparticles was characterized using a UV-Vis spectrophotometer with a wavelength range of 300-800 nm (Talita et al., 2018; Fathia, 2018). Silver nanoparticles are formed when colloidal silver nanoparticles show a maximum wavelength of 400 - 450 nm (Purnamasari, M.D., 2015).

b. Size/diameter and zeta potential of AgNPs with particle size analyzer (PSA)

Nanoparticles have a size range of 1-100 nm (Ananda et al., 2015). Two mL of colloidal silver nanoparticles were taken, put into a cuvette and then analyzed using a PSA instrument (Malvern, 2024).

c. Morphology and composition of AgNPs with SEM-EDS

The colloidal silver nanoparticles obtained was centrifuged at 10,000 rpm for 10 minutes, the resulting precipitate was then washed with 10 mL of demineralized aqua and centrifuged again, the precipitate obtained was dried in an oven at 50°C. The powder obtained was analyzed using SEM-EDS. For characterization using SEM-EDS, the morphology of the nanoparticles was obtained in a crystalline form at 10,000x and 20,000x magnification (Parisnawan, 2022).

d. Morphology of AgNPs by TEM

A drop of sample was placed on a copper grid and allowed to dry at room temperature and imaged. The size of the Ag nanoparticles was analyzed using an image software program that analyzed each sample from the TEM image. The histogram of the nanoparticle size distribution is processed using a frequency distribution table (Talita et al., 2018).

e. Functional group of AgNPs by FTIR

The colloidal silver nanoparticles and the coconut leaf water extract were analyzed using FTIR. The FTIR analysis was used to determine the functional groups of secondary metabolites in pure coconut leaf water extract and the functional groups in silver nanoparticles. Place the sample in the instrument and analysis with FTIR

Antifungal activity of silver nanoparticles

Testing the activity of silver nanoparticles as an antifungal for *Ganoderma boninense* was by using PDA media, where 9 mL of PDA media was poured into a Petri dish and then 1 mL of AgNPs was added with variations in the final concentration of the media mixture to 1.8; 2.4; 3.0; 3.6; 4.2; 4.8 mg/L. As a control (AgNPs 0 mg L^{-1}), 1 mL of sterile distilled water was used. The Petri dish was shaken orbitally so that the media mixture was homogeneous. After the media became solid, a pure culture of two weeks old *Ganoderma boninense* mycelia with a diameter of 10 mm was aseptically placed in the center of the Petri dish and then incubated at room temperature. Colony diameter of *G. boninense* was measured

every 3 days until the diameter of the control colonies covered the surface of the Petri dish. Each concentration had 3 replications. The percentage of *Ganoderma boninense* colony inhibition from each treatment was calculated using the following formula:

$$inhibition\ (\%) = \frac{(C-T)}{C}\ x\ 100\ \text{.....}\ (1)$$

C = diameter of *Ganoderma boninense* colonies on negative control media (cm)
T = diameter of *Ganoderma boninense* colonies on PDA media containing AgNPs (cm)

Results and Discussion

Coconut leaf water extract

Coconut leaf water extract is obtained by heating demineralized aqua containing coconut leaf powder at 80°C for 15 minutes. The extraction and dilution process of coconut leaf water extract uses demineralized aqua because it has polar properties so it can extract phenolic compounds contained in coconut leaves (Fajri et al., 2022). Aqua demineralisata is a solvent that is pure and non-toxic, it is certainly quite safe to use and the remaining waste is easily accepted by the environment so it does not pollute the environment. This process was chosen because it is easy, cheap, and does not require sophisticated equipment. Increasing the water temperature during the extraction process can increase the solubility of a compound contained in the plant extract (Marjoni, 2016).

Phytochemical screening results of coconut leaf water extract

The phytochemical screening test aims to determine the secondary metabolites contained in coconut leaf, which have the potential as bioreductors in the synthesis of silver nanoparticles. Phytochemical screening analysis for coconut leaf water extracts include the content of flavonoids, terpenoids and steroids, alkaloids, saponins and tannins. Based on the results of the qualitative phytochemical screening, the coconut leaf water extract contained flavonoid, terpenoid, saponin and tannin compounds. These results are in accordance with previous research conducted by Rakhil et al. (2023). The use of aqua demineralized water solvent which is polar can attract compounds with the same polarity so that it shows positive results in the testing for those compounds (Renda et al., 2023). Other factors that can influence the content of secondary metabolites in plants are internal factors and external factors. Internal factors are the genetic factors of the plant, while external factors include the intensity of sunlight, environmental temperature, humidity, soil pH, nutrient content in the soil and the altitude of the area where the plant grows (Susanti et al., 2022).

Synthesis of silver nanoparticles

The synthesis of silver nanoparticles in this research was carried out by mixing a solution of coconut leaf water extract with concentrations of 3% and 1% with 1 mM $AgNO_3$ solution in a ratio of 1:9 (v/v). The mixture is then heated at 60°C for 15 min until the color changes. The silver nanoparticle synthesis process involves coconut leaf water extract as a bioreductant because it contains compounds in the form of flavonoids, tannins, saponins and terpenoids which can help the biosynthesis process. Flavonoids are one of the phenolic compounds that are widely used in the synthesis process of silver nanoparticles. Flavonoid compounds have hydroxyl groups (OH) which can reduce by donating electrons to the Ag^+ ion from $AgNO_3$ to Ag^0 (Fajri et al., 2022). The general reaction mechanism for the formation of silver nanoparticles by flavonoids is illustrated in Figure 1.

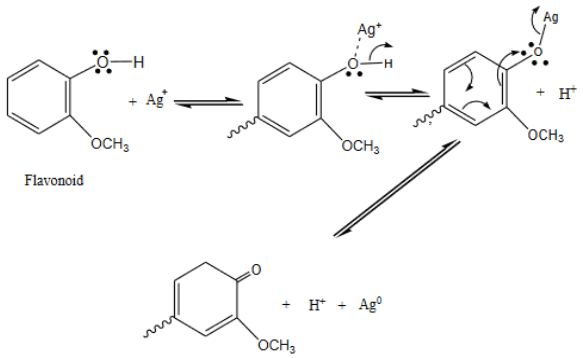

Figure 1. Estimated mechanism of silver nanoparticle formation by flavonoids (Bere et al., 2019)

Apart from being able to act as a reducing agent, flavonoid compounds can also act as stabilizers because they have hydroxyl (-OH) and carboxyl (-CO) groups which interact with silver particles and form an electric double layer (Bere et al., 2019). Based on researches carried out in the synthesis process, it appears that the color change of the $AgNO_3$ solution with coconut leaf water extract produces the same color change in the 1% and 3% extracts, from clear to brownish red after being heated, as in Figure 2. These changes are an indicator of the formation of silver nanoparticles. This is due to the reduction process reaction of silver ions to silver nanoparticles (Fabiani et al., 2018). Based on the research conducted, it shows that reaction time is very influential in the synthesis of silver nanoparticles. According to Jannah and Amaria (2020), the longer the reaction time, the more intense the color of the resulting solution. The influence of reaction time shows surface plasmon resonance (SPR) with different intensities.

Characterization of silver nanoparticles UV-Vis spectrophotometer

Characterization using UV-Vis spectrophotometer aims to determine whether silver nanoparticles have been formed. UV-Vis spectrophotometer analysis is used for qualitative or quantitative analysis of a compound. Before measuring the wavelength of AgNPs, wavelength measurements were carried out on the $AgNO_3$ solution and the coconut leaf water extract solution (Figure 3.). Based on the research results, an illustration of the maximum wavelength (λmax) of silver nanoparticles can be seen in Figure 3. where λmax is 430nm.

Each nanoparticle formed has a different character. The peak intensity of a nanoparticle that is visible in UV-Vis spectrophotometer analysis is represents the surface plasmon resonance (SPR). The formation of a brownish red color in the solution and the appearance of absorbance at a wavelength of 430 nm are some of the properties and characteristics of the SPR effect of silver nanoparticles. A greater absorbance value indicates that more nanoparticles are formed (Jannah & Amaria, 2020). This research is in accordance with research by Lestari et al. (2019) who found the wavelength of AgNPs to be between 400-450 nm. This proves that this research has succeeded in synthesizing silver nanoparticles because the SPR intensity meets the requirements for the formation of nanoparticles.

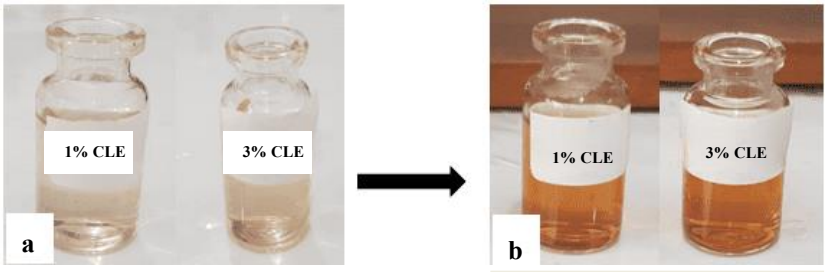

Figure 2. Formation of AgNPs colloids. (a) before AgNPs is formed, (b) after AgNPs is formed. CLE = coconut leaf extract

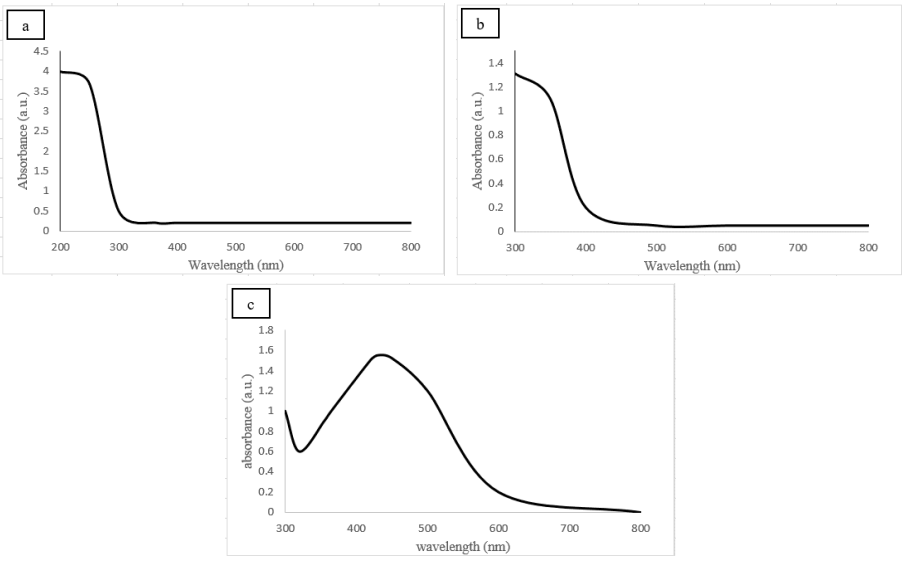

Figure 3. UV-Vis spectrum of (a) $AgNO_3$, (b) Coconut leaf extract, and (c) Silver nanoparticles

Particle size analyzer (PSA)

Particle size analysis aims to determine the particle size of colloidal nanoparticles. The measurement results using a particle size analyzer (PSA) are presented in Table 1. It can be seen that good AgNPs characteristics are obtained from the use of 3% extract of coconut leaf with a nanoparticle size of 63.29 nm, whereas 1% extract produces an average size exceeding 100 nm, namely 129.3 nm, so it is not included in the nanoparticle category, which should in the range of 1-100 nm. The polydispersity index value is a value that shows sample heterogeneity. A good PdI value under 0.5 indicates good long-term stability and shows a homogeneous or uniform particle size, while a polydispersity index value that exceeds 0.5 indicates the particles have a high level of heterogeneity (Taurina et al., 2017). Based on the research results, the nanoparticles formed from 3% extract have an average PdI value of 0.3361 so the sample is said to have a uniform size.

The zeta potential value is used to see the stability of nanoparticles to the effects of pH, temperature, and humidity. Nanoparticles with zeta potential values smaller than -30 mV or greater than +30 mV have higher stability (Juliantoni et al., 2020). The greater the zeta potential value, the more difficult it is for colloidal particles to flocculate or agglomerate. Based on the analysis carried out, results were obtained in the form of an average AgNPs zeta potential value from the 3% extract of -16.98 mV. These results indicate that the AgNPs colloidal particles formed have poor stability because they have a zeta potential value that does not match the standard. Meanwhile, the negative value (-) in the results indicates that the AgNPs layer formed is negatively charged.

Transmission electron microscopy (TEM)

Analysis with TEM aims to determine the morphology and size of the samples obtained. The size results range between 1-100 nm with round, oval, triangular, square or crystal particle shapes. Figure 4 shows the size and shape of AgNPs which has been successfully synthesized using a bioreductant from coconut leaf water extract as a bioreductant. Based on the research carried out, the shape of silver nanoparticles is round but less regular because there are several shapes that also look like triangles and also cubes (squares). According to Amin et al. (2020) nanoparticles formed from plant bioreductants have sizes ranging from 1-100 nm and show anisotropic morphology (balls, triangles, squares, and ovals).

Table 1. Particle size analyzer of AgNPs synthesized from different concentrations of coconut leaf extract

Concentration of coconut leaf extract (%)	Replication	Z-average (nm)	PdI	Zeta Potensial (mV)
	1	127	0.5235	-19.57
1	2	128.7	0.5321	-18.17
	3	132.2	0.4857	-18.46
Average		**129.3**	**0.5137**	**-18.73**
	1	63.22	0.3393	-16.37
3	2	63.44	0.3298	-16.59
	3	63.22	0.3393	-17.98
Average		**63.29**	**0.3361**	**-16.98**

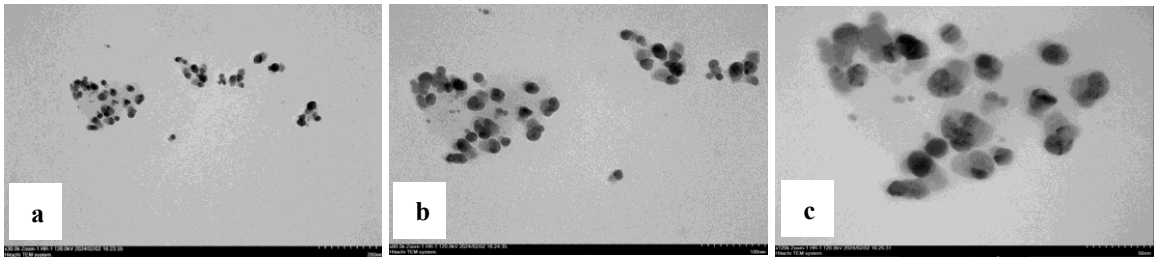

Figure 4. Morphological analysis of AgNPs using TEM (a) 30,000x magnification, (b) 60,000x magnification, (c) 120,000x magnification

Scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDS)

The SEM analysis is used to determine the morphology of the silver nanoparticles formed, while EDS analysis aims to determine the elemental composition of AgNPs. The morphology of AgNPs was observed using SEM at 10,000x, 2,000x and 400x magnification. Figure 5 shows the results of SEM measurements which provide an overview of the formation of silver particles with micro to nano. The SEM image at 400x magnification shows randomly shaped crystal particles with quite large sizes. This shows that the silver nanoparticles agglomerate so that the shape of the silver nanoparticles is not visible. At 2,000x and 10,000x magnification it shows random shaped particles such as balls, squares, triangles with very small sizes. Similar results were reported by Kasim et al. (2020) and Masakke et al. (2015) who observed that the morphology of silver nanoparticles shows random and varied shapes. According to Lestari (2019) and Paramesh et al. (2021), the results of their research show that nanoparticles analyzed using SEM are crystalline or round and agglomerated. The agglomeration that occurs causes large sized nanoparticles.

The results obtained from analysis using TEM and SEM gave similar results that the silver nanoparticles formed were very clearly visible. EDS spectrum analysis shows the elemental composition contained in the synthesized silver nanoparticles. The analysis results showed an energy peak of 2.983 keV where the presence of silver (Ag) atoms was detected. The results of this analysis show and confirm the existence of a strong signal in the peak area related to the formation of AgNPs. According to Murugan et al. (2017) AgNPs generally shows a typical optical absorption peak at 3 keV, due to the specific SPR properties of AgNPs.

The components detected in the synthesized AgNPs are carbon (C), oxygen (O), sodium (Na), chlorine (Cl), silver (Ag). Based on data on the elemental composition of silver nanoparticles, the mass percentage of Ag is 51.77%. Meanwhile, the carbon (C) and oxygen (O) elements have mass percentages of 16.64% and 23.22% respectively. These results are related to the biosynthesis process during the formation of nanoparticles, where the use of bioreductants from coconut leaf water extract will influence the results of the AgNPs elemental composition. Canang/coconut leaf water extract contains secondary metabolites, especially flavonoids and tannins which contain carbon (C) and oxygen (O) elements (Rakhil et al., 2023). Several other elements were also detected, possibly originating from other secondary metabolites in coconut leaf water extract.

Fourier transform infrared (FTIR)

FTIR analysis was carried out to determine components or groups that might be responsible for the AgNPs reduction or coating process (Murugan et al., 2017). The results of the characterization analysis of AgNPs showed some absorption in certain areas which indicated the presence of flavonoid compound characteristics (Figure 6). The

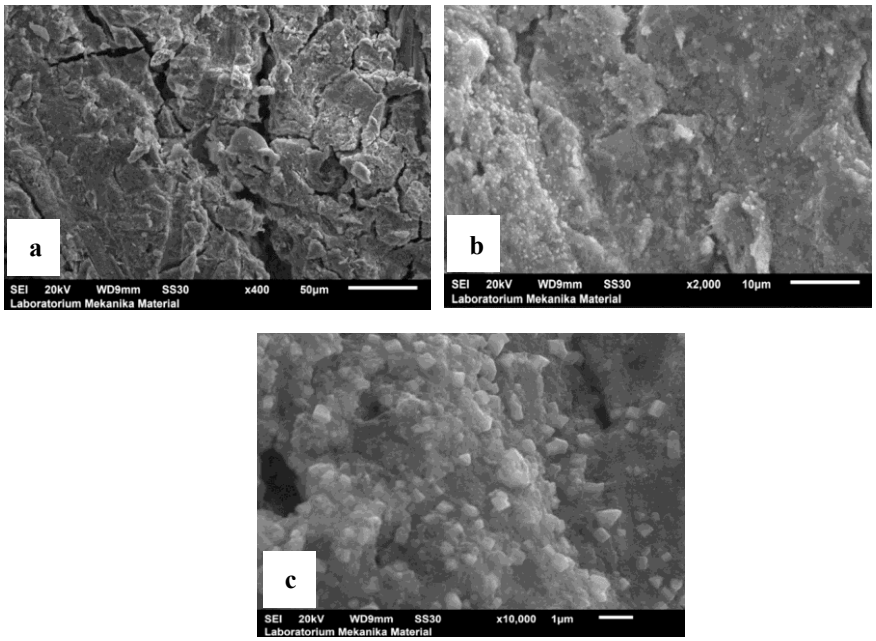

Figure 5. Morphological analysis of AgNPs using SEM (a) 400x, (b) 2,000x, (c) 10,000x magnification

presence of absorption in the 3445.32 cm^{-1} area indicates the presence of the -OH group which acts as a reducing agent. Apart from -OH, this area is also an aromatic C-H absorption area. The presence of absorption at a wave number of 2066.73 cm^{-1} indicates the presence of aromatic C=C, C-H groups (Adzani et al., 2020), the presence of absorption in the area of 1634.01 cm^{-1} indicates C=C absorption (aromatic and aliphatic) and C absorption =O (Skoog et al., 1996) and at a wave number of 562.64 cm^{-1} indicates the presence of Ar-H bonds (Dachriyanus, 2004).

Based on the results of FTIR analysis, it shows the presence of flavonoid compounds characterized by –OH groups which can donate electrons, so that silver nanoparticles can be formed. Previous research stated that the presence of hydroxyl groups (-OH) shown in the characterization of AgNPs can act as a capping agent by forming hydrogen bonds (Bhaumik, 2015; Mittal, 2014; Ghodake, 2020).

Antifungal activity of silver nanoparticles

The results of the antifungal activity test of AgNPs against the *Ganoderma boninense* fungus using several concentrations of silver nanoparticles, namely 1.8; 2.4; 3.0; 3.7; 4.3; 4.9 ppm, can be seen in Table 2. Based on these results, almost all concentrations of AgNPs can inhibit fungal growth. The growth of fungal colonies on PDA media containing AgNPs looked smaller than the growth on negative control media. These results also show that the higher the AgNPs concentration, the stronger the inhibitory effect on the growth of the *Ganoderma boninense* fungus. The results of the negative control for fungal colony growth can be seen in Figure 7 which shows that in the negative control there was no inhibition of fungal colony growth until the ten days. Based on the results of colony growth diameter obtained, it showed the highest percentage of inhibition of fungal colony growth, namely at a concentration of 4.9 ppm with an inhibition percentage value on day 10 was 68.37%.

The antifungal activity of AgNPs was observed from the growth of *G. boninense* mycelium on PDA media containing AgNPs at varying concentrations. Evaluation of antifungal activity includes visual observation and percentage of colony inhibition. *Ganoderma boninense* mycelia filled the surface of the control PDA media on day 10 with a diameter of 8 cm (Figure 7). Meanwhile, the growth of *G. boninense* mycelia tends to be hampered on PDA media containing AgNPs, especially on PDA media treatments containing 3.0 to 4.9 ppm AgNPs, with a mycelial diameter range of 2.53 - 5.03 cm (Table 2). The addition of AgNPs to PDA media showed that the greater the concentration of AgNPs added, the smaller the growth of *G. boninense* in the Petri dish. These results indicate the inhibitory effect of AgNPs on the mycelial growth of *G. boninense*. Smaller sized nanoparticles create more surface area in contact with *G. boninense*, so they can inhibit the growth of *G. boninense* (Raimondi et al., 2005; Faramitha et al., 2022).

Silver nanoparticles have been utilized in the inhibition of pathogens caused by bacteria, fungi, viruses, actinomycetes, nematodes, and other pathogens (Liang et al., 2018). The mechanism by which nanoparticles inhibit pathogens is by reducing the level of infection, inducing systematic resistance, attacking surface proteins, reducing cellular factors, inhibiting DNA replication, suppressing polymerase activity which can be seen as the mycelium grows smaller. According to Tripathi et al. (2017), AgNPs causes blocking of

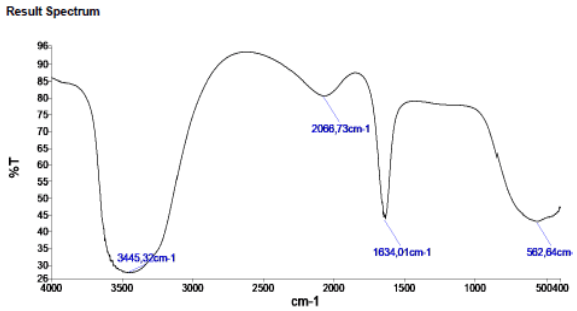

Figure 6. The FTIR spectrum results of silver nanoparticles

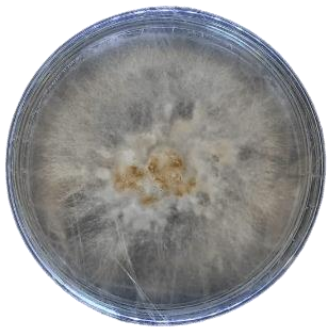

Figure 7. Negative control on PDA media without the addition of AgNPs (ten days)

Table 2. Percentage of inhibition of fungal colony growth

Concentration (ppm)	Average Percentage Inhibition of Fungal Colony Growth on Day 10 (%)
1.8	0
2.4	12.50 ± 3.13
3.0	37.08 ± 13.35
3.7	46.88 ± 3.13
4.3	61.46 ± 1.30
4.9	68.37 ± 0.72

apoplastic compounds by blocking them through the pores in the plasmodesmata or cell walls, as a result, limiting the flow of apoplastic nutrients and water, so that the fungus will shrink and eventually die.

The results above show that AgNPs is able to inhibit the growth of fungal colonies because there are antifungal compounds in silver nanoparticles. This is in accordance with research conducted by Fajar et al. (2020) which stated that AgNPs *Mikania micrantha* leaf extract has antifungal activity against the fungus *Aspergillus* sp with the inhibition diameter 11-14 mm. The mechanism for inhibiting the fungal growth process is through the process of denaturing protein bonds in the fungal cell membrane, so that the fungal cell membrane experiences lysis and AgNPs penetrates into the cell nucleus, which causes the fungus to not grow (Riza et al., 2014). The second mechanism is when AgNPs is able to penetrate into cells, so it can damage the DNA. According to Fajar et al. (2020) bacterial cells can live when the DNA within them is capable of replicating (doubling the double strand of DNA). However, when AgNPs penetrates into cells, the DNA molecules will turn thick, losing their replication ability and causing bacterial cells to die.

Conclusion

The water extract of coconut leaf (*Cocos nucifera* L.) is capable of synthesizing silver nanoparticles. Coconut leaf water extract contains flavonoids with a hydroxyl group (OH), which can be reduced by donating electrons to the Ag^+ ion from $AgNO_3$ to Ag^0. The silver nanoparticles obtained had a maximum wavelength of 430 nm, particle size of 63.29 nm, PdI value of 0.3361, and zeta potential value of -16.98 mV. The particle's shapes were round, oval, square, and triangle, with a mass percentage of Ag of 51.77%. These Silver nanoparticles had an antifungal activity with a percentage of mycelial growth inhibition up to 68.37% at 4.9 ppm of silver nanoparticles. The higher the concentration of silver nanoparticles, the better the antifungal activity against the growth of *Ganoderma boninense.*

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