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Cottonwood Honey (*Ceiba pentandra*) as Bioreductor for Preparation of AgNPs-mediated Chitosan-based Hand Gel Sanitizer

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ABSTRACT

Antimicrobial resistance is a critical issue where microorganisms develop resistance to the drugs intended to control them, thus posing a significant threat to global health. Silver nanoparticles (AgNPs) have various types of antibacterial mechanisms that can effectively overcome this problem. In this work, AgNPs were modified with water-soluble chitosan (oligochitosan) obtained by depolymerizing low molecular weight chitosan as a stabilizer to enhances the antibacterial activity of the nanoparticles. The synthesis of AgNPs was carried out through an environmentally friendly approach by mixing 0.1 M $AgNO_3$ with 3% cottonwood honey solution as a bioreductor for formulating a nonalcoholic hand sanitizer. The mixture was exposed to sunlight at temperatures between 26°C to 35°C with an intensity of 88,400-137,600 lux for 10 minutes. UV-Vis spectroscopic analysis showed a broad peak in the wavelength range of 330-550 nm, with the highest peak recorded at 450 nm. In the antibacterial activity test of the hand sanitizer gel containing AgNPs-mediated chitosan at concentrations of 5%, 7.5%, 10%, and 12.5%, the inhibition zones observed against *Staphylococcus aureus* bacteria were 12.58 mm, 14.35 mm, 14.66 mm, and 17.14 mm, while for *Pseudomonas aeruginosa* bacteria the inhibition zones were 11.41 mm, 12.33 mm, 12.99 mm, and 13.63 mm, respectively. AgNPs-mediated chitosan-based hand gel sanitizer demonstrates superior antibacterial efficacy compared to traditional 70% alcohol-based hand sanitizers. This innovative solution offers an alternative to continuous alcohol use, which can cause skin irritation, while also addressing concerns related to antimicrobial resistance.

***Keywords*:** antibacterial, chitosan, hand gel sanitizer, honey, silver nanoparticles

Introduction

Antimicrobial resistance (AMR) is the resistance of microbes to their antimicrobials. The overuse and continuous use of antibiotics have contributed to the increase of antibiotic-resistant bacteria.[1] One common, practical, and effective way to prevent the spread of dangerous microorganisms is through the use of hand sanitizer containing antiseptics. Typically, the hand sanitizers available to the public predominantly rely on alcohol as their active ingredient, with concentrations ranging from 62% to 95%. Alcohol within this standardized range is adept at efficiently disrupting microbial proteins and deactivating viruses. Nonetheless, the frequent use of alcohol-based hand sanitizers may give rise to additional concerns, including skin irritation and potential harm.[2] Gel-based hand sanitizers offer distinct advantages over other formulations due to their ability to create a protective layer on the skin's surface, ensuring longer-lasting protection, longer gel retention time, and a good moisturizing sensation.[3] Therefore, in this work, we endeavor developing a non-alcoholic hand sanitizer gel with active ingredients that effectively prevent the transmission and infection caused by harmful microorganisms.

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Nanotechnology is a branch of technology that manipulates individual atoms or molecules at the nanoscale level. This discipline involves the creation of nanoparticles using a variety of materials and delves into their vast potential across numerous sectors. Nanoparticles find diverse applications in fields such as healthcare, medicine, cosmetics, materials science, environmental science, electronics, industry, and energy.[4,5] One of the extensively utilized nanoparticles is silver nanoparticles (AgNPs), broadening their applications in the pharmaceutical, health, cosmetic, and food sectors. This popularity stems from their exceptional properties, particularly their remarkable biological activities, including antibacterial, antiviral, antifungal, antitumor, and anticancer.[6,7]

The synthesis of AgNPs can be accomplished through diverse techniques falling under physical, chemical, and biological methods. Physical methods encompass processes like pyrolysis and evaporation-condensation conducted within a tube furnace at atmospheric pressure. While physical methods for AgNPs synthesis are notable for their swiftness, use of radiation as a reducing agent, and avoidance of hazardous chemicals, they are afflicted by drawbacks including low synthesis yield, heightened energy consumption, potential solvent contamination, and less uniform distribution. On the other hand, chemical methods for AgNPs synthesis encompass chemical reduction, sono-decomposition, lithography, electrochemical reduction, and thermal decomposition. These methods tend to yield high quantities of AgNPs but involve the use of chemicals and generate hazardous by-products. However, both physical and chemical methods are encumbered with certain shortcomings and may not align with the principles of green chemistry.[8] To address these concerns, a biological approach has emerged in the synthesis of AgNPs by harnessing reducing agents derived from biological systems, such as bacteria, fungi, yeast, algae, and plant extracts. An example is the utilization of naturally occurring reducing sugars found in various

natural products. The advantages of AgNPs biosynthesis are that it is easier and simpler, inexpensive, environmentally friendly, and uses low energy.[9,10]

The biological synthesis method leverages the active compounds present in the plant extract to act as reducing agents.[11,12] Honey is a natural product with a high content of reducing agents in the form of glucose and fructose. It is reported that AgNPs synthesis mediated by honey offers several advantages, including rapid reaction kinetics, safety, biocompatibility, and cost-effectiveness. Honey serves a dual role as both a reducing agent and a stabilizing agent. Furthermore, the research highlights that the synthesis of AgNPs is notably influenced by direct exposure to sunlight. Synthesis via exposure to direct sunlight yields superior optical results and higher synthesis yields, evident through the heightened absorbance observed in UV-Vis testing.[13] In addition, AgNPs synthesized using honey demonstrate commendable antibacterial activity.[14]

As per the study findings, the antibacterial activity of AgNPs can be enhanced by coating them with various polymers, including poly-N-isopropylacrylamide[15,16], polyethylene glycol (PEG)[17], polymethyl acrylate (PMA)[18], polyvinyl alcohol (PVA)[19] and chitosan[20], because these polymers possess intrinsic antibacterial properties, which contribute to the heightened antibacterial effectiveness. Apart from increasing antibacterial activity, the polymer coating also serves as a stabilizing agent for AgNPs, preventing their aggregation, while facilitating interactions with bacterial cells[21]. Among these polymers, chitosan stands out as a cationic biopolymer with remarkable potential as a capping agent. This is due to its attributes including antibacterial activity, biodegradability, biocompatibility, reactivity, the ability to form a robust film, and its non-toxic nature.[22,23] Therefore, AgNPs-mediated chitosan holds promise as a formulation for non-alcoholic hand sanitizers, offering a potential reduction in reliance on alcohol-based options that can cause skin irritation. Moreover, this solution addresses concerns regarding antimicrobial resistance due to its multifaceted antibacterial mechanisms.

In light of the provided details above, our research involved the synthesis of AgNPs using honey as a bioreductor and modifying them with a chitosan coating. The resulting chitosan-modified AgNPs product was then incorporated as an active component in a hand sanitizer gel. Different concentrations of chitosan-coated AgNPs were systematically integrated into the hand sanitizer gel formulation to assess their impact on antibacterial activity. Characterization procedures were conducted using both Ultraviolet-Visible Spectrophotometry (UV-Vis) and Fourier Transform Infrared Spectrophotometry (FTIR) on AgNPs, chitosan, and AgNPs-chitosan. Further tests were performed, encompassing antibacterial assessments of AgNPs and AgNPs-chitosan. Additionally, the hand sanitizer gel underwent comprehensive testing, including pH analysis, syneresis evaluation, and antibacterial activity testing against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*) bacteria.

Method

Materials and Instrumentation

Silver nitrate ($AgNO_3$, 99%, Emsure), low molecular weight chitosan (deacetylation degree 75%–85%, Sigma Aldrich), acetic Acid (99%, Emsure), hydrogen peroxide (30%, Smart-Lab), thickening agent carbomer 940 (Anhui Newman) and methyl paraben (Golden Era), the test bacteria (*Staphylococcus aureus* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027) were obtained from the bacterial culture laboratory of the National Agency for Drug and Food Control of the Republic of Indonesia (Surabaya), infusion solution (NaCl 0.9%, PT Widatara Bhakti), nutrient agar (Merck), barium chloride ($BaCl_2$, Sigma Aldrich) and sulfuric acid (98%, Emsure). The bioreductor employed in AgNPs synthesis was Cottonwood (*Ceiba pentandra*) flower honey, sourced from Agro Tawon - Rimba Raya Farms, located on Bedali, Lawang District, Malang Regency, East Java, Indonesia (Coordinate of latitude: -7.830759 and longitude: 112.697098). The honey collection took place in the morning of February 2023. This timing allowed the bees to reorient and recognize their hive after the completion of the honey collection process. Ultraviolet-Visible Spectrophotometer (Model 1601 Shimadzu, Japan) was used to determine the surface plasmons of synthetic AgNPs.

Synthesis of AgNPs-mediated Chitosan

A 3% honey solution and a 0.1 M $AgNO_3$ solution were mixed in a 1:1 ratio and stirred for 10 minutes within the temperature range of 25-30°C. Subsequently, the solution was exposed to direct sunlight for 20 minutes. Identification of AgNPs was established based on the color transformation of the solution to a brownish after sunlight exposure. Characterization was then conducted using a UV-Vis spectrophotometer within a wavelength range of 200-800 nm.[13]

To prepare the chitosan solution for AgNPs coating, 2 grams of chitosan were dissolved in 50 mL of 1% acetic acid and stirred for 2 hours. Then, 50 mL of 10% H_2O_2 was added, and stirring process persisted for an additional 2 hours. The addition of H_2O_2 served to depolymerize the chitosan, enabling it to dissolve in water. Following this, the solution's pH was neutralized to reach a pH 7 by incorporating 10% NaOH. The remaining particulate matter was separated via filtration using Whatman No. 1 paper, yielding a water-soluble chitosan filtrate used as a coating for AgNPs.[20]

The AgNPs solution was mixed with water-soluble chitosan in a 1:1 ratio (v/v) and stirred for 2 hours, resulting in the formation of the AgNPs-chitosan solution. Once each of the individual solutions (AgNPs, chitosan, and AgNPs-mediated Chitosan) was prepared, a portion of the volume from each solution was subjected to the freeze-drying method for sample preparation. These dried samples were then characterized using an FTIR spectrophotometer.

Producing hand sanitizer gel

For preparation of hand sanitizer gel with different concentrations of AgNPs-mediated Chitosan, 4 beaker glass, labeled as 5%, 7.5%, 10%, and 12.5% (detail composition shown in Table 1), were set up. In each beaker glass, 0.2 g of carbomer was dissolved in 8 mL of distilled water until a homogeneous gel was formed. Then, 0.04 g of methyl paraben was dissolved in 2 mL of distilled water and smixed with carbomer gel, followed by stirring to get homogeneous mixture. Certain volumes of the AgNPs-mediated chitosan solution and distilled water were added to the mixture as outlined in Table 1, and stirring process was persisted until homogeneous mixture was attained. This process resulted in the formation of hand sanitizer gel with various concentrations of AgNPs-mediated Chitosan (5%, 7.5%, 10%, and 12.5%), each with a total volume of 20 mL.[20] Subsequently, the hand sanitizer products were tested, involving pH, syneresis, and antibacterial activity.

Table 1: Composition of 20 mL hand sanitizer gel with AgNPs-Chitosan concentration variations

Component	Volume			
	5%	**7.5%**	**10%**	**12.5%**
Carbomer + DW	0.2 g + 8 mL	0.2 g + 8 mL	0.2 g + 8 mL	0.2 g + 8 mL
methyl paraben + DW	0.04 g + 2 mL	0.04 g + 2 mL	0.04 g + 2 mL	0.04 g + 2 mL
AgNPs-Chitosan	1.0 mL	1.5 mL	2.0 mL	2.5 mL
DW	9.0 mL	8.5 mL	8.0 mL	7.5 mL

DW: Distilled water

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pH test

The pH test was conducted on each hand sanitizer gel with varying AgNPs-mediated chitosan concentrations of 5%, 7.5%, 10%, and 12.5%. pH measurements were performed using a pH meter at room temperature. It's essential for hand sanitizer gel products to have an ideal pH value within the range of 4-7. Deviations from this range can potentially disrupt the natural balance of skin activity and lead to skin damage when using the hand sanitizer gel.[3]

Syneresis Test

The syneresis test was performed on hand sanitizer gel products produced with various concentrations of AgNPs-mediated Chitosan. The samples were stored at a temperature of 5°C for a duration of 96 hours. At the end of the test, any alterations in the texture and weight of the hand sanitizer gel products were observed.[24]

Antibacterial Activity Test

Test samples were meticulously prepared, encompassing hand sanitizer gel with AgNPs-chitosan concentrations of 5%, 7.5%, 10%, and 12.5%, along with AgNPs solution (50%) and AgNPs-chitosan solution (100%). A commercially available hand sanitizer gel containing 70% ethyl alcohol (Aseptic Gel OneMed) served as the positive control. For the antibacterial activity test, the well diffusion method was employed using bacterial culture media in the form of agar with rejuvenated bacterial cultures. The types of bacteria used were gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*).

A certain amount of nutrient agar (NA) powder is dissolved in distilled water according to the instructions on the packaging (utilizing a ratio of 20 g of nutrient agar powder in 1 L of water), and bringing it to a boil. The mouth of the flask was sealed with cotton wool, and then the medium, which remained in a liquid form, 0.9% NaCl solution, and the test equipment were sterilized using an autoclave at 121°C and 15 lbs pressure for 15 minutes. This sterilization process was essential to eliminate microorganisms (bacteria, viruses, fungi, etc.) to prevent contamination by unwanted microorganisms during the growth of the desired bacteria.[25] The agar medium was cooled in a water bath to reach a temperature of 45°C while a bacterial suspension was prepared by dissolving one dose of bacteria in sterile 0.9% NaCl until its turbidity matched that of McFarland's 0.5 solution. If the bacterial suspension appeared clearer than McFarland's solution, additional bacteria were added. However, If the bacterial suspension was cloudier than McFarland's solution, sterile 0.9% NaCl was added until the turbidity matched. NaCl 0.9% was chosen as a solvent for bacterial suspensions because it is isotonic, ensuring that bacterial cells maintain their integrity and stability.

Next, 15 mL of agar media was poured into a Petri dish, and 100 µL of bacterial suspension was added, then the mixture was homogenized and allowed to stand until solidified. After the agar had solidified, a well was created in each dish using the base of a pipette (6 mm in size). The samples were then introduced into the wells, with a total sample volume of 20 µL for AgNPs and AgNPs-chitosan solutions, while for the hand sanitizer gel product sample, it was filled to the brim using a drop pipette. The Petri dishes were then incubated at 37°C for 24 hours. The antibacterial activity was determined based on the inhibition zone, as measured by calipers along three diameters (horizontal, vertical, and diagonal). The antibacterial activity test was conducted in triplicate for each bacterium. The quantity of hand sanitizer gel sample injected into the well was determined by specific gravity calculations.

Results and Discussion

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) in this work was carried out using the reduction method from Ag^+ ions to Ag^0 in $AgNO_3$ solution, using a honey bioreductor from Cottonwood flowers source. Honey primarily comprises glucose and fructose, which act as the key reducing agents in the reduction reaction responsible for the synthesis of AgNPs. In the open-chain structure of aldose, there exists an aldehyde group capable of donating electrons. The Ag^+ ions are reduced by sugars, accepting electrons from the aldehyde group, ultimately resulting in the formation of AgNPs[26] as depicted in Figure 1.

The synthesis process involves specific treatments, including stirring and exposure to sunlight. Stirring serves to facilitate interactions between particles and accelerate reactions. Simultaneously, exposure to sunlight is employed to activate the aldehyde group, enabling the reduction of Ag^+ to Ag^0. Additionally, the result indicates that light intensity and the duration of exposure are critical factors that impact the synthesis of silver nanoparticles. Higher light intensity and extended exposure time lead to a greater intensity of the absorbance peak in the resulting nanoparticles.[27,28] The formation of silver nanoparticles is visually indicated by a change in the color of the solution from colorless to yellow to brownish[29] as shown in Figure 2.

Characterization of Silver Nanoparticles using a UV-Vis Spectrophotometer

The silver nanoparticles (AgNPs) solution were subjected to characterization using a UV-Vis spectrophotometer, with measurements conducted within the wavelength range of 200-800 nm. Distilled water was employed as a blank reference. The primary objective was to identify the presence of AgNPs by examining the absorbance spectrum's peak as shown in Figure 3.

Consistent with prior research findings, AgNPs synthesized using honey bioreductor typically exhibit a maximum wavelength of 450 nm. This aligns with the data obtained in the current study, affirming the successful formation of AgNPs. Additionally, the width of the band around the maximum wavelength reflects variations in the size distribution of the formed silver nanoparticles.[30,31]

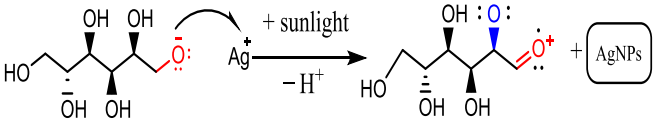

Figure 1: Mechanism of AgNPs formation with Cottonwood honey as a bioreductor

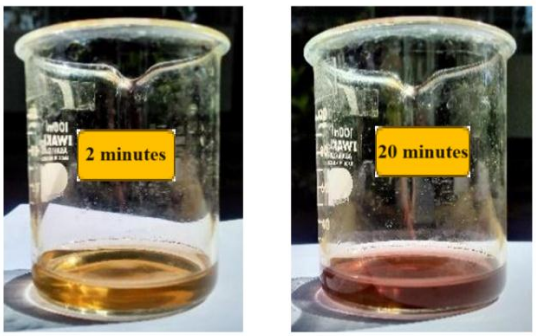

Figure 2: Change in color of the solution from yellow to brownish after exposure to direct sunlight for 2 (left) and 20 (right) minutes for the synthesis of AgNPs

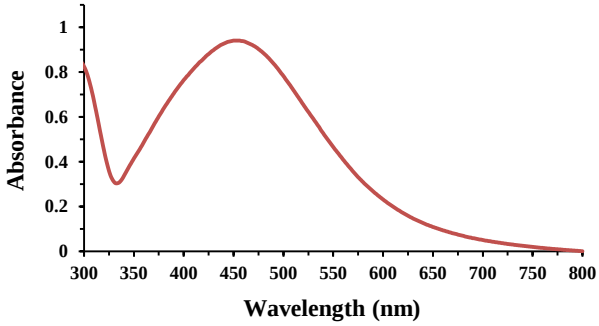

Figure 3: UV-Vis absorbance spectrum of silver nanoparticles

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The optical properties of AgNPs, including the maximum wavelength, are intricately tied to particle size. Larger particles, often due to aggregation, exhibit a higher absorbance wavelength, while smaller particles present a lower one, establishing an inverse relationship. [32] Beyond their optical attributes, the size of AgNPs also plays a pivotal role in their biological activity, especially in the context of antibacterial action. This relationship is associated with the specific surface area of silver nanoparticles. When the size of AgNPs decreases, the specific surface area increases. Consequently, the antibacterial activity is enhanced because smaller nanoparticles offer a higher likelihood for interaction with bacterial cells, effectively boosting their antibacterial efficacy.[33]

Coating Silver Nanoparticles with Chitosan

Silver nanoparticles (AgNPs) inherently possess a tendency to be less stable, often leading to aggregation and an increase in particle size. This aggregation results in a reduced specific surface area, compromising the desired properties of silver nanoparticles. To mitigate this issue, the synthesis of silver nanoparticles typically involves the use of a capping agent. One commonly employed capping agent is a polymer. The polymer becomes adsorbed onto the surface of the nanoparticles, encasing them, and subsequently decreasing the energy and surface tension of the nanoparticles. This process effectively prevents further aggregation.[1]

In the context of this research, the coating of AgNPs was accomplished using chitosan. When the AgNPs solution is combined with chitosan, the solution undergoes a transition from brownish to colorless, as depicted in Figure 4. This change in color signifies that the AgNPs have become dispersed within the chitosan matrix, with chitosan enveloping the entire surface of the nanoparticles.[20]

While honey can serve as both a reducing and capping agent for phenolic and other compounds, this study opted to coat silver nanoparticles with chitosan due to the relatively low phenolic compound content in honey. In addition to its role in stabilizing silver nanoparticles, chitosan boasts excellent biocompatibility and antibacterial properties.[34,35] As a result, it can synergize with AgNPs to enhance antibacterial activity, as highlighted in the Table 2. The inhibition zone, or the area where bacterial growth is suppressed, is notably greater in AgNPs-mediated chitosan when compared to AgNPs without chitosan coating. This distinction in the size of the inhibition zone between AgNPs and chitosan-mediated AgNPs is readily observable in the results of the antibacterial activity test, which are shown in Figure 5.

Producing AgNPs-mediated Chitosan Based Hand Sanitizer Gel

Hand sanitizer gel was manufactured with varying concentrations of AgNPs-mediated chitosan (5%, 7.5%, 10%, and 12.5%), with each batch measuring 20 mL. The ingredients used in this research serve specific functions within the hand sanitizer formulation. Carbomer 940 functions as a gelling agent responsible for providing the hand sanitizer with desired stability, viscosity, adhesion, and spreadability. It ensures that the hand sanitizer attains the desired consistency and serves as a medium to carry the active ingredient.[36] Methyl paraben serves as a preservative for the product due to its effective antimicrobial properties. It maintains stability and prevents alterations in the consistency, color, and odor of the product. The appearance of the hand sanitizer gel is depicted in Figure 6.

Notable properties that can be directly observed in the hand sanitizer gel include its colorlessness, slight cloudiness, a slightly sticky and moist texture upon application with slow drying, easy and even spreadability, and a faint odor of acetic acid. These properties are consistent across all variations of the hand sanitizer gel. Previous work has also reported that AgNPs-mediated Chitosan-based hand sanitizer gel exhibits characteristics such as clarity, colorlessness, homogeneity, ease of application, and even spreadability.[33] The turbidity observed in the hand sanitizer gel may arise from the chitosan filtration process when obtaining water-soluble chitosan, which may require filter paper with smaller pore sizes.

AgNPs-mediated Chitosan Hand Sanitizer Test

The pH measurements were conducted on four samples, specifically hand sanitizer gel with AgNPs-mediated Chitosan concentrations of 5%, 7.5%, 10%, and 12.5%. The measurements were executed using a pH meter, and the results are presented in Table 3. The pH values obtained for these samples were 3.5, 3.6, 3.7, and 3.8, respectively, starting with the sample containing the lowest AgNPs-mediated Chitosan concentration. These measurements demonstrate that the inclusion of AgNPs-mediated Chitosan leads to an increase in the pH value of the hand sanitizer gel.

Hand sanitizer gel is a product intended for application on the skin's surface, primarily on the hands. It is crucial that the pH value of hand sanitizer gel falls within an appropriate and applicable range to prevent potential skin damage resulting from the use of products with an unsuitable pH.

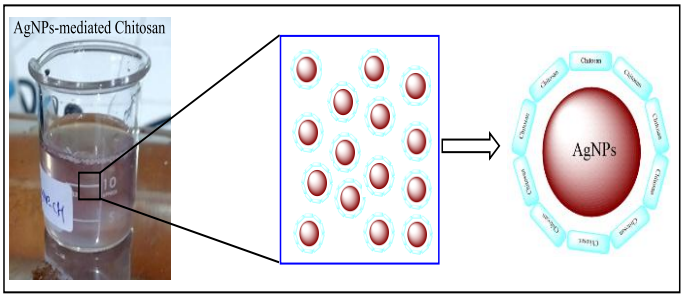

Figure 4: AgNPs-mediated Chitosan solution

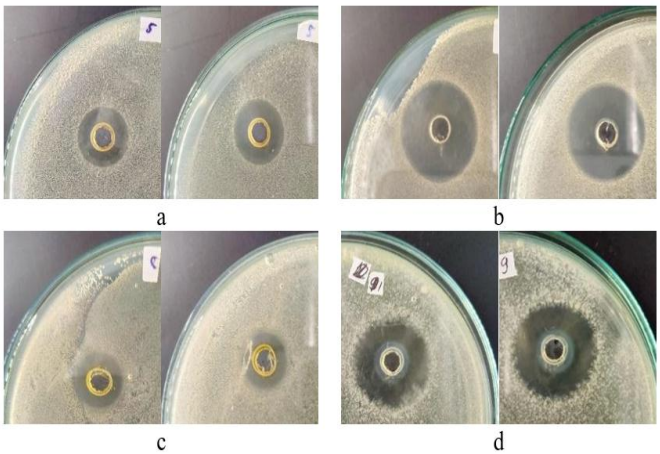

Figure 5: Antibacterial activity test results of (a) AgNPs against *S. aureus*, (b) AgNPs-mediated Chitosan against *S. aureus*, (c) AgNPs against *P. aeruginosa*, (d) AgNPs-mediated Chitosan against *P. aeruginosa*.

Table 2: Inhibition zone of antibacterial activity test

Sample	Inhibition zone (mm)					
	S. aureus			*P. aeruginzosa*		
	I	II	Average	I	II	Average
AgNPs	15.54	15.55	15.56	15.10	15.14	15.12
AgNPs-mediated Chitosan	27.59	27.55	27.51	21.07	21.15	21.11

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As per existing literature, the ideal pH range for skin is 4-6. Within this pH range, the skin functions optimally, including cell activities and skin layer functions, as well as the maintenance of the skin microbiome or flora, guarding against skin damage and related issues.[30] The adjustment of pH was achieved by introducing 10% NaOH into the chitosan solution during the preparation of water-soluble chitosan. The measurements demonstrated favorable results, with a pH of 4, for the hand sanitizer gel product across all concentrations of AgNPs-mediated Chitosan. Consequently, the product is considered safe for use on the skin's surface.[37]

The syneresis test conducted on gel hand sanitizer products is designed to assess alterations in the product's texture and weight under specific conditions. Syneresis in the gel refers to the contraction of the gel and the release of water from it. The test involved the examination of hand sanitizer gel products with different concentrations of AgNPs-mediated Chitosan both before and after storage at a temperature of 5°C for a duration of 96 hours. Based on observations, it was determined that the texture and appearance of all hand sanitizer gel samples remained unaltered. Subsequently, the results of the sample weight measurements are provided in Table 4.

The results of the syneresis test indicated a slight change in the weight of each sample, signifying that a low level of syneresis had taken place. The degree of syneresis is inversely related to the stability of the gel product against syneresis. In other words, the lower the syneresis, the better the product's stability against this phenomenon. This implies that the hand sanitizer gel product in this study exhibits a reasonable degree of stability in the syneresis test conducted at a temperature of 5°C. As presented in Table 4, the hand sanitizer gel with an AgNPs-mediated Chitosan concentration of 10% experienced the least syneresis, with a percentage of 1.08%. AgNPs-mediated Chitosan-based hand sanitizer gel demonstrates relative stability in the syneresis test under a temperature of 5°C. This is attributed to the presence of the chitosan biopolymer, which possesses amine groups that function as stabilizers and water retainers, effectively reducing the syneresis effect.[38]

The antibacterial activity test against *Staphylococcus aureus* as gram-positive bacteria and *Pseudomonas aeruginosa* as gram-negative bacteria was carried out by measuring the inhibition zones of hand sanitizer gel samples containing various concentrations of AgNPs-mediated chitosan of 5%, 7.5%, 10%, and 12.5% using the well diffusion method. Commercially available hand sanitizer gel possess an alcohol content of 70% was applied as a positive control (+). This test was conducted to determine the ability of the hand sanitizer gel product prepared in this work to inhibit bacterial growth. The wider the inhibition zone, the better the antibacterial activity properties of a sample.[39]

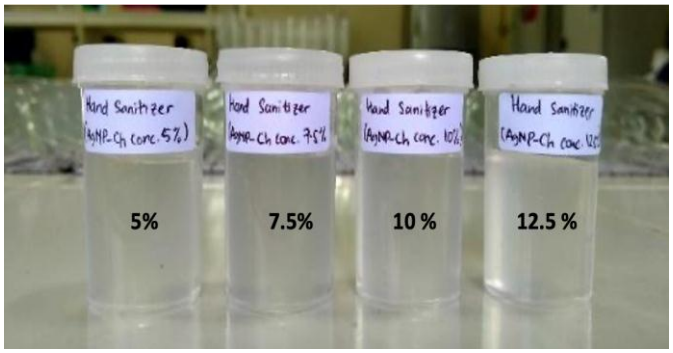

Figure 6: AgNPs-mediated chitosan based hand sanitizer gel at various concentration

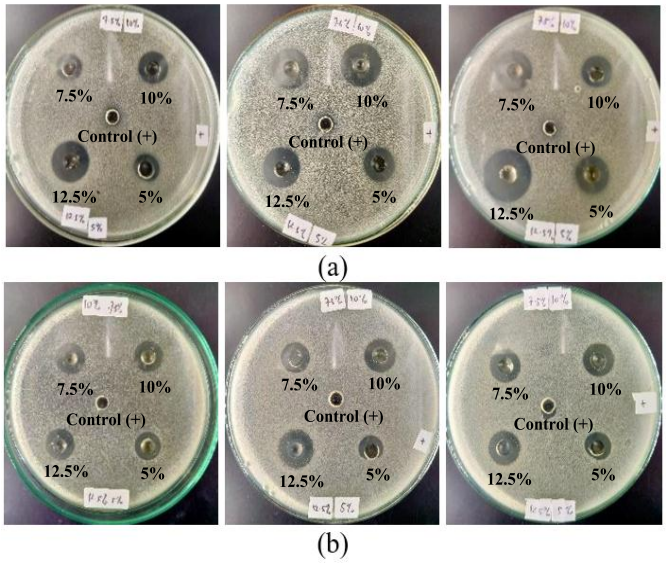

Figure 7: Antibacterial activity tests against bacteria (a) *Staphylococcus aureus* and (b) *Pseudomonas aeruginosa* at various concentration of AgNPs-mediated Chitosan.

Table 3: the pHs of hand sanitizer gel produced

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Concentration of AgNPs-mediated Chitosan in hand sanitizer product	pH I	II	III	pH mean
5%	3.5	3.5	3.5	3.5
7.5%	3.6	3.6	3.6	3.6
10%	3.7	3.7	3.7	3.7
12.5%	3.8	3.8	3.8	3.8

Table 4: Syneresis test results, mass lost during storage.

Concentration of Chitosan-coated AgNPs in hand sanitizer product	Initial Mass (g)	Final Mass (g)	Mass Loss (g)
5%	19.618	19.257	0.361
7.5%	19.624	19.292	0.332
10%	19.657	19.444	0.213
12.5%	19.623	19.361	0.262

Table 5: Inhibition zone of antibacterial test of AgNPs-mediated chitosan-based hand sanitizer gel

Bacteria	Sample	Inhibition Zone Diameter (mm)			
		I	II	III	Average
S. aureus	5%	10.92	12.50	14.32	12.58
	7.5%	11.38	16.03	15.63	14.35
	10%	13.15	17.50	13.33	14.66
	12.5%	15.93	15.70	19.78	17.14
	(+)	6.63	6.20	6.03	6.29
P. aeruginosa	5%	11.74	11.44	11.05	11.41
	7.5%	12.58	11.98	12.43	12.33
	10%	13.11	12.98	12.87	12.99
	12.5%	13.28	14.35	13.27	13.63
	(+)	6.12	6.00	6.68	6.27

The findings from the inhibition zone measurements are presented in Table 5, and antibacterial activity tests are documented in Figure 7. Table 5 represents the diameter of the clear zone including the diameter of the well (6 mm), with the amount of sample (hand sanitizer gel product) injected into the well is ± 80 µL. It was found that AgNPs-mediated chitosan-based hand sanitizer gel demonstrated remarkable antibacterial activity on both types of bacteria. The highest inhibition zone is resulted by a product containing AgNPs-mediated Chitosan concentration of 12.5%, showing 17.14 mm for *Staphylococcus aureus* bacteria and 13.63 mm for *Pseudomonas aeruginosa* bacteria.

In both bacteria, the area of the inhibition zone showed a higher value as the AgNPs-mediated chitosan concentration increased in the hand sanitizer gel. This phenomenon can be attributed to the larger surface area of nanoparticles at higher concentration of AgNPs-mediated chitosan. The larger surface area allows for increased interactions between AgNPs and bacterial cells, resulting in more effective prevention of bacterial growth, ultimately leading in superior antibacterial activity.[40]

The desired characteristic of a hand sanitizer which aims to prevent the spread of microbes is that it has good antibacterial activity, indicated by a larger area of inhibition zone (high diameter). This goal can be achieved by increasing the AgNPs-mediated chitosan concentration in hand sanitizer gel formulations, especially with the biocompatibility properties of chitosan-coated AgNPs.[40] However, the hand sanitizer gel formulation needs to be adjusted taking into account needs, efficiency and various factors.

The research findings in this work also reveal the superiority of the antibacterial activity of the AgNPs-mediated chitosan hand sanitizer gel products over the positive control (+), which only result in small inhibition zone of 6.29 mm for *Staphylococcus aureus* bacteria and 6.27 mm for *Pseudomonas aeruginosa* bacteria. The difference in the inhibition zone between the positive control and the product with the lowest AgNPs-mediated chitosan concentration (5%) was 6.29 mm in *Staphylococcus aureus* and 5.14 mm in *Pseudomonas aeruginosa*. This result implies that hand sanitizer gel containing AgNPs-mediated chitosan significantly outperforms the commercially available hand sanitizer gel in terms of antibacterial effectiveness. This evidence strongly indicates that hand sanitizer products utilizing AgNPs-mediated chitosan are exceptionally effective in combating antimicrobial resistance as proved by notably superior antibacterial activity when compared to commercially available 70% alcohol-based hand sanitizers.

It was also found that the diameter of the inhibition zone on *Staphylococcus aureus* was greater than that on *Pseudomonas aeruginosa* with a difference of difference of 1 - 4 mm, indicating the AgNPs-mediated chitosan hand sanitizer gel works better in inhibiting the growth of gram-positive bacteria than gram-negative bacteria, or in other words, gram-negative bacteria are more resistant than gram-positive bacteria.[41]

In the previous research,[20] the banana peel extract was used as a natural reducing agent for AgNPs formation, which were subsequently utilized in formulating chitosan-coated AgNPs hand sanitizer. However, the antibacterial test displayed efficacy primarily against gram-positive bacteria and exhibited reduced effectiveness against gram-negative bacteria. In this present study, the utilization of oligochitosan derived from depolymerized low molecular weight chitosan demonstrated significant antibacterial effectiveness against both gram-positive and gram-negative bacteria. This is attributed to oligochitosan's abundance of open amine groups ($-NH_2^+$), enabling easy interaction with the negatively charged cell wall ($-PO_4^{2-}$). This interaction leads to the breakdown of the cell wall, causing structural damage that ultimately results in bacterial death.

.Conclusion

The synthesis of AgNPs-mediated chitosan was successfully achieved through a green synthesis method utilizing honey as a reducing agent. The concentration of AgNPs-mediated chitosan in the hand sanitizer gel formulation has significant impacts on its pH, syneresis, and antibacterial properties where increasing the concentration of this active ingredients would increase the pH value, reduce the syneresis effect, and increase antibacterial activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa*) bacteria. These findings suggest that the formulation can be tailored to meet specific needs, optimizing its effectiveness and ensuring it complies with safety and performance standards. AgNPs-mediated chitosan-based hand sanitizer has better antibacterial activity than 70% alcohol-based hand sanitizer and can be a solution in the dependence on continuous use of alcohol that can irritate the skin and can be one of the solutions in dealing with the problem of antimicrobial resistance.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

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