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Nanosilver Synthesis of Bromelain Isolate from Pineapple Extract: A Green Approach for Antibacterial Applications

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KEYWORDS

Bromelain isolate; Silver nanoparticles; Antibacterial;

ABSTRACT:

Introduction: Silver nanoparticle technology has high bactericidal activity because silver ions can affect bacterial cell death, as well as nanoparticle size, which can increase drug bioavailability.

Objectives: This study aims to synthesize and characterize silver nanoparticles from bromelain isolate of pineapple fruit and test the antibacterial activity of silver nanoparticles.

Methods: Isolation was carried out to obtain bromelain enzyme isolates; isolates were tested for calculating bromelain content by spectrophotometry. The biosynthesis of silver nanoparticles was made by varying the number of isolates, silver nitrate solution, and PVA by ultrasonication method. Furthermore, characterization was carried out by visual observation, observation of wavelength, determination of particle size, polydispersity index, zeta potential, determination of functional groups by FTIR, and morphological evaluation by SEM. Then, the antibacterial activity of the diffusion method was tested against Staphylococcus aureus bacteria, and five formulations were obtained.

Results: The formation of nanoparticles was characterized by a change from colourless to brownish yellow, a wavelength of 537.0 nm, and an absorbance of 1.276. The particle size was 92.90 nm ± 1.15, the polydispersity index value was 0.379±0.028, and the zeta potential was –38.8 mV ± 0.64. The functional group formed indicates –OH, C=O, and N=O at wave numbers 3325.04, 1634.55, and 1370.06 cm^{-1}. It has a uniform cuboidal morphology. The results of the antibacterial activity test showed a clean and clear inhibition zone of 13.7 mm ± 1.5 against Staphylococcus aureus bacteria.

Conclusions: Silver nanoparticles synthesized by bromelain isolate of pineapple fruit have the requirements for suitable silver nanoparticles and have antibacterial activity.

1. INTRODUCTION

The incidence of antibacterial resistance is a health threat in the 21st century because it can reduce the effectiveness of antibiotics to treat infections [1]. It is estimated that by 2050, if new drugs are not developed, effective antibiotics will no longer be available, so alternative treatments are needed. The method commonly used to make antibiotics is bacterial or fungal fermentation, but preparation is complicated and takes a long time [2]. Resistance treatment can be carried out using readily available compounds with good antibacterial activity by increasing their conductivity effectively towards the target [3].

Natural-derived products have good potential and function as bioactive compounds that are effective as antibacterial agents [4]. Pineapple (*Ananas comosus* L. Merr.) contains lots of flavonoids, saponins, tannins and bromelain enzymes with aromatic hydroxyl groups, which have antibacterial properties [5]. Research by Loon et al. showed that the bromelain enzyme from pineapple extract was able to interact with the bacterial cell wall, affecting the permeability of the peptidoglycan cell wall and causing bacterial death [6]. Supported by

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Hidayat et al., the liquid extract of pineapple produces an inhibition zone of 15.5 - 20 mm on S. aureus, so it is suitable for antibacterial agents [7].

The development of superior nanotechnology in nanoparticles that can deliver drugs at an optimal dose range. Nano size can increase the therapeutic efficiency of drugs, reduce side effects, and improve patient compliance[8]. This type of metal nanoparticle can bind to protein molecules in microbial cells, disrupting metabolic activity and causing microbial death [9]. Chemical reducing agents form silver nanoparticles, but these compounds are more toxic, so they are overcome by the green synthesis method with a natural reducing agent, namely bromelain isolate from pineapple fruit [10].

Silver nanoparticles have previously been studied and developed as antibacterial, and pineapple fruit has been proven to be effective in killing microbes. Still, the combination of the two in strengthening the antibacterial effect has never been reported. Based on the above background, new research was carried out, namely the biosynthesis and characterization of silver nanoparticles from the bromelain isolate of pineapple (*Ananas comosus* L. Merr) as an antibacterial.

2. METHODS

Materials

Pineapple (*Ananas comosus* L. Mer.) from Ponggok plantation, Kediri, East Java, Indonesia, has a harvest age of 19 months. Aquades were purchased from OneMed (Sidoarjo, Indonesia). Staphylococcus aureus ATCC 2593 bacteria (Culti-Loops). Potassium phosphate monobase, NaOH, polyvinyl alcohol (PVA) 2.5%, Nutrient broth, Nutrient agar, Silver nitrate (AgNO3) (Merck) (Darmstadt, Germany). Blank disc paper and ciprofloxacin disc paper (Macherey Nagel). Sterile 0.9% NaCl were purchased from PT Widatra Bakti (Semarang, Indonesia). Biuret reagents were purchased from the ROFA Laboratorium Center (Bandung, Indonesia). Mc Farland standard (Himedia) Himedia Vivantis Phytotech.

Isolate Pineapples preparation

Pineapple fruit was mashed as much as 1,500 g, then mashed, squeezed, and filtered to obtain a clear liquid and stored at 4℃. Furthermore, 50 ml of clear pineapple liquid was separated with 50 ml of pH seven buffer, a total of 100 ml and stored at 4℃ for 3 hours. To the filtrate, 40% NaCl was added while stirring using a magnetic stirrer for 45 minutes and incubated overnight at 4℃ and then filtered with Whatman filter paper. The residue obtained was dried in a freezer dryer for 6 hours [11].

Synthesis Silver Nanoparticle

Methods for the synthesis of silver nanoparticles using high energy. Prepared pineapple isolate, then put it in a microtube. Taken with a silver nitrate micropipette with a volume according to Table 1, then put into a microtube that already contains pineapple bromelain isolate according to the predetermined formulation. Then, ultrasonication was carried out at a temperature of 30℃ with a pulser of 40 for approximately 4 minutes for each microtube. To speed up the reduction reaction, the five solutions of the formula can be incubated in a water bath at 50℃ for 5 hours.

Table 1. Silver Nanoparticle Formulation

Formulation	$AgNO_3$ (µL)	Pineapple bromelain isolation 8% (µL)	PVA (µL)
Formula 1	350	950	100
Formula 2	400	900	100
Formula 3	500	800	100
Formula 4	600	700	100
Formula 5	700	600	100

Charactheristics Silver Nanoparticle

Visual Observation of Color Change

Visual observations were carried out after 24 hours of making silver nanoparticles on the preparation results by seeing the colour change to brownish-yellow [12].

Observasi Panjang Gelombang Serapan UV-Vis

Measurement of wavelength using UV-Vis spectrophotometry: a solution of silver nanoparticles is poured into a quartz cuvette with an optical path length of 1 cm and read at a wavelength of 300-800 nm, and then the absorbance value is seen [13].

Determination of Particle Size, Polydyspesity Index and Zeta Potential with Particle Size Analyzer

Determination of particle size was carried out using a particle size analyzer (Horiba Scientific, Nano Particle Analyzer SZ-100) with variations in size results from the

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ratio of AgNO3 Zeta potential was analyzed using a zeta sizer. The preparation made is dissolved in water and then put into a cuvette. Then, the cuvette containing the sample is inserted into the holder, and the zeta potential menu for analysis is selected [14].

Functional Group Observation of Silver Nanoparticles with Fourier Transform Infrared

Samples were measured in the spectral range of 4000-400 cm^{-1} using Fourier transform infrared/FTIR (UATR Spectrum Two Perkin Elmer). Sample measurement was carried out using kBr pellets from kBr powder, which were pressed first and then dripped liquid samples so that the results could be read [15].

Morphological Observation of Silver Nanoparticles with Scanning Electron Microscope

The preparation of silver nanoparticles in the form of liquid was dried first with a freeze dryer; then, the dried samples were ready to be analyzed. SEM analysis was performed to evaluate the morphological visual images of AgNP biosynthesis. Samples were read using a scanning electron microscope/SEM (Phenom Pro X) in full BSD mode to determine the general morphology.

Antibacterial Activity Test

The antibacterial activity test was carried out using the diffusion method with three replications using nutrient agar (NA) and nutrient broth (NB) media. Diffusion begins with the transfer of the bacterial suspension to a petri dish medium, levelled on the surface with a sterile spreader. Blank disc paper, Ciprofloxacin antibiotic paper, paper containing silver nanoparticles and pineapple bromelain isolate were taken for five formulations. The paper is placed on the surface of the media. The placement of the paper discs must be arranged and spaced from one another. Then, it was incubated at 36°C for 24 hours. Then, the petri dish is taken from the incubator. The last step is to observe the results with a Scan-500 tool, which automatically measures the inhibition zone formed in the petri dish.

3. RESULTS

Pineapple Bromelain Isolate

Pineapple fruit filtrate was taken as much as 100 mL, and 50 mL of pH seven buffer was added. After that, it was stored at 4°C for 3 hours. Then 50 mL of 40% NaCl was added and stirred with a magnetic stirrer for 45 minutes, then stored at 4°C for 24 hours [11]. Then, the solution was dried with a freeze dryer for 8 hours, obtaining 38.275 g of pineapple fruit isolate powder with a characteristic white powder.

Visual Observation of Color Change

Visual observation of bromelain isolate silver nanoparticles from pineapple fruit was carried out by observing the colour changes of the entire formula that occurred at 0 minutes, 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours. The purpose of this observation is to qualitatively determine the formation of silver nanoparticles with a colour change. Samples that have been formed into silver nanoparticles will experience a colour change from a colourless solution to brown or brownish yellow [12]. The colour change in silver nanoparticles can be seen in Figure 1. The colour change that occurs indicates silver nanoparticles have been formed, from colourless to yellowish to brownish yellow [16]. Bromelain compounds from pineapple isolates play a role in reducing Ag+ à Ag0 [17]. The difference in the time of the biosynthesis process in each formula was due to variations in the number of pineapple bromelain isolates and silver nitrate. The absence of precipitation indicated that formula 1 to formula 5 remained stable in storage for 24 hours.

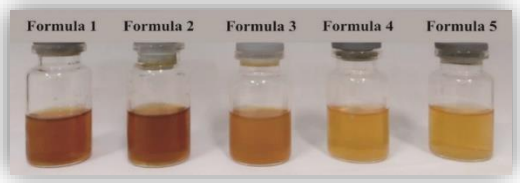

Figure 1. Visual observation of silver nanoparticles of pineapple bromelain isolate

Determination of Particle Size, Polydispersity Index, and Zeta Potential with Particle Size Analyzer

Observation of silver nanoparticle size of pineapple bromelain isolates using Particle Size Analyzer (Horiba Scientific, Nano Particle Analyzer SZ-100). The particle size and polydispersity index values (n=3) of the five formulations can be seen in Table 2. Of the five formulations formed, the result of the smallest particle size in Formula 3 is 92.90 ± 1.15 nm, and the polydispersity index value is 0.379 ± 0.028. The value obtained has entered into a suitable particle size range for AgNP, which is 10-200 nm [18], while the excellent

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polydispersity index (PI) is < 0.7 [19]. The polydispersity index value shows the uniformity of the size distribution in the nanoparticles; the smaller the polydispersity index value, the better the size distribution. Thus, it shows that the silver nanoparticle formulation from pineapple bromelain isolate did not experience sedimentation due to the narrow size distribution and showed good physical stability [20]. Meanwhile, the zeta potential value of formulas 1 to 5 shows that silver nanoparticles have a potential zeta value of more than -30 mV; this can be interpreted as the repulsion of the charge between the dispersion particles is more significant than their attractiveness [21]. Thus, the synthesis of silver nanoparticles with bromelain-reducing compounds from pineapple fruit bromelain isolates is a stable preparation. The stability of silver nanoparticles can be seen by measuring the zeta potential value. A good zeta potential value is more favorable than +30 mV or more damaging than -30 mV [22].

Table 2. Mean ± SD Particle size, polydispersity index and zeta potential value

Sample	Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
F 1	125,20 ± 0,78	0,554 ± 0,006	- 48,2 ± 1,92
F 2	117,67 ± 1,31	0,496 ± 0,025	- 39,5 ± 0,40
F 3	92,90 ± 1,15	0,379 ± 0,028	- 38,8 ± 0,64
F 4	98,67 ± 0,82	0,418 ± 0,032	- 40,6 ± 1,31
F 5	112,27 ± 0,91	0,547 ± 0,101	- 41,0 ± 0,40

Description: F = Formula

Functional Group Observation of Silver Nanoparticles with Fourier Transform Infrared

Functional group testing needs to be done because the active group has a role in reducing Ag+ with bromelain isolate from pineapple to be able to form silver nanoparticles [23]. Spectrum results of silver nanoparticles for formula 3 and 8% pineapple bromelain isolate can be seen in Figure 2.

Figure 2. IR spectrum of silver nanoparticles of pineapple bromelain isolate

The results of the Infrared (IR) spectrum of silver nanoparticles were obtained, including the wave number 3325,04 cm^{-1} indicates a bond O–H (alcohol) and –NH (amine) [24], derived from pineapple bromelain isolate and silver nanoparticles, then there was a strain shift in the wave number 2115,53 cm^{-1} show bond stretching C–H (alkane) [25], this group has a slightly sloping graph that comes from compounds other than bromelain [26], wavenumber 1634,55 cm^{-1} indicates a bond C=O (carbonyl) from pineapple bromelain and silver nanoparticles [27], as well as bond C=C (alkane) [4], and wavenumber 1370,06 cm^{-1} indicates a bond N=O (nitrogen group) derived from silver nitrate [24].

Morphological Observation of Silver Nanoparticles with Scanning Electron Microscope

Observation with SEM aims to determine the morphology of silver nanoparticles. The formula analyzed by SEM was chosen for formula three because it is the best formula. The results of the morphology of silver nanoparticles are shown in Figure 3. From the image obtained, it can be seen that the silver nanoparticles synthesized by the pineapple bromelain isolate tended to be in the form of a cube with almost uniform size. However, visually, the measure remained varied. This is in accordance with the characteristics of silver nanoparticles that have been carried out [18]. It can also be seen that the silver nanoparticles are not aggregated because there are no lumps [28]. Thus, silver nanoparticles from pineapple fruit bromelain isolates had a good morphology and were suitable.

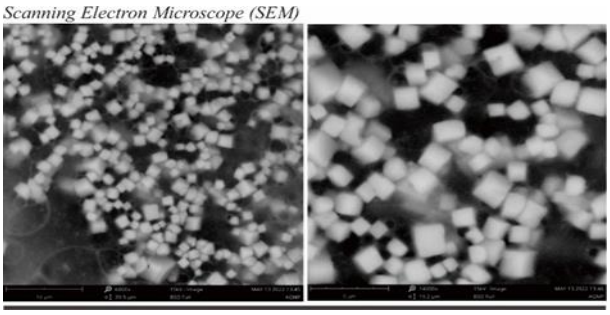

Figure 3. Morphology of silver nanoparticles isolated from pineapple fruit bromelain Scanning Electron Microscope

Antibacterial Activity Test

The results of the antibacterial activity of silver nanoparticles using the diffusion method with three replications, the five formulas compared the inhibition

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zone formed against Staphylococcus aureus ATCC 2593 with silver nitrate (AgNO3 one mM) and the antibiotic Ciprofloxacin as positive controls. These results can be seen in Table 3, and of the five formulations obtained, a clean and clear inhibition zone. The largest diameter of the inhibition zone produced by formula 2 was 13.7 ± 1.5 mm, with silver nitrate (11.9 ± 0.7 mm) and pineapple bromelain isolate 8% (10.6 ± 0.3 mm).

Table 3. The results of the inhibition zone of silver nanoparticles of pineapple bromelain isolate

Sample	Replication Inhibition Zone (mm)			Average Obstacles zone (mm)
	1	2	3	
F 1	12,8	13,3	13,4	13,2 ± 0,3
F 2	12,7	15,4	13,1	13,7 ± 1,5
F 3	12,4	13,8	12,9	13,0 ± 0,7
F 4	13,6	13,0	12,6	13,1 ± 0,5
F 5	13,5	11,9	13,8	13,1 ± 1,0
Ag	11,7	12,6	11,3	11,9 ± 0,7
PI	10,6	10,9	10,3	10,6 ± 0,3
C	34,6	37,7	37,7	36,7 ± 1,8

Description:
F : Formula
Ag : $AgNO_3$ 1 mM
PI : Pineapple Isolation 8%
C : Control (+)

The five formulations produced an inhibition zone value greater than AgNO3 and only 8% of pineapple bromelain isolates. This proves that the incorporation of silver nitrate and pineapple bromelain isolate formulated into silver nanoparticles can increase antibacterial activity [15]. A comparison of the size of the inhibition zone for each formulation showed antibacterial action with a difference in the number of 8% pineapple bromelain isolates and AgNO3 in AgNP synthesis [21 It can be concluded silver nanoparticles synthesized by pineapple bromelain isolates have antimicrobial ability because they have inhibitory power for bacterial growth in the range of 10-20 mm [29 with the same conditions when replicating the test using the diffusion method. The results of the inhibition zone of silver nanoparticles can be seen in Figure 4.

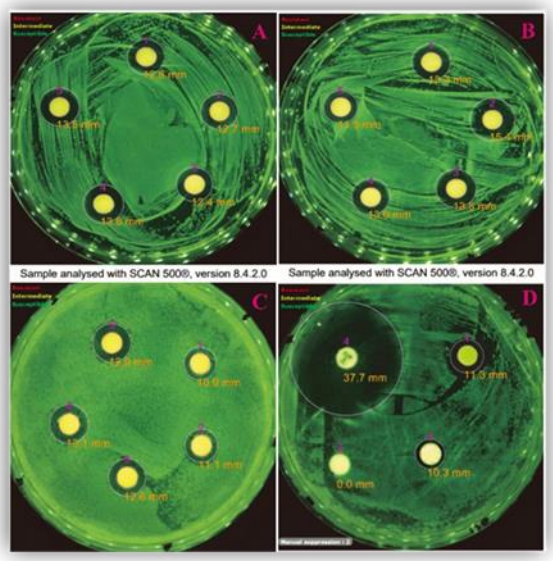

Figure 4. Inhibition zone of silver nanoparticles (A) Replication of 1 formula 1, 2, 3, 4 and 5. (B) Replication of 2 formulas 1, 2, 3, 4 and 5. (C) Image of 3 procedures: 1, 2, 3, 4 and 5. (D) Additional 1: AgNO3 solution, 2: 8% solution of pineapple bromelain isolate, 3: negative control (-) and 4: positive control (+).

4. CONCLUSION

The biosynthesis of silver nanoparticles with pineapple fruit bromelain isolate has good characterization. Changes from colorless to brownish yellow, with wavelength, absorbance, particle size, polydispersity index value, zeta potential value, functional group, and morphology that have met the requirements. The antibacterial activity test showed a clean and clear inhibition zone against Staphylococcus aureus bacteria. Thus, the preparation of pineapple bromelain isolate silver nanoparticles has met the requirements of suitable silver nanoparticles and has antibacterial activity.

5. ACKNOWLEDGEMENT

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