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SILVER NANOPARTICLES – ARABICA COFFEE (COFFEA ARABICA) LEAF EXTRACT AND ITS ANTIOXIDANT ACTIVITY TEST

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ABSTRACT

Nanotechnology has become an increasingly attractive research area in the development of functional products, especially in the food, beverage and drug delivery sector. Nanotechnology in this research is used to produce Ag-NPs using active compounds extracted from Arabica coffee leaf as reducing agent. This research aims to determine the characteristics of silver nanoparticles using Arabica coffee leaf extract and determine the effect of silver nanoparticles on antioxidant activity. The process of synthesis of nanoparticles was carried out by adding Arabica coffee leaf extract to a 10 mM $AgNO_3$ solution with a ratio of 1:35, 2:35, dan 3:35. Silver nanoparticles were characterized using a UV-Vis spectrophotometer, Particle Size Analyzer (PSA), and Scanning Electron Microscope – Energy Dispersive X-Ray (SEM-EDX). The research results show that the absorbance value will increase as the contact time increases. Maximum absorption was obtained at a wavelength of 419 nm using a UV-Vis spectrophotometer. The size of silver nanoparticles obtained based on the results of PSA analysis is 35.71 nm with a zeta potential value of - 14.34 mV and a PI value of 0.5078. The result of synthesis silver nanoparticles analyzed using SEM-EDX produced crystalline forms with material compositions namely silver (21.44%), nitrogen (19.27%), carbon (7.12%), and oxygen (5.76%). Arabica coffee leaf extract silver nanoparticles have the potential as a very strong antioxidant with an IC50 value of 10.4233 ppm, while Arabica coffee leaf extract has an IC50 value of 13.3828 ppm.

KEYWORDS: Silver nanoparticles, Arabica Coffee Leaves, Antioxidant.

INTRODUCTION

A drug is a substance that brings about changes in biological functions through its chemical effects. Living organisms grow with complex tissues, which can make it difficult for drug molecules to reach their targets. Therefore, drug delivery systems continue to develop in order to create a formulation with an efficient delivery system. This system aims to minimize side effects on certain organs. Advances in nanotechnology have great potential for drug delivery. This has led to the development of drug delivery formulations using nanoparticles.

Nanoparticles have the potential for further development based on their size, distribution, and molecular morphology. Broadly, nanoparticles can be produced using physical methods (top-down) and chemical methods (bottom-up). However, both methods have disadvantages, such as excessive use of chemicals, environmental pollution, and high costs. Another technique used in producing silver nanoparticles is the green synthesis method. This method synthesizes metal nanoparticles with the help of natural materials derived from plants. One of the most promising nanoparticles for development is silver nanoparticles. Therefore, silver nanoparticles using plant extracts can be an alternative due to their environmentally friendly and safe reaction mechanisms, as well as the low concentrations used.

One of the plants that can be used for the biosynthesis of nanoparticles is the coffee plant. The most widely distributed coffee plant in society is Arabica coffee. In this study, the leaves of the Arabica coffee plant were used. Arabica coffee leaves are often considered waste and have not been widely utilized as food products or natural ingredients. In fact, Arabica coffee leaves contain many secondary metabolite compounds such as alkaloids, flavonoids, phenolics, saponins, tannins, steroids, and terpenoids (Adzkiya and Hidayat, 2022). Several researchers have also shown the use of Arabica coffee leaves as antioxidants (Oktavia and Sutoyo, 2021), antibacterial (Anggraeni, 2014), antidiabetic (Shiyan *et al.*, 2017), and anti-inflammatory (Wenas *et al.*, 2020).

Silver nanoparticles extracted from plants, specifically Arabica coffee leaves, show potential as antioxidants. Antioxidants are compounds that can neutralize free radicals, when excessive in the body can increase oxidative stress and trigger degenerative diseases by damaging cells and tissues. According to Retnaningtyas *et al.*, (2017), plants with high phenol content, such as flavonoids have high antioxidant activity due to their ability to scavenge free radicals through hydroxyl groups. These phenolic compounds can donate hydrogen to ROS (Reactive Oxygen Species), neutralize reactive oxygen, and prevent cellular damage. The research by Retnaningtyas *et al.*, (2017) demonstrated that the ethanol extract of Arabica coffee leaves has antioxidant activity with an IC_{50} value of 19.856 ppm, reinforcing the antioxidant potential of silver nanoparticles extracted from Arabica coffee leaves.

One method for testing antioxidant activity is the DPPH (2,2-diphenyl-1-picrylhydrazyl), which is based on the ability of a substance to neutralize free radicals. The mechanism of this method involves the antioxidant compound donating an H^+ atom to the hydrogen radical, so that all electrons in the DPPH radical pair up to form a stable molecule. This method is the simplest, fastest, easiest, most accurate, affordable, and capable of measuring various components that act as free radicals. Several studies related to the use of silver nanoparticles from plant extracts and their antioxidant activity tests have been conducted. Abdel *et al.*, (2015) synthesized silver nanoparticles using young Kozan fruit as a bioreductor and obtained silver nanoparticles sized 30-50 nm, with an IC_{50} value for antioxidant activity testing of 13.720 ppm. Thomas *et al.*, (2018) synthesized silver nanoparticles using Coleus vettiveroids plant extract as a bioreductor, obtaining silver nanoparticles with a diameter of 5 nm and an IC_{50} value for antioxidant activity testing of 71.90ppm.

Based on the description above, this study was conducted to synthesize silver nanoparticles using Arabica coffee leaf extract. The use of Arabica coffee leaf extract as a bioreductor in the synthesis of silver nanoparticles has not been previously explored, making it highly potential as a new bioreductor in silver nanoparticle synthesis.

MATERIAL AND METHODS

Materials

The materials used in this study include Arabica coffee leaves sourced from Ulian Village, Kintamani District, Bangli Regency, Bali. Other materials used are silver nitrate ($AgNO_3$) powder, distilled water, methanol (CH_3OH), DPPH powder, Meyer's reagent, ammonia (NH_3), hydrochloric acid (HCl) 37%, sulfuric acid (H_2SO_4) 98%, ferric chloride ($FeCl_3$), ethanol (CH_3CH_2OH) 96%, magnesium (Mg) powder, anhydrous acetic acid ($C_4H_6O_3$), and chloroform ($CHCl_3$).

Equipment

The equipment used in this study includes a set of glassware, rotary vacuum evaporator, grinder, magnetic stirrer, test tube rack, vial bottles, filter paper, spectrophotometer UV-Vis, Particle Size Analyzer (PSA), and Scanning Electron Microscope – Energy Dispersive X-Ray (SEM-EDX).

Procedures

Preparation of Arabica Coffee Leaves

The plant used in this study is Arabica coffee leaves obtained from Ulian Village, Kintamani District, Bangli Regency, Bali. The Arabica coffee leaves are washed with running water to remove dirt until clean. The leaves are then cut into small pieces and dried by air-drying at room temperature. Once dried, the leaves are ground and sifted to obtain a fine powder. furthermore, the moisture is determined.

Preparation of Arabica Coffee Leaf Extract

The extraction is performed using the maceration method. 300 grams of Arabica coffee leaf powder are weighed and placed into a beaker, then 2000 mL of 96% ethanol is added and soaked for 24 hours. Every 6 hours during soaking the mixture is stirred with a stir bar. The resulting extract is filtered using filter paper into a bottle (Filtrate I). The remaining solid is then remacerated. The solid is added to 1000 mL of 96% ethanol and soaked for another 24 hours. Every 6 hours during soaking the mixture is stirred with a stir bar and the resulting extract is filtered using filter paper (Filtrate II). All obtained filtrates are combined and evaporated using a rotary vacuum evaporator at 50˚C until a thick extract is obtained. The yield is calculated using the following equation.

$$\%\text{Yield} = \frac{\text{Weight of Extract (gram)}}{\text{Weight of Simplisia (gram)}} \times 100\%$$

Phytochemical Test

Phytochemical screening is a preliminary step that provides an overview of the specific compounds present in the natural material being studied. This phytochemical screening includes tests for alkaloids, phenolics, flavonoids, saponins, terpenoids, and steroids.

Synthesis of Silver Nanoparticles – Arabica Coffee Leaf Extract

Arabica coffee leaf extract volumes of 1, 2, and 3 mL were added to 35 mL of a 10 mM $AgNO_3$ solution in a 100 mL Erlenmeyer flask. The mixture was stirred with a magnetic stirrer for 15 minutes until the color changed from clear to yellow-brown and then stored in a glass bottle. Color changes were observed over 1, 2, 3, and 4 days. The solution was analyzed using a UV-Vis spectrophotometer in the 200-800 nm wavelength range. The volume of extract that remained stable over time was chosen. The solution was then centrifuged at 10,000 rpm for 15 minutes to collect a silver nanoparticle precipitate. This precipitate was dried in an oven at 80°C for 24 hours. The resulting silver nanoparticles were

characterized using a Particle Size Analyzer (PSA) and a Scanning Electron Microscope – Energy Dispersive X-Ray (SEM-EDX).

Determination of Antioxidant Activity

A 1000 ppm stock solution is prepared by dissolving 25 mg of silver nanoparticles from Arabica coffee leaf extract and 25 mg of Arabica coffee leaf extract separately in 25 mL of distilled water. Each stock solution is then diluted to concentrations of 50 ppm, 100 ppm, 150 ppm, and 200 ppm with distilled water.

For the assay, 1 mL of each concentration is mixed with 2 mL of methanol and 1 mL of 0.1 mM DPPH in methanol. The mixture is vortexed and left for 30 minutes at room temperature in the dark.

Absorbance is measured at 516.5 nm using a UV-Vis spectrophotometer. Methanol serves as the blank, and the control is 1 mL of 0.1 mM DPPH with 2 mL of methanol. The percentage inhibition of DPPH absorption is then calculated. The percentage inhibition of DPPH absorption is calculated using the following equation.

$$\% \text{ Inhibition} = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100\%$$

Keterangan:

Abs. Control = DPPH Radical Absorption

Abs. Sample = DPPH Sample Absorbance

RESULTS AND DISCUSSION

Preparation of Arabica Coffee Leaves

The Arabica coffee plant leaves obtained from the weeding process are washed with running water to remove any dirt adhering to them. The leaves are then cut into small pieces to facilitate the drying process at room temperature. This drying is intended to prevent damage to secondary metabolites that are not resistant to high temperatures and to prevent the growth of microorganisms such as fungi and bacteria. The dried leaves are then ground into a fine powder using a blender.

Figure 1: Preparation of Arabica Coffee Leaves.

Moisture Content of Arabica Coffee Leaves

The determination of moisture content is performed to measure the amount of water present in the material. The method used to determine moisture content is the drying method (Thermogravimetry). This method can measure the change in mass of a material due to temperature effects, which can influence the material's mass. The average moisture content measurement results for Arabica coffee leaves are presented in Table 1. According to SNI (2016), the specified moisture content limit is <10%. The obtained result shows a moisture content of 4.93%.

Table 1: Moisture Content of Arabica Coffee Leaves.

Repitition	% Moisture Content
I	5,53 %
II	5,17 %
III	4,09 %
Average	4,93 %

Extraction of Arabica Coffee Leaves

The extraction method used is the maceration method. The principle of this method involves soaking and stirring the plant material at room temperature. This approach is designed to ensure adequate contact time between the solvent and the cell walls of the plant material. The maceration method is chosen because it can effectively extract a greater amount of active compounds through soaking without heating, this avoiding damage to secondary metabolites. The solvent used is ethanol. This solvent is selected because it is volatile and polar. The polarity is due to the presence of polar (OH) and non-polar is ethyl (CH3CH2-) groups. The short carbon chain imparts a polar nature to ethanol. The extraction yields a thick, dark brown extract of Arabica coffee leaves, amounting to 79.79 grams with a yield percentage of 26.60%. The results of the extract yield are presented in Table 2.

Table 2: Results of Arabica Coffee Leaf Extraction.

Weight of Fresh Leaves (gram)	Weight of Simplisia (gram)	Ethanol Solvent (mL)	Results of Maceration (mL)	Weight of Extract (gram)	Yield of Extract (%)
1000	300	3000	2500	79,79	26,60

Phytochemical Test

Qualitative analysis is performed through phytochemical screening to identify secondary metabolites and provide an overview of the types of compounds present in Arabica coffee leaves. The results of the phytochemical screening for Arabica coffee leaves are presented in Table 3.

Table 3: Results of Screening Phytochemical.

Compound Identification	Reagent	Information
Alkaloid	Meyer	(+)
Phenolic	FeCl3	(+)
Flavonoid	Mg Powder and 37% HCl	(+)
Saponin	Distilled Water and 37% HCl	(+)
Terpenoid	Lieberman – Buchard	(+)

Synthesis of Silver Nanoparticles – Arabica Coffee Leaf Extract

The formation of silver nanoparticles can be observed by the color change of the solution from clear to yellowish-brown. The color change in a solution is influenced by the chemical reduction process. This occurs when Ag^{+} ions are reduced to Ag^{0}. The uncharged silver particles will collide with each other, forming clusters. The formation of these clusters is indicated by the change in color a deeper color suggests that more organic compounds are oxidized. This reduction process of silver is called aggregation. This happens because silver atoms interact through their metallic bonds and collide to form nanoparticles, increasing the size of the clusters. The aggregation of these clusters can be influenced by secondary metabolites that act as capping agents.

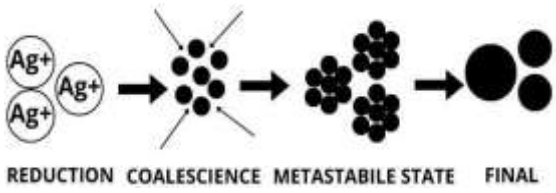

Figure 2: Formation of Silver Nanoparticles.

Additionally, this color change is caused by the phenomenon of surface plasmon resonance (SPR). SPR occurs because atom silver with their electron configuration in the d orbital, are highly reactive. This reactivity causes electrons in silver atoms to be excited, transitioning from low to high energy levels by absorbing energy. These electrons can be polarized due to the influence of the electric field from light. The color indicating the formation of nanoparticles can be observed in Figure 3.

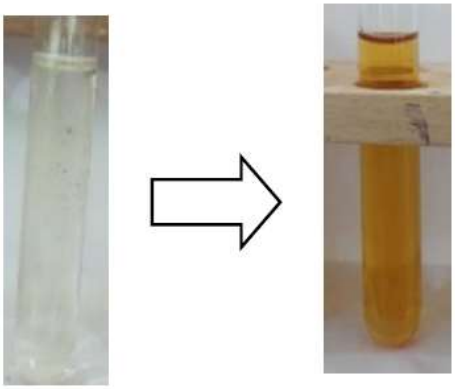

Figure 3: Color of Silver Nanoparticles Solution.

The process of nanoparticles formation is carried out by adding 1 mL of Arabica coffee leaf extract to a 10 mM $AgNO_3$ solution in a 1:35 ratio. This volume of extract is chosen to balance the amount of secondary metabolites with the concentration and composition of the $AgNO_3$ solution, compared to using a larger volume of leaf extract. The Arabica coffee leaf extract contains secondary metabolites such as alkaloid, phenolic, flavonoid, saponin, and terpenoid, which function as reducing agents to convert silver ions (Ag^{+}) into uncharged silver (Ag^{0}). This is because these secondary metabolites have hydroxyl (-OH) groups. Additionally, these secondary metabolites help stabilize the silver nanoparticles to prevent easy aggregation. The formation of silver nanoparticles is indicated by an increase in wavelength and absorbance of the nanoparticles over time. The mechanism of silver nanoparticles formation using a bioreductor can be seen in Figure 4.

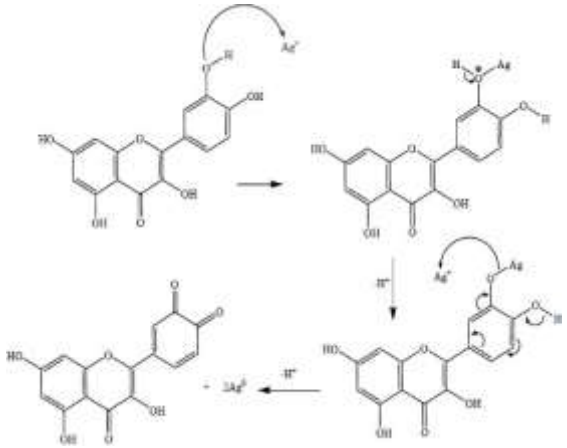

Figure 4: Mechanism of Silver Nanoparticles Formation from Arabica Coffee Leaf Extract.

Morphology of Silver Nanoparticles

The morphology of silver nanoparticles was characterized using Scanning Electron Microscope – Energy Dispersive X-Ray (SEM-EDX) at a magnification of 5 µm, with the complete results shown in Figure 5. The SEM-EDX analysis of the sample indicates that the silver nanoparticles are predominantly crystalline in shape. The varying particle shapes can be

influenced by factors such as the concentration of AgNO3 solution, the compositionof the AgNO3 solution, the amount of extract, and the reaction time. However, the most important factor is the secondary metabolites present in the Arabica coffee leaf extract, which act as capping agents. A higher amount of secondary metabolites leads to greater stability in the size of the produced silvernanoparticles (Yusof *et al.*, 2018).

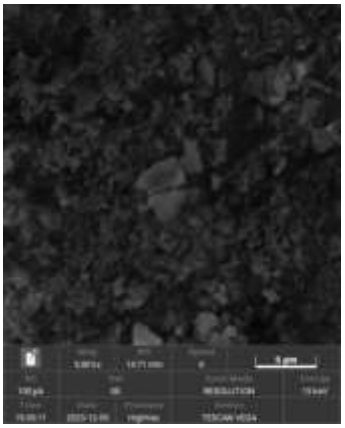

Figure 5: Morphology of Silver Nanoparticles.

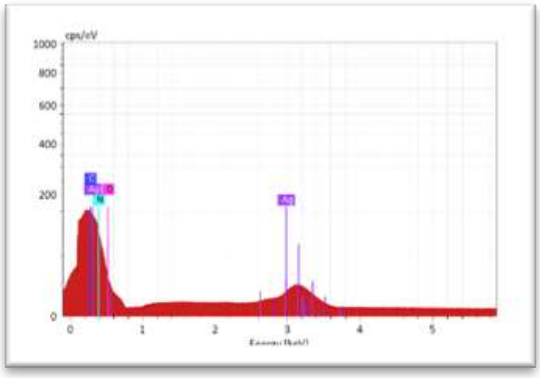

Figure 6: Energy of Silver Nanoparticles.

Furthermore, EDX is used to determine the composition of materials contained within silver nanoparticles. Figure 6 shows the EDX spectrum of silver nanoparticles synthesized using Arabica coffee leaf extract, indicating the presence of silver (Ag). Figure 6 confirms the presence of silver (Ag) with an absorption peak value at an energy level of 2.9 – 3 keV. Typically, metallic silver nanocrystals appear at an absorption peak value of 2.938 keV due to Surface Plasmon Resonance (SPR) (Taqhizadeh *et al.*, 2018). SPR occurs due to the interaction between light and the induced electrons from silver atoms (Ag). These electrons can be polarized due to the influence of the electric field from the light. Additionally, SPR can be affected by the type of material, size, shape, and density of the nanomaterial. Other material compositions found in the silver nanoparticle sample include carbon, nitrogen, and oxygen. The material composition of the silver nanoparticles is presented in Table 4.

Table 4: Material Composition of Silver Nanoparticles.

Material	Percentage Mass of Silver Nanoparticles (%)
Silver (Ag)	21,44
Nitrogen (N)	19,27
Carbon (C)	7,12
Oxygen (O)	5,76

Size and Zeta Potential of Silver Nanoparticles

The Particle Size Analyzer (PSA) is used to determine the particle size and zeta potential values of the sample. The size of the nanoparticles ranges from 1 to 100 nm (Oktavia and Sutoyo, 2021). The measurement results are presented in Table 5.

Table 5: Size and Zeta Potential Silver Nanoparticles.

Size(nm)	Potensial Zeta (mV)	Polydispersity Index(PI)	Area(%)
35,71	-14,34	0,5078	100

The obtained result for the particle size of the silver nanoparticles is 35.71 nm, indicating that nanoscale size has been achieved. The polydispersity index (PI) represents the distribution of particle sizes formed. According to Nengsih et al., (2013), a PI value greater than 1.0 indicates that the particle size distribution is non-uniform, while a PI value less than 1.0 indicates a uniform particle size distribution. The result shows a PI value of 0.5078, meaning that the particle size formed is uniform (homogeneous). Additionally, the zeta potential value represents the surface charge of a particle. Measuring the zeta potential aims to estimate the surface charge of the colloid solution and can indicate the stability of nanoparticles in the colloid (Khatun et al., 2023). The obtained zeta potential value is -14.34 mV, indicating that the stability of the silver nanoparticles is considered good. The size and zeta potential values can be influenced by the reaction kinetics. The parameters of reaction kinetics during the synthesis of silver nanoparticles include the concentration of AgNO3 solution, the composition of AgNO3 solution, and the plant extract.

Antioxidant Activity

Antioxidant activity can be assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test method, which is based on a substance's ability to scavenge free radicals. Antioxidant activity is tested using a UV-Vis spectrophotometer. The wavelength used is 516.5 nm, which is determined by measuring the maximum absorbance of DPPH. The blank used is methanol, which serves as a calibration solvent or for correcting the absorbance measurements. The control used is 0.1 mM DPPH in methanol. Methanol is chosen because it is a suitable solvent for DPPH, as it does not interfere with the DPPH reaction or absorbance measurement. The results of the antioxidant activity measurements using the spectrophotometer are presented in Table 6 and 7.

Table 6: Antioxidant Activity of Silver Nanoparticlesfrom Arabica Coffee Leaf Extract.

Concentration (ppm)	Absorbance	%Inhibition	IC50 (ppm)	Antioxidant Activity
50	0,2460	32,3060		Very Strong IC50 <50 ppm (Tristiantini, 2016)
100	0,2409	33,7094	10,4233	
150	0,2303	36,6263		
200	0,2267	37,6169		
Control	0,3634	-		

Table 7: Antioxidant Activity of Arabica Coffee LeafExtract.

Concentration (ppm)	Absorbance	%Inhibition	IC50(ppm)	Antioxidant Activity
50	0,2736	24,7111		Very Strong
100	0,2673	26,4447		IC50 <50 ppm (Tristiantini, 2016)
150	0,2564	29,4441	13,3828	
200	0,2525	30,5173		
Kontrol	0,3634	-		

The sample of silver nanoparticles from Arabica coffee leaf extract exhibits a better inhibition percentage compared to the Arabica coffee leaf extract without silver nanoparticles, as shown in Table 6 and 7. The antioxidant activity measured by IC50 values, shows that the silver nanoparticles from Arabica coffee leaf extract have an IC50 of 10.4233 ppm, while the Arabica coffee leaf extract without silver nanoparticles has an IC50 of 13.3828 ppm. Based on these results, both the silver nanoparticles from Arabica coffee leaf extract and the Arabica coffee leaf extract without silver nanoparticles fall into the very strong category. This is influenced by the presence of antioxidant compounds such as alkaloids, phenolics, flavonoids, saponins, and terpenoids. Additionally, the nano-scale size significantly enhances its ability to capture free radicals, resulting in a greater reduction of free radicals by the antioxidant compounds. The mechanism of free radical scavenging by silver nanoparticles from Arabica coffee leaf extract is illustrated in Figure 7.

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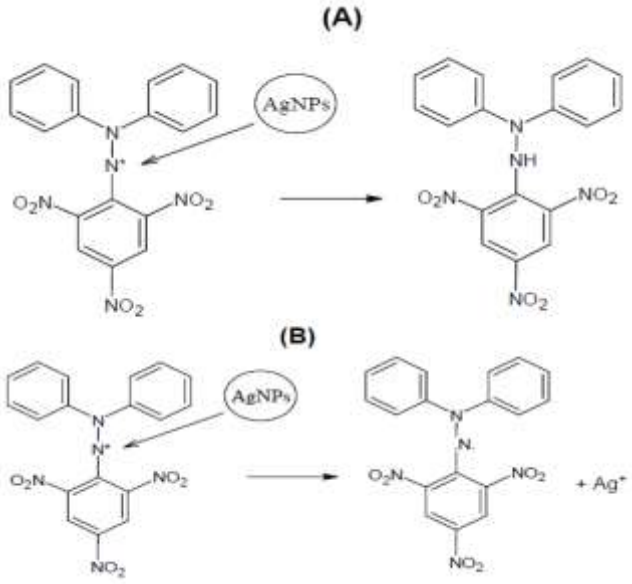

Figure 7: Mechanism of DPPH Radical Scavenging by Silver Nanoparticles.

The reaction depicted in Figure 7 involves the donation of electrons and hydrogen atoms from the antioxidant substance, which in this case is silver nanoparticles synthesized using Arabica coffee leaf extract to the DPPH radical. Silver nanoparticles have antioxidant potential because they can donate an unpaired valence electron to the DPPH radical. In Figure 7a, after the stabilizing agent in the silver nanoparticles donates a hydrogen atom to the DPPH radical, the DPPH radical becomes stabilized. This stabilization occurs because the free electron on the DPPH radical pairs up with the hydrogen atom donated by the stabilizing agent in the silver nanoparticles from the Arabica coffee leaf extract. In Figure 7b, after the silver nanoparticles donate a valence electron to the DPPH radical, the radical becomes stabilized. This stabilization is due to the donation of a single electron from the silver nanoparticles made from Arabica coffee leaf extract.

CONCLUSION

The research concluded that silver nanoparticles were successfully synthesized using Arabica coffee leaf extract. The characteristics of the synthesized nanoparticles showed are crystalline with a composition of silver (21.44%), nitrogen (19.27%), carbon (7.12%), and oxygen (5.76%), average size to be 35.71 nm, and they have a zeta potential of -14.34 mV, suggesting good stability, and demonstrated enhanced antioxidant effectiveness with an IC50 value of 10.4233 ppm, outperforming the extract alone, which had an IC50 value of 13.3828 ppm.

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