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Green Synthesis and Characterization of Silver Nanoparticles Derived from Ethanol Extract of Sappan Wood

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ABSTRACT: Silver nanoparticles had been synthesized through the reduction process using an ethanol extract of sappan wood (*Caesalpinia sappan* L.) as a bioreductor. The variation of the formula used is a mixture of the ethanol extract of sappan wood with a 2 mM $AgNO_3$ solution in a volume ratio of 1:1, 1:2, 1:3, and 1:4. The results showed that the UV-Visible spectrophotometry confirmed that the volume ratio of 1:1 was the optimum formula with an absorbance value of 1.243 at the maximum wavelength of 446 nm. Particle size analysis using Zetasizer Nano ZS revealed that the synthetized silver nanoparticles had a particle size of 618.1 nm. The hydroxyl group (OH) had a important role in the nanoparticle formation process, according to functional group characterization using FTIR. Due to silver nanoparticles tendency to agglomerate, the SEM image of the silver nanoparticles showed a variety of sizes and forms.

KEYWORDS: Bioreductor, Ethanol extract, Silver nanoparticles, Sappan wood

INTRODUCTION

In recent years, the growth of science and industry worldwide has increasingly centered on nanotechnology. Nanoparticles are a subfield of nanotechnology that is presently under development. Because of their numerous advantages and diverse uses in the areas of environment, health, agriculture, textiles, electrochemistry, optics, and energy, nanoparticles are a fascinating technological development to study. Nanoparticles are a technical design that uses nanoscale material structures with sizes ranging from 1-100 nm. However, materials with a diameter smaller than 1 µm or 1000 nm can still be classified as nanoparticles [1].

Physical methods and chemical methods are common methods used in the nanoparticle synthesis process. Although both processes provide pure particles, they have significant disadvantages, including the potential for environmental degradation and high manufacturing costs. To solve this issue, a different approach that is more affordable, straightforward, and ecologically friendly has been created. This is built on the concept of green chemistry, namely, biosynthesis.

In this process, plants and microbes serve as stabilizers, reducing agents, or both to create nanoparticles. The synthesis of silver nanoparticles uses organic compounds from a class of secondary metabolites found in plants [2]. Especially some secondary metabolites that have antioxidant activity include terpenoids, phenolics, flavonoids, tannins, saponins, and alkaloids that can act as reducing agents and capping agents. It can produce silver nanoparticles by reducing Ag^+ ions [3,4]. Silver ions will be reduced into silver nanoparticles by the hydroxyl (OH) and amine (NH_2) groups of secondary metabolites in plants [5].

Sappan wood is one of the plants with significant antioxidant activity. Secondary metabolites of sappan wood that have antioxidant activity include flavonoids, alkaloids, phenolics, and tannins [3,7,8]. These secondary metabolites have the potential as natural reducing agents in the synthesis of silver nanoparticles. Many studies have reported the use of plant ethanol or methanol extracts as bioreductor. However, there has never been reported the synthesis of silver nanoparticles using ethanol extract of sappan wood as a bioreductor.

In this work, silver nanoparticles were synthesized using an ethanol extract of sappan wood as a bioreductor, and they were then characterized using a Zetasizer Nano ZS, UV-Vis spectrophotometer, FTIR spectrophotometer, and scanning electron microscopy (SEM).

MATERIAL AND METHODS

A. Material and Tools

The materials were required for this study: sappan wood, technical ethanol, ethanol pa (Merck), $AgNO_3$ (Merck), aquabidest, aluminum foil, filter paper (Whatman no. 42), and cling wrap.

The equipment needed in this research is a set of maceration tools, rotavapor (Buchi R-300), vacuum pump (Gast DOA-P504-BN), centrifuge, magnetic stirrer (Heidolph), freeze dryer (Martin Christ Alpha 1-2 Ldplus), oven (Heraeus ST-5042), analytical balance (Adventurer Ohaus), Buchner funnel, UV-Vis spectrophotometer (Shimadzu UV-1800), Zetasizer Nano ZS (Malvern), infrared spectrophotometer (Shimadzu FTIR-8400S), Scanning Electron Microscope (SEM) (FEI Inspect S50), and glassware available in the laboratory.

B. Methods

1) Sappan Wood Sample Preparation

Sample of sappan wood used in this research was obtained from UPT Materia Medica, Batu, East Java, Indonesia. The sample was identified at LIPI, Purwodadi Botanical Gardens, Pasuruan, East Java, Indonesia, before further study. The samples were then cleaned, divided into smaller pieces, and allowed to dry at room temperature. The dried sample is ground into a fine powder for use in the extraction process.

2) Sappan Wood Sample Extraction

Sappan wood dried powder (500 g) was macerated three times for one day at room temperature in 1 L of technical ethanol solvent. The maceration solution was filtered using a Buchner funnel to obtain an ethanol extract of sappan wood. The combined solvent of the macerated extract was evaporated using a rotary evaporator to obtain a concentrated ethanol extract of sappan wood. The extract was dried for 8 hours using a freeze dryer to obtain a dry ethanol extract of sappan wood [8].

3) Preparation of Sample Solution

The dried ethanol extract of sappan wood was weighed to a maximum of 1,0 gram, 40 mL of ethanol was added, and the mixture was agitated until homogenous before being placed onto a funnel lined with filter paper (Whatman no. 42) for dialing. The obtained solution was stored in a refrigerator [9].

4) Preparation of 2 mM $AgNO_3$ Solution

A total of 0.085 g of $AgNO_3$ was dissolved with aquabides in a 250 mL volumetric flask to the mark and shaken until homogeneous to obtain a solution of $AgNO_3$ 2 mM [9].

4) Optimization of the Ratio of the Amount of Extract Solution with $AgNO_3$ 2 mM Solution

In four separate beakers, the ratios of the sappan wood ethanol extract solution to the 2 mM AgNO3 solution were 1:1 (10 mL:10 mL), 1:2 (10 mL:20 mL), 1:3 (10 mL:30 mL), and 1:4. (10 mL: 40 mL). Using a magnetic stirrer, the mixture was agitated for 15 minutes. The UV-Vis spectrum was then examined in the wavelength range of 300-700 nm to ascertain the sample solution's maximum wavelength and absorbance to determine the ideal ratio of sappan wood ethanol extract to $AgNO_3$ [10].

5) Silver Nanoparticle Synthesis

The ideal ratio of sappan wood ethanol extract solution and 2 mM $AgNO_3$ solution were made again as much as 40 mL and stirred using a magnetic stirrer for 15 minutes. Part of the mixture was centrifuged and then separated between the filtrate and residue. Silver nanoparticle solution was analyzed with Zetasizer Nano ZS to determine the particle size while the pellets were dried using a freeze dryer so that solid silver nanoparticles were obtained. Furthermore, solid silver nanoparticles were analyzed with the FTIR instrument to determine the functional groups and their changes and SEM to determine the surface morphology [10].

RESULT AND DISCUSSION

A. Sappan Wood Extract

The dried powder of sappan wood (500 g) was macerated using technical ethanol solvent (1 L) for 1 day at room temperature, repeated three times. The use of polar solvent ethanol is intended to extract secondary metabolites of phenolic types contained in sappan wood. Ethanol can degrade the sappan wood's cell walls, allowing the phenolic chemicals it contains to dissolve in ethanol and be more easily extracted from the sappan wood cell [11]. The maceration solution was filtered using a Buchner funnel to obtain an ethanol extract of sappan wood without residue. Then the combined solvent extracted from the maceration was evaporated using

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a rotary evaporator to obtain concentrated sappan wood ethanol extract. It is dried using freeze dryer for 8 hours to produce a solid ethanol extract of dark red sappan wood that weighs 21.745 grams (4.35 percent).

B. Optimization of the Composition of the Ethanol Extract of Sappan Wood Extract with 2 mM. $AgNO_3$ Solution

Sappan wood ethanol extract solution was added with 2 mM $AgNO_3$ solution in 4 different beakers with various compositions, namely 1:1 (10 mL:10 mL), 1:2 (10 mL:20 mL), 1:3 (10 mL:30 mL), and 1:4 (10 mL:40 mL). Each mixture was stirred for 15 minutes with a magnetic stirrer, then the UV-Vis spectrum was measured in the wavelength range of 300-700 nm to determine the peaks of plasmon resonance typical of silver nanoparticles [12]. The results of the UV-Vis spectrophotometer analysis of the four formulas are presented in Table 1 and Figure 1.

The volume ratio of the ethanol extract of sappan wood extract and the 2 mM $AgNO_3$ solution was analyzed using UV-Vis spectrophotometry, and the results indicated an absorption peak at a wavelength of 446 nm. The appearance of the UV absorption peaks indicated that silver nanoparticles have been formed because its peak at a wavelength between 410-480 nm [13]. If a peak occurs at a wavelength of 320 nm or between 200-250 nm, it indicated that silver nanoparticles have not yet been formed [14,15,16,17]. Silver nanoparticles can also be identified organoleptically. The solution resulting from the synthesis of silver nanoparticles was initially pale yellow, turning darker and then brownish red [18,19].

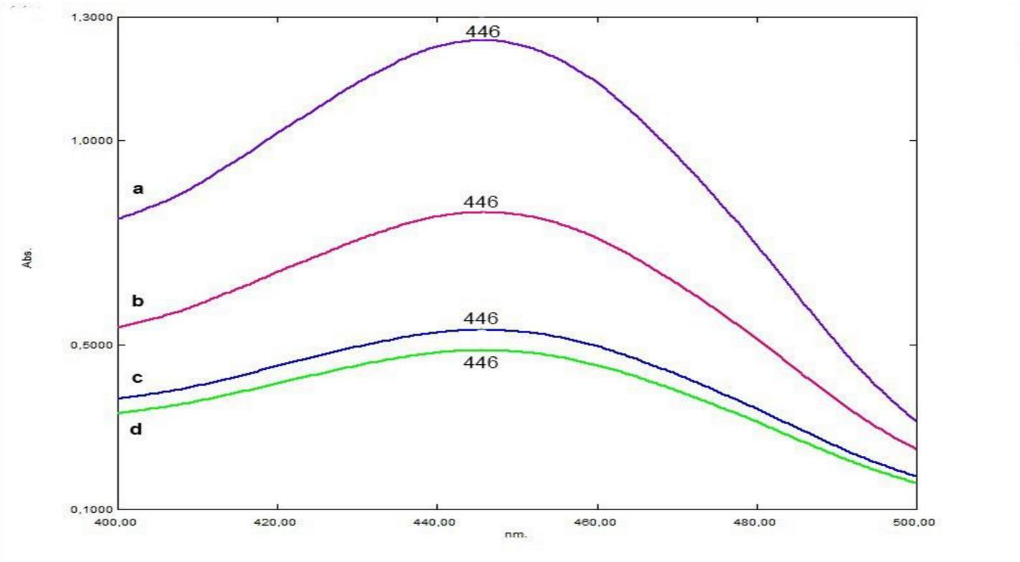

Figure 1. The UV-Vis Spectra of Combination of Sappan Wood Ethanol Extract Solution with Silver Nitrate Solution

Table 1. Maximum Wavelength and Absorbance of Silver Nanoparticles

No.	Composition of Ethanol Extract Solution and $AgNO_3$ Solution	Max λ (nm)	Absorbance
1	1:1	446	1.243
2	1:2	446	0.824
3	1:3	446	0.537
4	1:4	446	0.487

Based on Figure 1 and Table 1, silver nanoparticles have been formed in the four combinations of sappan wood ethanol extract solution and silver nitrate solution, as shown by the appearance of a peak at a wavelength of 446 nm, which satisfies the requirements for the maximum UV absorption of silver nanoparticles, which is between 410-480 nm. The mixture with a volume combination of ethanol extract of sappan wood extract with 1:1 silver nitrate solution has a maximum wavelength of 446 nm with

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the highest absorbance value of 1.243. Thus the mixture with this combination is the most optimum for producing silver nanoparticles [10, 20].

C. Silver Nanoparticle Synthesis

The ideal composition of a combination of ethanol extract, sappan wood extract, and silver nitrate solution was established with a volume ratio of 1:1 based on the analysis of UV absorption using a UV-Vis spectrophotometer. The optimum composition was made again as much as 40 mL and stirred for 15 minutes using a magnetic stirrer, followed by centrifugation and separation between the filtrate and the residue. Zetasizer Nano ZS was used to measure the particle size in the filtrate, and the residue was dried using a freeze dryer so that FTIR and SEM could be used to characterize the functional groups and morphology. The freeze dryer produced brownish-red solid silver nanoparticles.

D. Silver Nanoparticle Size Characterization

The Zetasizer Nano ZS (Malvern) equipment was used in this research to measure the particle size of nanosilver produced using sappan wood's ethanol extract as a bioreductor. Silver nanoparticles were measured using the optimal mix of sappan wood ethanol extract solution and silver nitrate solution (1:1). According to the measurement results, the formed silver nanoparticles had a particle size of 618.1 nm with a polydispersity index (PdI) of 0.363.

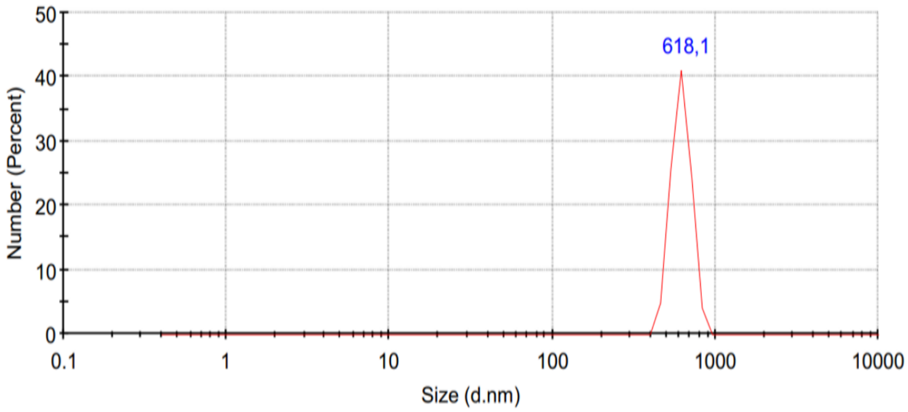

Figure 2. Measurement Results of Synthesized Silver Nanoparticles Using Zetasizer Nano ZS

Based on Figure 2, silver nanoparticles synthesized from ethanol extract of sappan wood have an average particle size of 618.1 nm. The size obtained from the analysis still meets the requirements for nanoparticle size in the range of 1-1000 nm so it can be said that the synthesis of silver nanoparticles using a sappan wood bioreductor has formed nanoparticles [1,21]. The size of the silver nanoparticles obtained is still smaller than the results of research by Rahim et al [20] with a green tea leaf extract bioreduction which produced silver nanoparticles with a diameter of up to 740,899 nm, but still larger than the results of Fabiani et al [22] with an average diameter of 544.1 nm which was produced with the help of a bioreductor of Pucuk Idat leaf extract. The large size of silver nanoparticles was caused by the tendency of silver nanoparticles to agglomerate so that the particle size was bigger. The addition of stabilizers can be done to prevent agglomeration [23].

E. Functional Group Characterization of Silver Nanoparticles

Analysis of functional groups that play a role in the synthesis of silver nanoparticles was carried out by FTIR, by comparing the spectra of the ethanol extract of sappan wood with silver nanoparticles. This is based on the principle of secondary metabolites in sappan wood such as phenolics, alkaloids, flavonoids, and tannins which were thought to be able to synthesize silver nanoparticles [24,25]. The hydroxyl (OH) group of phenolic compounds contained in the bioactive compound of sappan wood will reduce Ag^+ ions to silver nanoparticles (Ag^0) by donating electrons [26]. This analysis was used to determine what groups were involved in reducing Ag^+ ions to Ag^0. A comparison of the spectra of sappan wood ethanol extract with synthesized silver nanoparticles is presented in Figure 3 and the wave number and %T values are shown in Table 2.

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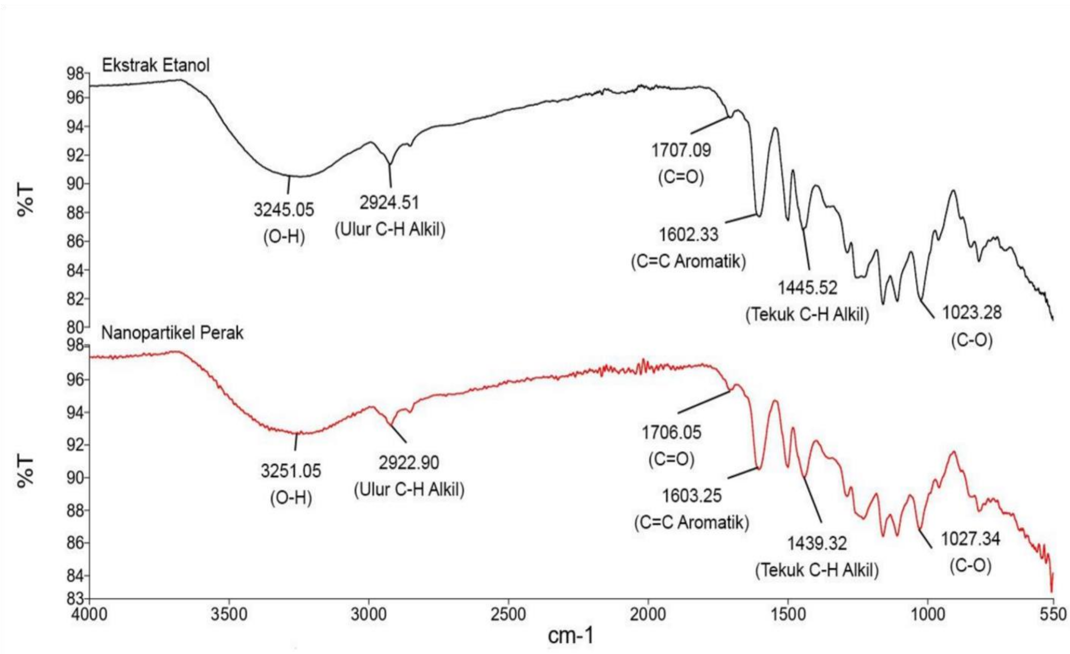

Figure 3. FT-IR Spectrum of Secang Wood Ethanol Extract and Synthesized Silver Nanoparticles

Based on the FTIR spectra (Figure 3), it can be stated that there has been a process of formation of silver nanoparticles due to the reduction of silver ions by the OH group in the ethanol extract of sappan wood which is indicated by an increase in the percentage of transmittance (%T) of the hydroxyl group of the sappan wood ethanol extract compared to the synthesized silver nanoparticles (from 90.51% to 92.67%). The higher the transmittance percentage value, the less the number of hydroxyl groups contained in silver nanoparticles. Another thing that also supports the process of forming silver nanoparticles is the interaction of functional groups which is characterized by a shift in wavenumber [10].

Table 2. Functional Groups of Secang Wood Ethanol Extract and Synthesized Silver Nanoparticles

No	Functional groups	Sappan wood ethanol extract		Synthetized silver nanoparticles	
		Wave number (cm^{-1})	%T	Wave number (cm^{-1})	%T
1	OH	3245.05	90.51	3251.05	92.67
2	CH alkyl (stretch)	2924.51	91.38	2922.90	93.21
3	C=O	1707.09	94.65	1703.05	95.40
4	C=C aromatic	1602.33	87.73	1603.25	90.50
5	CH alkyl (bend)	1445.52	86.80	1439.32	90.04
6	C-O alcohol	1287-1107	85.22	1287-1108	88.87

Figure 3 shows that the peak of the OH group of silver nanoparticles is gentler than in the ethanol extract of sappan wood because it is used to reduce Ag^+ to Ag^0. The oxidation of the hydroxyl groups of the phenolic compounds contained in the ethanol extract to carbonyl groups (C=O) caused a decrease in the absorption intensity of the C-O alcohol and C=C aromatic groups with an indication of the increasing percentage of transmittance. Meanwhile, the absorption intensity of the carbonyl group (C=O) increased in silver nanoparticles compared to the ethanol extract of sappan wood [27].

F. Silver Nanoparticle Morphology

In this research, a Scanning Electron Microscopy (SEM) device was used to characterize the morphology of the synthesized silver nanoparticles. The morphology of silver nanoparticles using SEM is shown in Figure 4.

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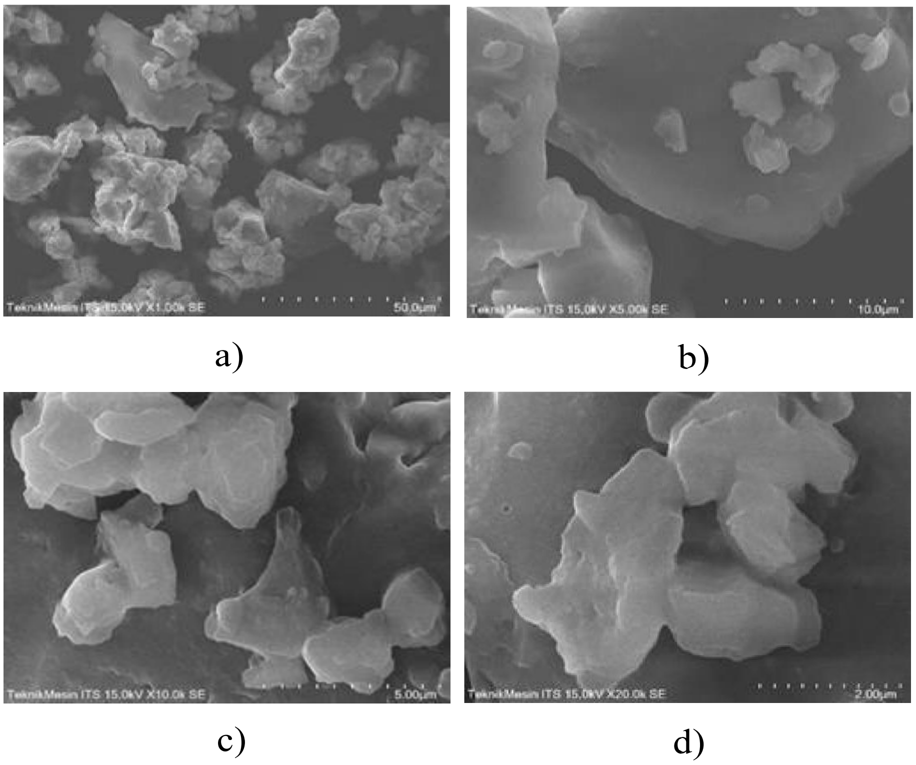

Figure 4. Morphology of Synthesized Silver Nanoparticles with Magnification: (a). 1000x(b). 5000x(c). 10,000x (d). 20,000x

Figure 4 shows the morphology of the synthetized silver nanoparticles, that have a smooth surface structure with a variety of shapes and particle sizes. The effect of nanoparticle aggregation caused of the van der Waals forces of silver nanoparticle molecules in solution gives variety of forms and sizes of silver nanoparticles [20].

CONCLUSION

Based on the results of research can be concluded that silver nanoparticles had been synthetized using sappan wood ethanol extract as bioreductor. For the synthesis of silver nanoparticles, the optimum volume ratio of sappan wood ethanol extract solution and silver nitrate solution is 1 : 1. The synthetized silver nanoparticles had a particle size of 618.1 nm and UV absorbance at a maximum wavelength of 446 nm. The FTIR spectrum of silver nanoparticles showed the presence of absorption bands for OH (hydroxyl), CH alkyl, C=O (carbonyl), C=C aromatic, and C-O alcohol. The increase in the percentage of transmittance of the hydroxyl group of silver nanoparticles compared to the ethanol extract of sappan wood supported the reduction of silver ions into silver nanoparticles. The SEM measurements showed that silver nanoparticles' surface morphology had a variety of shapes because of their tendency to aggregate.

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