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Antioxidant and Antibacterial Activities of Silver Nanoparticles Biosynthesized by Leaf Foeniculum vulgare Mill. Ethanolic Extract

Nindita Clourisa Amaris Susanto[1,*], Dian Eka Ermawati[1], Sholicha Rohmani[1], Fitria Lidini Hanifah[1], Fadhila Dian Nafisa[1], Echa Hana Prastia[1], Siti Nurkhasanah[1], Anis Laela Megasari[2], Aptika Oktaviana Trisna Dewi[3]

[1] Department of Pharmacy, Vocational School, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[2] Departement of Nurse Anesthesia, Vocational School, Universitasf Sebelas Maret, Surakarta, Central Java, Indonesia.
[3] Department of Pharmacy, Politeknik Indonusa, Surakarta, Central Java, Indonesia.

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Corresponding Author:
Nindita Clourisa Amaris Susanto
nindita_clourisa@staff.uns.ac.id

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Abstract: Silver nanoparticles can be synthesized using biosynthesis methods assisted by plant extracts and can be applied as antibacterials. Hence, this study aimed to synthesize silver nanoparticles from different concentrations of $AgNO_3$ precursor solutions using ethanol extract from fennel leaves. The influences of various $AgNO_3$ concentrations and the volume ratios of $AgNO_3$ solutions and leaf extracts on the activities were observed. The samples were investigated using a UV-Vis spectrophotometer, and the Surface Plasmon Resonance (SPR) absorbance was attained at 410-415 nm, representing the silver nanoparticle formation. The findings showed that the optimal ratio of precursor solution and extract was 10:1. In contrast, the optimal temperature was at 70 ° C. The size of nanoparticles was estimated using the Particle Size Analyzer (PSA), exhibiting that the different $AgNO_3$ concentrations did not significantly change their sizes. The yields demonstrated that the antioxidant activities for nanoparticles synthesized with $AgNO_3$ concentrations of 0.001 and 0.005 M were 153.56 ± 0.09 µg/mL and 0.005 M 111.42 ± 0.15µg/mL, respectively. The results showed higher inhibition zones of antibacterial activity obtained by silver nanoparticles with 0.005 M $AgNO_3$ as compared to those of 0.001 M $AgNO_3$.

Keywords: Antibacterial; Antioxidants; Fennel leaves; Silver nanoparticles.

Introduction

The synthesis of silver nanoparticles can be done through physical and chemical techniques. However, these techniques generate harmful substances and require high temperatures, making them environmentally unfriendly and expensive (Yousaf et al., 2020). Both methods can be replaced by biosynthesis methods using plant extracts. Plant extracts can be used as bioreductors working as reducing agents for Ag+ ions in solution to Ag0 (Adeyemi et al., 2022; Gayesa et al., 2o24; Selvam et al., 2024). The use of plant extracts provides advantages, as the properties of plants can also be combined and found in nanoparticles. Plant phytochemical compounds provide antioxidant and antibacterial activity so that both activities can improve the properties of nanoparticles (Keshari et al., 2020).

Silver nanoparticles pose no toxicity to eukaryotic cells, yet they are highly toxic to prokaryotic cells like microorganisms such as bacteria (Mirda et al., 2021). They have unique chemical, optical, mechanical, and magnetic characteristics. Therefore, silver nanoparticles have been extensively studied for their applications as antibacterials (Chandhru et al., 2022; Daphedar et al., 2024), antioxidants (Khan et al., 2023), and colorimetric detection (Yusnaidar et al., 2023).

A number of plants have been identified as sources for silver nanoparticle synthesis, including carrots

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(Fareed et al., 2023). These plants contain phytochemical compounds such as flavonoids that can bind and decline metal ions in nanoparticles because of their large number of hydroxyl and carbon groups (Adeyemi et al., 2022). Prior research exposed that silver nanoparticles synthesized from plant extracts showed activity in reducing radicals from 2,2-diphenyl-1-picrylhydrazyl (Ansar et al., 2020; Abdellatif et al., 2021; Abdel-Aty et al., 2023).

Foeniculum vulgare Mill. leaves also are composed of phytochemical compounds, including flavonoids, alkaloids, polyphenols, saponins, steroids, and terpenoids. Fennel leaves are reported to have antioxidant and antibacterial activity (Badgujar et al., 2014; Partonowati et al., 2021). Differing from prior research, this study synthesized silver nanoparticles AGNO3 precursor solutions using the biosynthesis method with the help of ethanol extract from fennel leaves. This study further observed the effects of various AgNO3 concentrations and the volume ratios of AgNO3 solutions and leaf extracts on the antioxidant activity and antibacterial activity of silver nanoparticles.

Method

Tool and Materials

The raw materials were Silver Nitrate (AgNO3) (Darmstadt, Germany), Mueller Hinton Agar (MHA), and fennel leaves (Foeniculum vulgare Mill.) (obtained from Selo, Boyolali, Central Java). Other materials were concentrated HCl (Merck KgaA, USA), MgSO4 (Merck KgaA, USA), Dragendroff's reagent, FeCl3 (Merck KgaA, USA), H2SO4 (Merck KgaA, USA), and paper disc (Oxoid, England). Besides, this study employed Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Staphylococcus epidermidis ATCC 12228, and Escherichia coli ATCC 25922 bacteria from the Microbiology Laboratory of the Faculty of Medicine, Sebelas Maret University.

Research Methods

Fennel Leaf Extract

Fresh fennel leaves were wet sorted, then cleaned and dried in an oven. They were powdered, and the powder was weighed as much as 250 grams and soaked in 1000 mL of 96% ethanol. The extraction was done by stirring the mixture for 3 days. The mixture was subsequently filtered through Whatman No.1 paper to isolate the filtrate and residue. The filtered fennel leaf extract was stored in the refrigerator.

Phytochemical Test

Flavonoid test

Fennel leaf extract was was placed in a small test tube, and $MgSO_4$ (magnesium sulfate) was added. 1 mL of HCl (hydrochloric acid) was then transferred to the test tube. A positive reaction if the color changes to orange, green, yellow, or red points to the existances of flavonoids (Handarni et al., 2020).

Alkaloid test

A few drops of reagent, Dragendroff reagent, were poured into 1 mL of extract. Positive results indicating the existence of alkaloids are represented by the formation of orange to reddish-brown deposits (Handarni et al., 2020).

Saponin test

0.5 mL of extract was transferred into a test tube, and 5 mL of boiling aquadest was introduced into it. The tube was shaken for 60 seconds. A positive result indicates saponin exists when a stable foam is formed with a height of about 1 cm (Handarni et al., 2020).

Phenol test

The extract is poured into a small test tube, and 3 drops of 1% $FeCl_3$ (iron (III) chloride) are poured. If the colors become purple, green, or blue, it denotes the presence of phenol (Handarni et al., 2020).

Steroid/Terpenoid Test

5 mL of extract was placed in a test tube, and 2 mL of chloroform was added and homogenized. Then, Salkowsky's reagent or concentrated H_2SO_4 (sulfuric acid) was added. Positive steroid/terpenoid results are revealed if a red ring is formed (Handarni et al., 2020).

Optimization of Volume Ratio and Temperature of Silver Nanoparticle Synthesis

$AgNO_3$ solutions with concentrations of 0.001 M and 0.005 M were given in measuring flasks. The solutions and fennel leaf extract were then mixed in beakers with volume ratios (mL: mL) (AgNO3:leaf extract) of 8.5:2.5, 9:2, 9.5:1.5, 10:1, and 10.5:0.5 mL. The mixtures were homogenized with a magnetic stirrer for 60 minutes.

The optimum volume ratio obtained was then used for temperature optimization of the silver nanoparticle synthesis. The temperature variations used in this study were 30, 60, 70, and 80ºC. The resulting silver nanoparticle colloid was then assessed with a UV-VIS spectrophotometer in the wavelength range of 300–800 nm. The optimum volume and temperature ratio were applied for the synthesis of silver nanoparticles and continued for antioxidant and antibacterial tests.

Antioxidant Test

10 mg of silver nanoparticles were mixed with 10 ml of ethanol in a 50 mL measuring flask, and the ethanol volume was added until the limit mark (1000

ppm). Then, a series of solutions were made with concentrations of 12.5, 25, 50, 75, and 100 ppm. The solutions with these concentrations were poured into 10 ml measuring flasks, and ethanol was added to the limit mark. For antioxidant testing, 3 ml of each solution was added to 2 ml of 0.05 mM DPPH and then left for 30 minutes in a dark place. The absorbance was observed at maximum wave (Bedlovičová et al., 2020). The results of antioxidant determination were compared with ascorbic acid as a positive control.

IC50 was calculated by plotting the sample concentration against the percentage of inhibition on the x and y axes, respectively, using a linear regression model. The IC50 value is expressed by the y value of 50, and the x value to be attained is considered IC50. The IC50 value denotes the concentration of the solution required to decrease DPPH free radicals by 50%.

Antibacterial Test

Antibacterial testing was conducted using the disc technique, and the diameters of the inhibitory power against Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, and Escherichia coli bacteria were measured. The prepared MHA media was transferred into a petri dish and let to solidify. The bacterial suspension was inoculated into the solidified MHA using the swab method. Separately, 6 mm diameter paper discs were moistened with silver nanoparticle solutions with a concentration of $AgNO_3$ of 0.001 M and 0.005 M, each as much as 20 µL, and placed on the MHA media. The media is incubated in an incubator at 37°C for 24 hours, and the diameter of the inhibition zone (clear zone) is determined with a vernier caliper (Nurhayati et al., 2020).

Result and Discussion

Extraction was performed using the maceration technique with 96% ethanol as the solvent. Maceration is a technique that isolates or takes active components from solutions of solids of raw materials by soaking the materials with solvents. During the maceration, the cell walls and membranes are disrupted because of the pressure imbalance inside and outside the cell, causing secondary metabolites in the cytoplasm to dissolve in organic solvents (Chairunnisa et al., 2019). The yields of the maceration process in this study were green liquid extracts of fennel leaves. According to the results of the phytochemical test, the liquid extracts of fennel leaves were positive for the flavonoid, alkaloid, saponin, phenol, and steroid/terpenoid compounds.

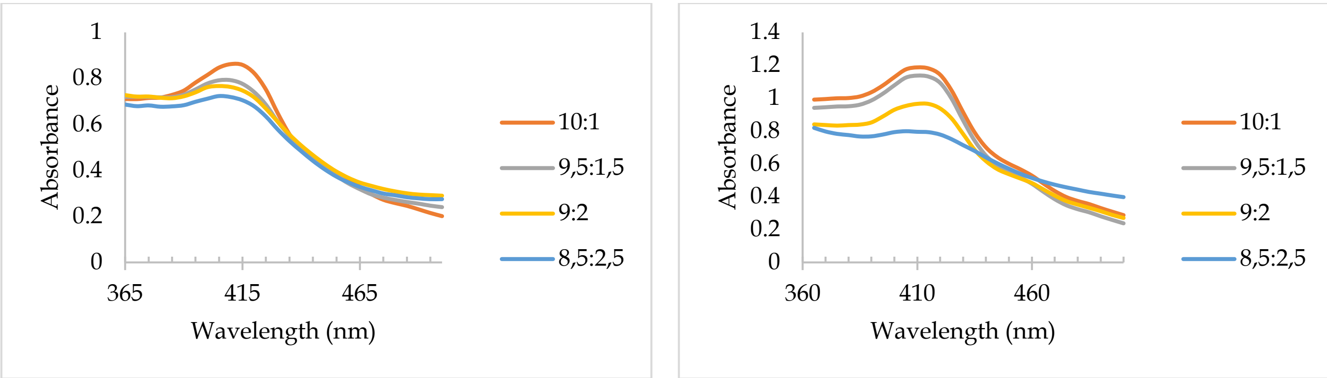
UV-Visible Study of the Effect of AgNO3 Solution Volume Comparison with Extract and Temperature on Silver Nanoparticle Synthesis

The characteristic features of silver nanoparticles are revealed by the presence of pronounced and broad peaks in the visible spectrum called SPR. The findings revealed that the maximum wavelength range of silver nanoparticles was about 410-415, which aligns with prior research. Dwiastuti et al. (2020) stated that the formation of silver nanoparticles occurred with the emergence of SPR intensity at a wavelength of 400-450 nm.

Silver ions were sourced from a silver nitrate ($AgNO_3$) solution at varying concentrations of 0.001 M and 0.005 M reacted with fennel leaf extracts. The results demonstrated that the higher concentration of silver nitrate induced a faster reduction process. This is because the higher concentration induces more Ag ions in the solution, so natural reducers easily interact or bind with Ag ions (Selvam et al., 2024). Silver nanoparticles can be synthesized with bioreductors due to the existence of hydroxyl groups in plant extracts, which contribute to the reduction of Ag^+ to Ag^0 and as a capping agent (Adeyemi et al., 2022). The nanoparticle fabrication with fennel leaf extract bioreductor resulted in a solution with a color change from green to brownish yellow (Figure 1). The color change indicates that silver nanoparticles have been formed (Gayesa et al., 2024).

Figure 1. Intermediate Solution in Silver Nanoparticle Synthesis

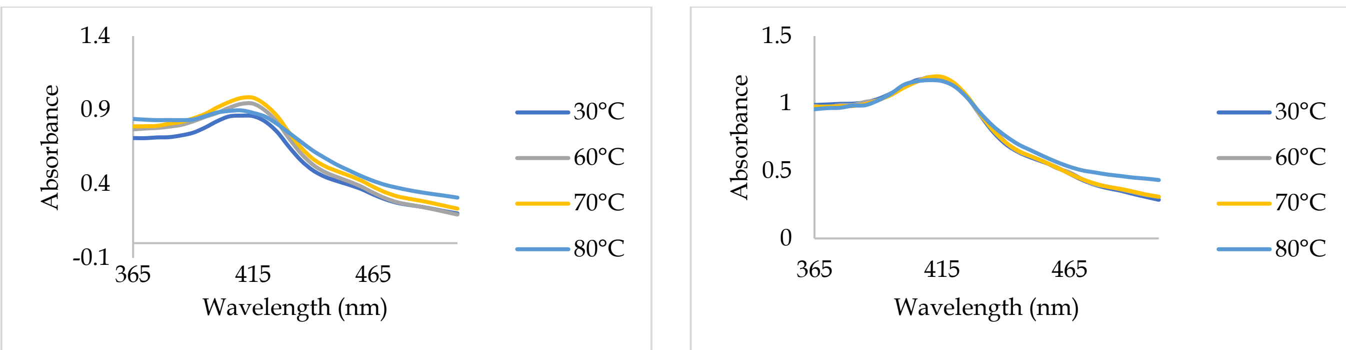
The maximum absorptions of the prepared nanoparticles with various volume ratios of AgNO3 solution and fennel leaf extract of 10:1, 9.5:1.5, 9:2, and 8.5:2.5 were at wavelengths of 410 and 405 nm, and the highest absorbance was produced at a ratio of 10:1 (Figure 2). High absorbance values indicate a greater number of silver nanoparticles formed (Yusnaidar et al., 2023). When the extract volume is added to the solution composition, the absorbance value decreases due to more organic compounds surrounding the nanoparticles, which causes the particles to settle in huge quantities. Hence, the characteristic peaks of the SPR do not emerge in the desired wavelength range (Dwiastuti et al., 2020).



(a) (b)

Figure 2. UV-Vis spectra of silver nanoparticles with various volume ratios of AgNO3 solution and fennel leaf extract and AgNO3 solution concentrations of (a) 0.001 M and (b) 0.005 M

Temperature is a key factor that can accelerate the reaction rate. In this study, temperatures were varied to obtain the optimal value for the silver nanoparticle synthesis. The yields exhibited that higher temperatures produced faster color changes in the solution. Hence, it can be said that the higher temperature induces the faster silver nanoparticle formation (Liu et al., 2020).



(a) (b)

Figure 3. UV-Vis spectra of the samples with distinct reaction temperatures and AgNO3 concentrations of (a) 0.001 M and (b) 0.005

Figure 3 presents the impacts of distinct reaction temperatures on the UV-Vis spectra of the samples with 0.001 and 0.005 M of AgNO3. All samples demonstrated improved absorbance values as the temperature increased. The highest absorbance value was obtained when the reaction during the synthesis was carried out at 70°C. The increasing absorbance value denotes the greater number of silver nanoparticles formed (Razali et al., 2022). However, at 80°C, the absorbance value decreased because excessive temperature could damage the active compounds in the extract (Bere et al., 2019). Thus, the optimal temperature for the synthesis reaction in this study was 70°C.

The compounds suggested to contribute to the biosynthesis process in this study are flavonoid and phenol since they have carbonyl and hydroxyl groups and high levels in fennel leaf extract (Pambudi et al., 2017). Phenolic compounds have hydroxyl groups that can bind metals because they have large nucleophilic properties from the aromatic ring. Meanwhile, flavonoids are a significant group of polyphenolic compounds that strongly bind and reduce metal ions into nanoparticles (Fajri et al., 2022). A reduction reaction occurs between Ag^+ ions and polyphenol compounds in which Ag^+ ions as reactants act as catalysts. Polyphenol compounds undergo a group change to RO- from the R-OH groups. The RO-Ag group is created by the binding of Ag^+ ions. In this reaction, the polyphenol chain will break because the bound and released Ag^+ ions form Ag° (silver nanoparticles). The mechanism of the nanoparticle formation is illustrated in Figure 4.

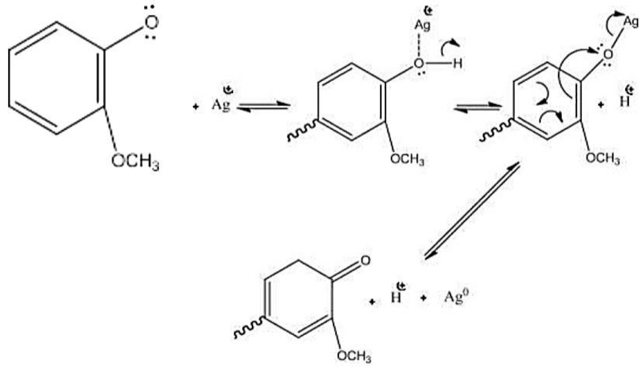


Figure 4. Mechanism of Silver Nanoparticle Formation

Silver nanoparticles obtained from optimal conditions were subjected to particle size measurement using PSA. The results were data on particle size and polydispersion index, presented in Table 1. It reveals that different silver precursor concentrations affected the sizes of silver nanoparticles. Higher precursor concentrations provide larger particle sizes. The polydispersion index values of the samples are in the range of 0.08 - 0.7. This range is the upper range where the distribution algorithm operates appropriately. The polydispersion index > 0.7 indicates an extensive distribution of particle size, allowing sedimentation to occur (Karim et al., 2021).

Table 1. Particle Size and Polydispersion Index of nanoparticles

Sample	Size (nm)	PDI
Silver Nanoparticles from Fennel Leaf Extract (0.001 M)	104.7	0.206
Silver Nanoparticles from Fennel Leaf Extract (0.005 M)	105.3	0.217

Antioxidant Test

The DPPH (2,2-diphenylpicrylhydrazyl) free radical scavenging assessment was employed to evaluate the antioxidant activity of the synthesized silver nanoparticles, using ascorbic acid as a reference. The color of DPPH will alter from purple to yellow when it is reduced (Geyesa et al., 2024).

Table 2 shows the IC50 values of ascorbic acid, fennel leaf ethanol extract, and silver nanoparticles. The lower IC50 value indicates higher antioxidant activity. This study found that the antioxidant activity of leaf extract was lower than that of silver nanoparticles. Conversely, the IC50 values of silver nanoparticles with both AgNO3 concentrations were lower than ascorbic acid. Similar results were reported by Rajivgandhi et al. (2020). They found that silver nanoparticles increased antioxidant activity and scavenged DPPH free radicals to a higher level than the extract.

Nanoparticles exhibit antioxidant performances due to the interaction of antioxidant compounds in fennel leaf liquid extract. This interaction facilitates bioreduction and the subsequent removal of silver salts. Previous studies research has demonstrated that silver nanoparticles synthesized using Zingiber officinale leaf extracts displayed strong free radical scavenging capabilities. (Wang et al., 2021). The antioxidant properties of silver nanoparticles offer several benefits over conventional antioxidant delivery systems, such as encapsulation of the antioxidant agent, enhanced bioavailability, and precise, controlled delivery.

Table 2. Antioxidant activity of the synthesized silver nanoparticles

Sample	IC50 value (µg/ml)	Antioxidant Power
Ascorbic Acid	5.51 ± 0.02	Very strong
Fennel Leaf Extract	292.61 ± 0.11	Weak
Silver Nanoparticles with 0.001 M AgNO3	153.56 ± 0.09	Weak
Silver Nanoparticles with 0.005 M AgNO3	111.42 ± 0.15	Moderate

Antibacterial Test

The antibacterial activity of silver nanoparticles towards Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli bacteria was tested, and the results were indicated by the inhibition zones demonstrated. The antibacterial activity is categorized as weak, moderate, strong, and very strong if the inhibition zone diameters are ≤ 5 mm, 6-10 mm, 11-20 mm, and ≥ 21 mm, respectively (Geyesa et al., 2024).

The results of the antibacterial test in this study are shown in Table 3. It reveals that the inhibition zones were different for each bacteria and affected by distinct concentrations of $AgNO_3$. Based on Table 3, the inhibition zones against the bacteria demonstrated by silver nanoparticles with 0.005 M $AgNO_3$ were higher than those presented by 0.001 $AgNO_3$. It can be considered that the higher $AgNO_3$ concentration produces more silver particles contained in the solution, resulting in a larger inhibition zone (Suryadi et al., 2022). Further, the higher $AgNO_3$ concentration can improve the antibacterial activity of the silver nanoparticles formed.

This potential antibacterial activity is thought to originate from the tendency of silver nanoparticles to release silver ions. Because silver ions have reactive properties, they can damage proteins and nucleic acids from cell membranes, thereby triggering DNA damage (Keshari et al., 2020; Selvam et al., 2024). Hence, the higher concentration of the $AgNO_3$ precursor solution can induce a larger bacterial inhibition zone. From this study, it can be infered hat silver nanoparticles from ethanol extract of fennel leaves demonstrate antibacterial activity.

Table 3. Antibacterial activity against various bacteria a (S.epidermidis), b (S.aureus), c (P.aeruginosa), d (E.coli)

Silver nanoparticles with different AgNO3 concentrations	Inhibition zones against Bacteria (mm)			
	a	b	c	d
0.001	5.83 ± 0.03	6.13 ± 0.22	6.28 ± 0.15	4.13 ± 0.13
0.005	11.4 ± 0.52	7.89 ± 0.23	7.21 ± 0.14	6.26 ± 0.20

Conclusion

Silver nanoparticles can be obtained from the biosynthesis method using a fennel leaf ethanol extract as a bioreductor. The ratio of $AgNO_3$ solution as a precursor and fennel leaf ethanol extract and temperature plays a vital role in controlling the absorbance intensity of silver nanoparticles. The absorbance peaks of silver nanoparticles with 0.001 M and 0.005 $AgNO_3$ were obtained at 410-415 nm, categorized in the SPR range of silver nanoparticles. Silver nanoparticles produced in this study have exhibited antioxidant activity and antibacterial activity. Further, the yields revealed that different concentrations of $AgNO_3$ precursor solutions affected the antioxidant activity and antibacterial activity.

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Author Contributions

Conceptualization, N.C.A.S.; D.E.E. and S.R.; methodology, N.C.A.S.; F.L.H.; F.D.N.; E.H.P.; amd S.N.; writing—original draft preparation, N.C.A.S.; writing—review and editing, A.L.M. and A.O.T.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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