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CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY TEST OF CHITOSAN-SILVER NANOPARTICLE GEL AND BINAHONG LEAF EXTRACT *(Anredera cordifolia (Tenore) Steen)* AGAINST *Staphylococcus aureus*

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Abstract: Binahong leaf is one of the medical plants that contain alkaloids, saponins, and flavonoids, which directly act as antibiotics. Additionally, chitosan and silver nanoparticles also possess antibacterial properties. This study aims to determine the antibacterial activity of a gel preparation against *Staphylococcus aureus bacteria.* It was a true experimental study with data collection carried out at the inorganic level. This study's results show that the gel preparation's organoleptic test meets the requirements for skin pH of 4.5-6.5. The spreading test meets the need for good distribution on the skin, which is 5-7cm. The antibacterial activity test showed that the largest diameter of the inhibition zone was observed at concentration F5 (5% binahong leaf extract), which was 13.4 mm.

Keywords: *Binahong Leaf (Anredera cordifolia (Tenore) Steen), Chitosan, Silver Nanoparticles, Staphylococcus aureus, gGel, Antibacterial*

INTRODUCTION

A wound is a form of tissue damage on the skin that results in the loss of its complex structure. When a wound occurs, the blood vessels have been damaged, causing the wounded area to bleed [1]. There are two types of wounds: open wounds and closed wounds. One example of an open wound is a cut. A cut occurs due to the sharp cutting of an instrument [2]. Infection in a cut is usually caused by *Staphylococcus aureus bacteria*, characterized by the formation of pus which can lead to pneumonia, meningitis, sepsis, and endocarditis. *Staphylococcus aureus bacteria* are pathogenic bacteria that usually reside on the skin of infected individuals due to sharp instrument injuries.

The treatment of wound infections caused by *Staphylococcus aureus* bacteria can be addressed by administering antibiotics. Antibiotics are known to damage and even kill bacteria [3]. Improper use of antibiotics, including overuse, can lead to the development of antibiotic resistance. Antibiotic resistance can cause the body to become vulnerable to bacterial infections of the same type. The ability of active compounds in the drug to kill bacteria will decrease due to excessive use.

Herbal medicine is one alternative to overcome the problem of antibiotic resistance that is currently occurring. One of them is binahong (Anredera cordifolia (Tenore) Steen). This plant is a type of climbing plant in the Basellaceae family. The most commonly used part in traditional medicine is its leaves which contain alkaloids, flavonoids, polyphenols, tannins, saponins, terpenoids, and essential oils. Flavonoids function as antibacterials by producing complex compounds against extracellular proteins that damage bacterial cell membrane integrity. Alkaloids can be antibacterials by inhibiting the formation of peptidoglycan in bacterial cells, which is considered a process that prevents the formation of a complete cell wall and causes cell death. To prevent wounds from becoming infected, saponins, which are protein compounds, act as antiseptics to eliminate or inhibit the growth of bacteria typically present in wounds [1]. Polyphenolic compounds have antibacterial properties by disrupting the bacterial cell membrane, and their antigenic compounds can induce the formation of complex compounds with enzymes or microbial substrates that can increase their toxic properties [4].

With the increasing advancement of technology, innovation related to wound healing is needed, one of which is the addition of an active substance that can be antimicrobial, namely silver nanoparticles. Silver nanoparticles can potentially become antimicrobial agents that can fight against 650 types of bacteria [5]. Silver nanoparticles are classified as nano-sized particles if they have a size range of 1-100 nm. However, in synthesizing silver nanoparticles, several factors can affect their size, such as solution temperature, salt concentration, reducing agent, and reaction time [6]. The synthesis of silver nanoparticles can be achieved through chemical reduction using a chemical reducer such as sodium borohydride (NaBH4), which functions effectively as a reducer. However, according to Kundu et al. (2012), NaBH4 is relatively reactive and has some negative environmental impacts. Therefore, using a reducer as an alternative for producing environmentally friendly nanoparticles (green synthesis) is necessary. One of the reducers that can be used is chitosan, which is stable and effective.

Chitosan has many properties, one of which is its antimicrobial activity. It is non-toxic, biocompatible, biodegradable, and soluble in water [7]. Chitosan is produced through the deacetylation of chitin, which is the main component in the shells of crustaceans such as crabs and shrimp. Chitosan has been widely used in the chemical, food, and

pharmaceutical industries. It is also expected to help products achieve size uniformity on a nanometer scale.

Treatment for incision wounds can be done using topical medication, as oral and parenteral medication cannot penetrate the tissue affected by the incision wound. Reducing infection in the wound can be done by giving topical medication precisely and effectively [7]. One of the topical medications is a gel, which has the advantage of being transparent, leaving a light-transmitting film when applied, easy to clean, and has a long shelf life [8].

Thus, a thorough investigation of the characteristics and antibacterial properties of chitosan-nanoparticles extracted from binahong leaves (*Andrographis paniculata)* and their effects on *Staphylococcus aureus* bacteria should be conducted.

RESEARCH METHODS

This study is a true experimental study. The study was conducted at the Inorganic Chemistry Laboratory of Universitas Negeri Surabaya, Organic Chemistry Laboratory of Universitas Negeri Surabaya, Solid State Laboratory of the Physics Department at Institut Teknologi Sepuluh Nopember, Material Engineering Laboratory of Institut Teknologi Sepuluh Nopember, and Institut Tropis. The study also occurred at the Disease Research Laboratory of Universitas Airlangga from February to April 2023. This study aims to investigate the effect of the activity of chitosan-nanoparticle gel and *Anreidea cordifolia* (Tenore) Steen leaf extract on Staphylococcus aureus bacteria.

The research design used is a *one-shot case study* design with a single group given a treatment, followed by observation of the desired variables.

The objective variable in this study is to measure the inhibitory effect on the antibacterial activity test by chitosan-nanoparticle gel and *Anreidea cordifolia* (Tenore) Steen leaf extract on *Staphylococcus aureus bacteria.* The collected data will be analyzed quantitatively.

The data analysis technique used is quantitative descriptive analysis, which describes the results of parameter testing using numerical data.

The tools used in this study include a glass topley, a stirring rod, an analytical balance (Ohaus), chemical glassware (Pyrex), aluminum foil, plastic wrap, chemical glassware (Iwaki), test tubes (Pyrex), pipettes, test tubes holder, tripod stand, funnel, spirit burner, magnetic stirrer (Dglab), measuring glass (Pyrex), spatula, volumetric pipette, volumetric flask (Pyrex), glass plate, needle holder, test tube rack, incubator, autoclave, freezer, filter paper, Petri dish, and forceps.

The materials used in this research are Binahong leaf powder, 96% ethanol, concentrated hydrochloric acid (HCl), concentrated sulfuric acid, 2N sulfuric acid (H_2SO_4), 1% ferric chloride ($FeCl_3$), chloroform, ammonia, magnesium metal, 60-80% methanol, 70% ethanol, Liebermann-Burchard reagent, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, chitosan powder, silver nitrate solution, sodium borohydride solution, ice cubes, Binahong leaf extract, propylene glycol, chitosan-nanoparticle gel and Binahong leaf extract xanthan gum, sodium benzoate, chitosan-nanoparticle gel solution, *Mueller Hinton* media, nutrient agar media, sodium chloride solution, *Staphylococcus aureus bacteria*, and distilled water.

Procedure for Research

In making Binahong leaf extract, a maceration method was used with three stages of maceration, namely the first and second maceration stages, with a Simplisia and ethanol ratio of 750g:2250 ml. In contrast, the third maceration stage used a ratio of 750g:1500 ml [9]. Each maceration was carried out for 24 hours. The maceration resulted in a filtrate which was then evaporated using *a rotary evaporator* (Büchi) until a greenish solid extract was obtained.

Phytochemical Test

1. Phenol Test

The phenol test weighed 0.5 g of the extract and added a few drops of $FeCl_3$ solution. The presence of phenols was indicated by a color change from blue-black to dark brown [9]

2. Tannin Test

To conduct the tannin test, weigh 0.5 g of the extract, add a few ml of boiling water, and then add 1% $FeCl_3$ solution drops. The presence of tannins is indicated by a dark green color [10].

3. Flavonoid Test

Weigh 0.5 g of the extract and add 5 ml of ethanol for the flavonoid test. Then add a few drops of concentrated HCl solution and 0.2 g of magnesium powder, and heat the mixture for approximately five minutes. The presence of flavonoids is indicated by a color change to blackish-green, yellow, or orange [11].

4. Saponin Test

Weigh 0.5 g of extract, add a few ml of chloroform, a few ml of ammonia, and a few drops of H_2SO_4 solution for the saponin test. Shake the mixture, and two layers will be formed. Three test tubes, each with a volume of 2.5 ml, are used to collect the H_2SO_4 layer produced. Three solutions are tested with Mayer's, Drageendorff's, and Wagner's reagents. The formation of a white precipitate indicates a positive result for Mayer's reagent, while Wagner's reagent produces a brown precipitate, and Drageendorff's reagent produces a red or orange residue [11].

5. Testing of Steroids and Terpenoids.

Weighing the extract up to 0.5 g, putting it into a test tube, and adding a few ml of concentrated H_2SO_4 solution can be used to examine steroids and terpenoids. Then the solution is shaken gently and left for a few minutes. A positive steroid test shows a blue

to green color, while a positive terpenoid test shows a reddish-brown to purple color [12].

Synthesis of Nanoparticles with Chitosan as a Perak Precipitant

The synthesis of silver nanoparticles using chitosan is carried out in a nitrogen gas environment. Then, 50 mL of chitosan solution is added to a few mL of AgNO3 solution and stirred for 10 minutes until homogenous. After that, the mixture is reduced with 1 mL of freshly prepared $NaBH_4$ solution in water (0.42 grams of $NaBH_4$ dissolved in 10 mL of water). The addition of the $NaBH_4$ solution is done by dropwise addition while stirring until the solution turns light purple.

Formulation of Gel Nanoparticles Perak-Kitosan and Binahong Leaf Extract

The gel formulation starts by heating 10 ml of water and binahong leaf extract to 75°C. Then, xanthan gum and sodium benzoate are added to a beaker above a stirrer and stirred quickly until dissolved and homogeneous. The temperature is then lowered to 65°C using a beaker containing a mixture of xanthan gum, sodium benzoate, and binahong leaf extract while continuously stirring until homogeneous. After reaching 65°C, propylene glycol is added and stirred until homogeneous. Once homogeneous, the stirrer is turned off, and the nanoparticle chitosan gel solution is added to the beaker and stirred for 30 minutes until homogeneous.

Table 1. Formulation of Chitosan-Nanoparticle Gel and Extract of Binahong Leaves

Material	Total (%) (b/v)								Function
	F_0	F_1	F_2	F_3	F_4	F_5	F_6	F_7	
Ethanol extract Binahong leaves	0	1	2	3	4	5	6	6	Active ingredient
Nanoparticle Chitosan kit	25	25	25	25	25	25	1	2	Active ingredient
Xanthan gum	3	3	3	3	3	3	3	3	Gelling agent
Propylene glycol	15	15	15	15	15	15	15	15	Humektan
Benzoat	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	preservative
Aquades ad	100	100	100	100	100	100	100	100	solvent

Organoleptic test

Organoleptic testing was carried out by visualizing and directly observing each gel formulation's appearance, color, and texture. The testing was replicated three times for each formula.

Homogeneity Test

One gram of the solution is placed on a transparent glass. After that, the solution is observed, showing a homogenous arrangement without any visible coarse particles.

Dispersion Test

Weigh 0.5 grams of the sample and place it in the center of a transparent glass plate, then place another glass plate on top of the sample. Let it stand for 1 minute, then add 50 to 250 grams of weight to the glass plate every 1 minute. The spreadability of the formulation is usually between 5 and 7 cm. [13] & [14]. The test is performed three times on each formula.

pH test

The purpose of the pH test is to evaluate the formulation's safety so that it can be used without causing skin irritation. Use 10 ml of distilled water to dissolve 1 gram of the gel formulation. The quality product pH range is between 4.5 to 6.5, the same range as the skin's pH [14], [13] & [5]. The testing is conducted three times for each formula.

Characterization with Particle Size Analyzer (PSA)"

Using Particle Size Analyzer (PSA), the pegylated nanoparticles are evaluated as the active ingredient in the gel formulation to determine their particle size and size distribution.

Characterization with Fourier Transform Infrared (FT-IR)

Identification of functional groups in binahong leaf extract compounds is conducted using FTIR spectroscopy

Antibacterial activity test

1. MHA Media Preparation

To make *Mulleir Hinton Agar* (MHA) media, 0.9 grams of MHA was weighed, dissolved in 100 mL of water in an Eirleinmeiyeir flask, then boiled until homogeny. Then autoclaved for 15 minutes at 121°C. Pour the media into 20 mL Petri dishes and allow to solidify.

2. Preparation of Test Bacteria Suspension

Colonies from the NA solid medium were transferred into test tubes containing 9 ml of physiological NaCl to make suspensions of Staphylococcus aureus test colonies. The turbidity of the test colony suspension was calibrated with 0.5 McFarland standard (approximately 1.5 x 108 CFU/mL). In 15 minutes, the suspension must be used as an inoculum.

3. Antibacterial test with the disc method.

Bacterial suspensions were inoculated with 0.1 ml of MHA media, then spread with a hockey stick and left to dry. Filter paper discs that had been soaked in chitosan-nanoparticle extract from binahong leaves for 15 minutes were aseptically placed on the surface of the media. The resulting zones of inhibition around the disc were observed [15].

Data analysis

The zone of inhibition, which represents antibacterial activity, was measured three times at points that differed slightly, as shown in the figure, and the values were averaged [16].

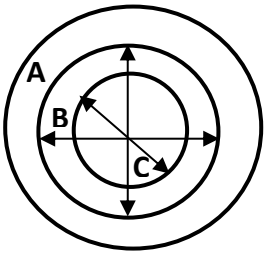

Figure 1. Measurement of bacterial inhibition zone

Information:
A : Petri dish
B : inhibition zone
C : disk paper
↔ : measurement of inhibition zone

RESULTS AND DISCUSSION

Binahong Leaf Extraction Process

Samples in the form of fine powder from binahong leaves Anredera cordifoilia (Tenore) Steen the first and second soaking using 96% ethanol solvent with 750g binahong leaf powder and 2250 ml solvent; in the third soaking, as much as 750g powder with 96% ethanol solvent as much as 1500 ml. Then, it was left in a tightly closed container to prevent solvent evaporation. Each immersion was carried out for 24 hours. The maceration results were filtered using filter paper, resulting in a concentrated green ethanol extract of binahong leaves and black residue. The ethanol extract was evaporated using a rotary vacuum at ± 65 ℃ within ± 60 minutes until the ethanol solvent evaporated. This process produces a thick green extract of as much as 15 1904 grams to obtain the yield as in the table below. One method to determine the amount of secondary metabolites present in an active ingredient is through phytochemical screening. Meanwhile, some identified substances may benefit industries, health, beauty, and other purposes in Table 2.

Table 2. Binahong Anredera cordifoilia (Tenore) Steen Leaf Extraction Yield Results

Tanaman	Berat Simplisia	Berat Ekstrak Etanol	Rendemen
Daun binahong *Anredera cordifoilia* (Tenore) Steen	750 g	15.19 g	2.02%

Binahong leaves were extracted and macerated before phytochemical screening was conducted. The extract obtained from the binahong leaves was dark green. In addition, a qualitative analysis of phytochemical screening was carried out using color tests. The results of the phytochemical screening of the bin along leaves are presented in Table 3.

Table 3. Results of Phytochemical Screening of Binahong Leaves Using 96% Ethanol as Solvent

Chemical Content	Testing Method	results	Information
Flavonoid	Wilstater	Green Colour	+
Saponin	Forth	Foam for approximately 30 minutes	+
Alkaloid	Dragendroff	White Precipitate	+
Tanin	$FeCl_3$ 1%	Green, brownish coloration	+
Steroid	$CHCl_3$ + Liebermen-Burchard	dark green	+

Information:
(+) = There are chemical contents

Based on the results of phytochemical screening in Table 2, it is known that the binahong leaves contain Flavonoids, Saponins, Alkaloids, Tannins, and Steroids. This research result is consistent with previous studies that showed that binahong leaf extract contains flavonoids, steroids, and tannins [17], flavonoids, triterpenoids, and saponins [18]. In another study, it was mentioned that binahong leaf extract

contains coumarin [19]. The difference in the results of phytochemical screening tests from several studies indicates the presence of triterpenoids, steroids, saponins, and coumarins in binahong leaves. It could be due to variations in the testing procedures, the growing environment of binahong plants, and the solvents used to extract the binahong leaves.

The Wilstateir method can be used on binahong leaves to screen for the presence of flavonoids and produce promising results. Magnesium (Mg) and 2N HCl solution are used in the Wilstateir test to identify substances containing the α-benzopyrone nucleus. [10]. A positive flavonoid test is indicated by the formation of flavilium salt in the test solution with reagents [20].

Based on the phytochemical screening results using the foam test method, it was found that the extract of binahong leaves contains saponin. The foam produced during the test is a glycoside hydrolyzed into glucose and other compounds, resulting in a foamy appearance (Rusdi in [17].

The test results for alkaloids using Dragendorff and 2N H_2SO_4 reagents on the extract of binahong leaves showed a positive result. It indicates the formation of potassium alkaloid precipitate due to the covalent bond between nitrogen and K^+ metal ion.

The phytochemical examination of the content of tannins using 1% $FeCl_3$ added to the extract of binahong leaves showed positive results with the production of a blackish-green color. Tannins in the extract react with the Fe^{3+} ions from the reagent to form a complex compound [21].

The steroid test result using the extract of binahong leaves and Liebermann-Buchard reagent showed a green ring. This phenomenon indicates the occurrence of oxidation reactions in the group of steroid compounds.

Organoleptic Test

The sensory evaluation encompasses the aroma, color, and texture of chitosan-nanoparticle peak and binahong leaf extract, which the panelists visually observe. The samples' sensory evaluation results can be seen in Table 4.

Sensory evaluation was conducted on the gel samples from each formulation, which varied from being odorless to strongly pungent with binahong leaf extract. The texture of the gel samples from all formulations was slightly solid/semi-solid. As for the color of the gel, it varied significantly depending on the concentration of binahong leaf extract used, ranging from colorless to dark green. The odor and color of the gel samples were derived from the binahong leaf extract.

Table 4. Results of Sensory Evaluation

Formulation	Color	Smell	Texture
0	Colorless	Odorless	Slightly solid
1	Leaf green	Non-pungent	Slightly solid
2	Green	Slightly pungent	Slightly solid
3	Green	Slightly pungent	Slightly solid
4	Green	pungent	Slightly solid
5	Dark Green	pungent	Slightly solid
6	Dark Green	Very pungent	Slightly solid
7	Dark Green	Very pungent	Slightly solid

Homogeneity Test

The purpose of the gel homogeneity test is to evaluate whether all gel components are well-mixed or not. The results of the gel homogeneity test are shown in Table 5.

Table 5. Results of the Homogeneity Test

Formulation	Homogeneity
0	homogenized
1	homogenized
2	homogenized
3	homogenized
4	homogenized
5	homogenized
6	homogenized
7	homogenized

Each gel formulation showed no visible presence of coarse particles on the glass slide during observation and had uniform color. These gels are considered homogenous.

Antibacterial Test

The spreading power test aims to determine how easily the gel can be applied or used. The results of the spreading power test are shown in Table 6.

As a result of the gel's strong spreading ability and wider skin contact, active ingredients are absorbed more quickly. The chitosan-nanoparticle gel and binahong leaf extract have an average spreading power ranging from 6.22 - 6.28 cm. The results meet the requirements for the gel's spreading power. It is because the gel's size is already within the required range of 5 to 7 cm [8] & [20]. Therefore, this gel can be applied to infected skin.

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Table 6. Results of the Spreading Power Test

Formulation	Spreading Power (cm) week to				Average
	1	2	3	4	
0	6. 28	6. 23	6.23	6.15	6.22
1	6. 31	6. 31	6. 27	6. 25	6. 28
2	6. 30	6. 30	6. 30	6. 28	6. 31
3	6. 32	6. 31	6. 30	6. 30	6. 31
4	6. 21	6. 21	6. 16	6. 12	6. 17
5	6. 42	6. 42	6. 42	6. 40	6. 41
6	6. 37	6. 36	6. 35	6. 34	6. 35
7	6. 32	6. 30	6. 27	6. 24	6. 28

pH Test

The pH test aims to determine the solution's acidity so that the topical pH can be adjusted accordingly. The results of the pH test for the solution are shown in Table 7.

Tab 7. The results of the pH Test

Formulation	pH week to				Average
	1	2	3	4	
0	5	5	5	5	5
1	5	5	5	5	5
2	5	5	5	5	5
3	5	5	5	5	5
4	6	5	5	5	5.25
5	5	5	5	5	5
6	6	6	5	5	5.5
7	6	6	6	5	5.75

The chitosan-nanoparticle gel and binahong leaf extract have an average pH ranging from 5 to 5.75, which meets the standard pH of the skin. When the pH of the gel is below 4.5, it becomes acidic and can irritate the skin. When the pH of the gel is above 6.5, it becomes alkaline and can cause dryness and scaling of the skin [14] & [13].

Characterization with Particle Size Analyzer (PSA)

Characterizing particle size is crucial to determine the particle size of the ionic gel solution. The testing was conducted using the ionic nanoparticle gel solution at a concentration of 50 ppm. The testing conducted at this concentration yielded results as shown in Figure 2.

From Figure 2, the concentration of the examined nanoparticle solution from the peak has nanoparticles with a size of 31.16 nm. Nanoparticles are tiny particles with a diameter between 1 and 100 nm. Nanoparticles are used in various industries, such as environmental remediation, technology, and medicine [2]. A maximum size of 200 nm is recommended for using nanoparticles in medicine [22] & [4]. Based on the general explanation of the size of peak nanoparticle concentration, nanoparticles with a size of 31.16 nm can be applied in medicine.

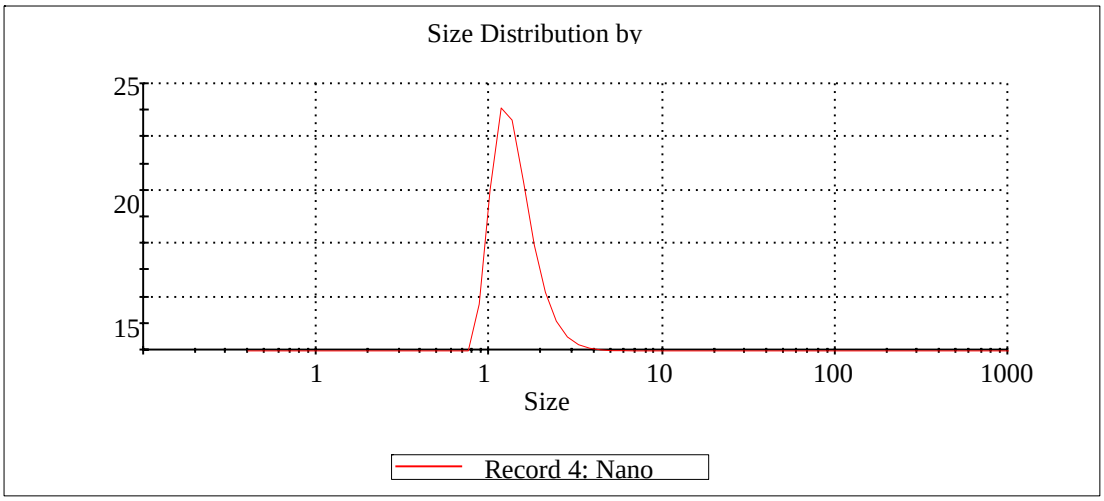

Figure 2. The result of the particle size analysis of the silver nanoparticle test

Characterization with Fourier Transform Infrared (FT-IR)

The infrared spectroscopy analysis results obtained spectrum data, as shown in Figure 2. The spectrum reveals that the extract contains several functional groups, such as the O-H group, indicated by a broad peak at a wavelength of 3370 cm-1. Also, alkane C-H groups at wavelengths of 2921.03 cm^{-1} and

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2850.39 cm^{-1}. There is also an aromatic C=C group at a wavelength of 1636.38 cm^{-1} and a C-O group at 1363.20 $cm^{-1.}$ The spectrum also shows the presence of the epoxy C-O-C group at a wavelength of 1042.34 cm^{-1}. Furthermore, there is a -(CH2)n group at a wavelength of 591.45 $cm^{-1.}$

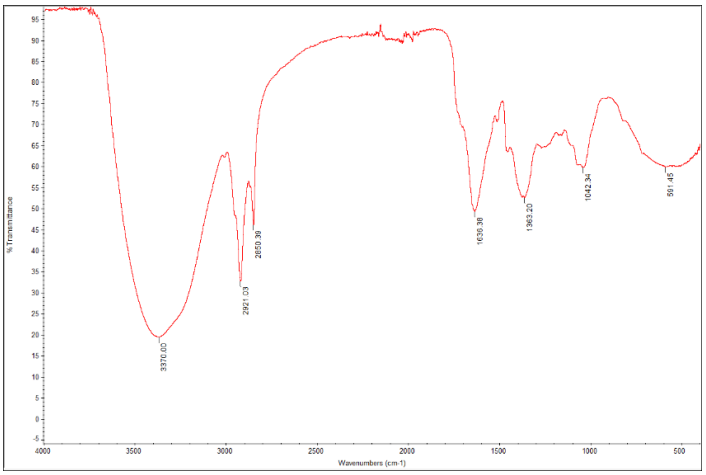

Figure 3. Infrared spectrum of Anreideira cordifoilia (Teinorei) Steiein Binahong leaf extract

Antibacterial Activity Test

Testing the antibacterial activity of chitosan-nanoparticle solution and *Anreideira cordifolia (Tenore*) Steen leaf extract with various concentrations of 1%, 2%, 3%, 4%, 5%, and 6% of nanoparticle solution, and 6% chitosan using the disk diffusion method against the pathogenic bacteria, Staphylococcus aureus.

The research showed that *Anreideira cordifolia* (Tenore) Steen leaf extract and chitosan-nanoparticle solution could limit the growth of the tested microorganisms. The inhibition zone of the bacteria, which was affected by *Staphylococcus aureus*, is presented in Figure 4.

The antibacterial capacity was measured based on the inhibition zone around the paper plate. The diameter of the inhibition zone was measured three times. The results of measuring the inhibition zone diameter of *Staphylococcus aureus* bacteria are shown in Table 7.

Table 7 shows that chitosan-nanoparticles and *Anredera cordifolia (Tenore)* Steen leaf extract have bactericidal properties against *Staphylococcus aureus*. The inhibition zones produced were larger and more varied. The antibacterial test results showed that with an increase in extract concentration, the inhibition zones created by chitosan-nanoparticles and Anredera cordifolia (Tenore) Steen leaf extract are relatively strong.

Table 8. Antibacterial Test Results Against *Staphylococcus aureus*

No	Sample	Concentration (%)	Measurement of inhibition zone (mm) Concentration (%)			Average
			1	2	3	
1	Gel	1	10.78	11.42	10.35	10.85
2	Chitosan-	2	12.98	12.59	10.12	11.89
3	nanoparticles	3	10.99	10.36	14.66	12.03
4	Silver and	4	10.78	12.94	11.55	11.75
5	Ekstract	5	12.76	14.15	12.22	13.04
6	Leaf Binahong	6	6.34	8.82	9.31	8.15
7		6	8.70	8.03	9.34	8.69
8		Control +	20.73	23.24	22.57	22.18
9		Control -	0	0	0	0

Table 8 shows that the treatment obtains the largest bacterial inhibition zone with a 5% concentration of binahong leaf extract, which is about 13.04 mm in diameter. The higher the concentration of the extract, the larger the inhibition zone tends to be.

Based on the results shown in Table 7, *Staphylococcus aureus* is resistant to the antibacterial effects of geil chitosan-nanoparticles and binahong leaf extract. The active compounds in these substances are responsible for their activity. One of the active compounds is the secondary metabolite content found in binahong leaf extract, chitosan, and nanoparticle silver. Flavonoids found in binahong leaf extract can break down bacterial walls, allowing internal components to leak out and kill the bacteria and inhibiting protein production. Tannin can damage the

cell parts that bind them, while alkaloids can disrupt protein function [23].

On the other hand, chitosan damages the bacterial cell membrane, thereby killing the bacteria. Meanwhile, nanoparticles function by releasing their ions is react with the peptidoglycan. These small nanoparticles can change shape and release particles of silver ions. These ions penetrate the outer part of the bacteria and disrupt it, leading to the formation of new cells [24].

CONCLUSION

The synthesis of a gel extract from Binahong leaves with a combination of chitosan and silver nanoparticles was characterized through organoleptic testing, which was observed directly for color, aroma, and texture. The expected characteristics of the gel, including spreadability, pH, and homogeneity, resulted in a quick distribution of the drug and an effective therapeutic effect when applied to the incised skin. Meanwhile, the characterization based on functional group identification of Binahong leaf extract showed the presence of OH functional group, alkane C-H, aromatic C=C, C-O, the stretching of the epoxy C-O-C group, and $–(CH_2)n$. The combination of silver nanoparticles, Binahong leaf extract, and chitosan in various forms can effectively fight Staphylococcus aureus in an antibacterial test, particularly with gel formulations. Strong results were shown by a gel concentration of 5% that had a higher proportion of Binahong leaf extract than other gel concentrations, with an inhibitory zone of 13.04 mm. It is because chitosan nanoparticles and silver are two active components in every Binahong leaf extract.

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