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Antibacterial Efficacy of $AgNO_3$ Combined with *Cymbopogon citratus* Extract and Chitosan Nanocomposite Against *Pseudomonas aeruginosa*

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ABSTRACT

Infections caused by pathogenic bacteria such as *Pseudomonas aeruginosa* present significant challenges in clinical practice, particularly due to rising resistance to conventional antibiotics. The development of environmentally friendly, nanotechnology-based antibacterial agents is considered a promising alternative. This study aimed to evaluate the antibacterial activity of a nanocomposite comprising silver nitrate ($AgNO_3$), *Cymbopogon citratus* leaf extract, and chitosan against *P. aeruginosa*. The nanocomposite was synthesized using a green synthesis method, with plant extract serving as a natural reducing agent and chitosan as a nanoparticle stabilizer. Antibacterial activity was assessed via disk diffusion against five concentrations (6.25, 12.5, 15, 25, and 50 mg/mL), and compared to positive (chlorhexidine) and negative (acetic acid) controls. The results showed that the 6.25 mg/mL concentration produced the largest inhibition zone (average 11 mm), although it did not surpass the effectiveness of chlorhexidine. The inhibition zones remained stable for up to 72 hours, indicating sustained antibacterial activity. A decline in efficacy at 50 mg/mL was observed, likely due to nanoparticle aggregation and biological saturation. These findings support the potential of $AgNO_3$–*C. citratus*–chitosan nanocomposite as a natural-based alternative antibacterial agent. Further studies are recommended to characterize its physicochemical properties, elucidate its mechanism of action, and evaluate its toxicity and applicability in pharmaceutical and biomedical contexts.

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ABSTRACT

Infeksi yang disebabkan oleh bakteri patogen seperti *Pseudomonas aeruginosa* merupakan tantangan besar dalam praktik klinis, terutama karena meningkatnya resistensi terhadap antibiotik konvensional. Pengembangan agen antibakteri berbasis nanoteknologi dengan pendekatan ramah lingkungan menjadi alternatif strategis. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri nanokomposit berbasis perak nitrat ($AgNO_3$) yang dikombinasikan dengan ekstrak daun *Cymbopogon citratus* dan kitosan terhadap pertumbuhan *P. aeruginosa*. Nanokomposit disintesis melalui metode green synthesis, dengan ekstrak tanaman sebagai agen pereduksi alami dan kitosan sebagai penstabil nanopartikel. Aktivitas antibakteri diuji menggunakan metode difusi cakram terhadap lima variasi konsentrasi (6,25; 12,5; 15; 25; dan 50 mg/mL), serta dibandingkan dengan kontrol positif (chlorhexidine) dan kontrol negatif (asam asetat). Hasil menunjukkan bahwa konsentrasi 6,25 mg/mL menghasilkan zona hambat tertinggi (rata-rata 11 mm), meskipun belum melampaui efektivitas chlorhexidine. Zona hambat tetap stabil hingga 72 jam, menunjukkan potensi aktivitas yang persisten. Namun, efektivitas menurun pada konsentrasi 50 mg/mL, yang kemungkinan disebabkan oleh agregasi nanopartikel dan kejenuhan biologis. Hasil ini mendukung potensi nanokomposit $AgNO_3$–*C. citratus*–kitosan sebagai kandidat alternatif antibakteri berbasis bahan alam. Penelitian lanjutan diperlukan untuk karakterisasi fisikokimia, klarifikasi mekanisme aksi, serta evaluasi toksisitas dan aplikasinya dalam bidang farmasi dan biomedis.

Kata Kunci: Antibakteri; Kitosan; *Cymbopogon citratus*; Nanokomposit, *Pseudomonas aeruginosa*

1. Introduction

Nosocomial infections are a major public health concern, particularly in hospital environments such as intensive care units and dental clinics, where the incidence and severity are notably high. Among the most problematic pathogens is *Pseudomonas aeruginosa*, a Gram-negative bacterium known for its intrinsic resistance to multiple antibiotics and its role in recurrent infections, particularly in wounds and the respiratory tract [1],[2],[3]. This pathogen can contaminate water sources and medical equipment, making infection control increasingly challenging [1].

One commonly used antiseptic in dental care is chlorhexidine, owing to its broad-spectrum antimicrobial activity. However, long-term use of chlorhexidine has been associated with adverse effects, including tooth discoloration, altered taste sensation, and mucosal irritation [4],[5]. These limitations have driven the search for alternative, natural antibacterial agents with fewer side effects.

Silver-based nanomaterials have emerged as promising candidates due to their potent antimicrobial, antioxidant, and wound-healing properties [6],[7]. The biosynthesis of silver nanoparticles (AgNPs) using plant extracts such as *Cymbopogon citratus* (lemongrass) offers a green and sustainable approach, reducing reliance on toxic chemical reducers [8]. *C. citratus* contains bioactive compounds including flavonoids and phenolic constituents, which not only serve as reducing agents in nanoparticle synthesis but also exhibit therapeutic potential [9].

However, the stability of silver nanoparticles remains a major concern due to their tendency to aggregate, leading to reduced bioactivity. To overcome this, chitosan-a natural polysaccharide derived from chitin-can be used as a stabilising and capping agent. Chitosan provides functional amino ($-NH_2$) and hydroxyl (-OH) groups that can chelate silver ions and enhance nanoparticle stability [10], [11]. The integration of $AgNO_3$, *C. citratus* extract, and chitosan in a single nanocomposite formulation is hypothesised to result in a synergistic antibacterial effect, combining the physicochemical stability of chitosan with the bioactivity of silver and lemongrass phytochemicals.

Previous studies have shown that AgNP-chitosan nanocomposites synthesised using natural reducers such as Allium sp., Musa paradisiaca, and Azadirachta indica exhibit enhanced antibacterial activity against various pathogenic bacteria [9],[10],[11]. Nevertheless, the use of *C. citratus* combined with chitosan in the biosynthesis of silver

nanocomposites specifically targeting *P. aeruginosa* remains underexplored. Therefore, this study aims to evaluate the antibacterial efficacy of $AgNO_3$-*Cymbopogon citratus*-chitosan (AgChCc) nanocomposites against *P. aeruginosa* and assess their potential as natural, sustainable alternatives to conventional antiseptics.

2. Methods

Materials

The materials used in this study included silver nitrate ($AgNO_3$; Sigma-Aldrich, USA), *Cymbopogon citratus* ethanol extract (obtained from BPSI TROA, West Java, Indonesia), and chitosan derived from the Faculty of Marine and Fisheries, IPB University. Additional reagents included 70% ethanol (SAE Alcohol), 1.5% acetic acid solution, Brain Heart Infusion (BHI) broth (Himedia), nutrient agar (NA), and distilled water (SmartLab, Indonesia). Chlorhexidine 0.2% (Minosep, Jakarta) was used as the positive control, and distilled water and acetic acid served as negative controls.

Tools and Equipment

The study employed several instruments, including a rotary evaporator, autoclave (GEA Steriliser), hot plate with magnetic stirrer, centrifuge (Hermle Z 446 K, Germany), incubator (LIB-080M, Labtech), and biosafety cabinet (Class II A2, Biobase). Additional tools included micropipettes, sterile Petri dishes, inoculating loops, sterile tweezers, filter paper (Whatman), disc paper (6 mm), and standard laboratory glassware (test tubes, beakers, Erlenmeyer flasks).

Preparation of *Cymbopogon citratus* Extract

Fresh lemongrass leaves were washed, air-dried for two weeks, and cut into small pieces. The dried leaves were macerated in 70% ethanol at a ratio of 1:10 (w/v) for 72 hours at 27°C with manual agitation every 15 minutes for 8 hours per day. The resulting extract was filtered through Whatman filter paper and concentrated using a rotary evaporator at 50-60°C [12].

Synthesis of $AgNO_3$-*Cymbopogon citratus*-Chitosan Nanocomposite (AgChCc)

A total of 1.70 g of silver nitrate was dissolved in 100 mL of distilled water and heated at 40°C for 30 minutes. Separately, 1.5 g of chitosan was dissolved in 30 mL of 1.5% acetic acid. The chitosan solution was then added to the $AgNO_3$ solution and adjusted to a final volume of 100 mL. The mixture was stirred and heated again at 40° C for 30 minutes and then incubated in the dark at room temperature for 24 hours. A colour change from colourless to yellowish-brown indicated the successful synthesis of silver nanoparticles [13],[14].

Preparation of Test Samples

The synthesized nanocomposite was centrifuged at 3,000 rpm for 3 minutes. The supernatant was collected and diluted using 1.5% acetic acid to obtain concentrations of 6.25, 12.5, 15, 25, and 50 mg/mL for antibacterial testing.

Bacterial Inoculum and Media Preparation

The test organism, *Pseudomonas aeruginosa* ATCC 10145, was cultured in Brain Heart Infusion broth. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. Nutrient agar media were prepared according to manufacturer instructions and sterilised at 121° C for 15 minutes.

Antibacterial Activity Test (Disc Diffusion Method)

The antibacterial effect of AgChCc nanocomposites was assessed using the disc diffusion method. Sterile cotton swabs were used to inoculate the surface of solidified NA with the bacterial suspension. Sterile paper discs (6 mm in diameter) were placed onto the agar, and 20 µL of each sample concentration was applied to the discs. Plates containing chlorhexidine (positive control), acetic acid, and distilled water (negative

controls) were also prepared. The Petri dishes were incubated anaerobically at 37°C for 24, 48, and 72 hours [15].

Measurement of Inhibition Zone

After each incubation period, the diameter of the inhibition zones was measured using a digital ruler. The average inhibition zone was calculated using the following formula:

$$\text{Zone of Inhibition (mm)} = \frac{p+q}{2}$$

where p represents the longest diameter and q the shortest diameter of the inhibition zone [15].

Statistical Analysis

All experimental data were presented as mean ± standard deviation (SD). The normality of the data was tested using the Shapiro-Wilk test. If the data were normally distributed (P > 0.05), they were analysed using One-Way ANOVA, followed by Tukey's post hoc test to determine significant differences between groups. A significance level of P < 0.05 was considered statistically significant. Statistical analysis was conducted using SPSS version 29 [14], [15].

3. Results and Discussion

Inhibition zone observation results at 24, 48 and 72 hours

Research on the antibacterial activity of AgCh nanocomposites synthesised with *C. citratus* with the use of chitosan stabiliser against *P. aeruginosa* inhibition zone formation at concentrations of 6.25 mg/mL, 12.5 , 15 mg/mL, 25 mg/mL and 50 mg/mL, observed at 24 hours, 48 hours and 72 hours.

The results of the normality test with Shapiro Wilk on observations for 24 hours, 48 hours, and 72 hours showed a value > 0.05 in **Table 1**. The statistical test was then continued with a parametric test using oneway ANOVA. The results of the oneway ANOVA test showed a value of P > 0.05, indicating that there were significant differences that occurred in the study group so that further testing was continued using Post Hoc Tukey.

Table 1. Shapiro-Wilk Normality Test Results for Inhibition Zone Data at 24, 48, and 72 Hours

Group	Normality Test (Shapiro-Wilk)		
	P Value		
	24 hours	48 hours	72 hours
Composite 6.25 mg/mL	1.000	1.000	1.000
12.5 µg/mL Composite	1.000	0,984	1.000
15 µg/mL Composite	1.000	0,988	0,988
25 µg/mL Composite	1.000	1.000	0,991
50 µg/mL Composite	1.000	0,989	1.000
Positive control *chlorhexidine*	1.000	1.000	0,992
Acetic acid negative control	1.000	1.000	0,989

Zone observation results at 24 hours

The results of further tests on the inhibition zone of *P. aeruginosa* at 24 hours showed significant differences with a value of P< 0.05 in the study group. In the negative control group, there was a significant difference (P< 0.05) with the positive control group and AgCh nanocomposite synthesised with *C. citratus* (AgChCc) concentrations of 6.25, 12.5, 15, 25, and 50 mg/mL. The inhibition zone results at 24 hours observation can be seen in **Figure 1**.

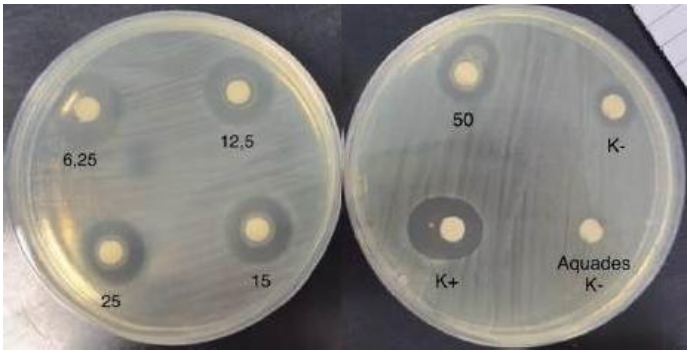


Figure 1. Inhibition Zone Diameter of P. aeruginosa Treated with AgChCc Nanocomposites at 24 Hours

The negative control group had the smallest inhibition zone when compared to the other groups. The positive control group showed no significant difference, P = 0.360 with the AgChCc group of 6.25 mg/mL concentration and the AgChCc group of 25 mg/mL concentration with a value of P = 0.068. The positive control group showed significant differences (P < 0.05) in the negative control, 12.5, 15, and 50 mg/mL AgChCc groups. The positive control group showed the largest inhibition zone formation compared to the other groups. The results of the 24-hour time zone observation can be seen in **Table 2**.

Table 2. Results of the zone of inhibition of *P. aeruginosa* at 24 hours observation

Group	Number of samples	Zone of Inhibition (mm) ± SD	P Value
Acetic acid negative control	3	5.20± 0.99^{d}	P< 0.05
Positive control *chlorhexidine*	3	12.18± 0.75^{a}	P< 0.05
Composite 6.25 mg/mL	3	11.11± 0.11^{ab}	P< 0.05
12.5 mg/mL Composite	3	10.50± 0.35^{b}	P< 0.05
Composite 15 mg/mL	3	9.59± 0.47^{bc}	P< 0.05
25 mg/mL Composite	3	10.59± 0.62^{ab}	P< 0.05
50 mg/mL Composite	3	8.62± 0.51^{c}	P< 0.05

a-d in different columns show significant differences

Zone observation results at 48 hours

The observation of the inhibition zone at 48 hours showed that there was a significant difference (P< 0.05) between the negative control group and the positive control group, AgChCc 6.25, 12.5, 15, 25 and 50 mg/mL groups. The results of the inhibition zone observation at 48 hours can be seen in **Figure 2**.

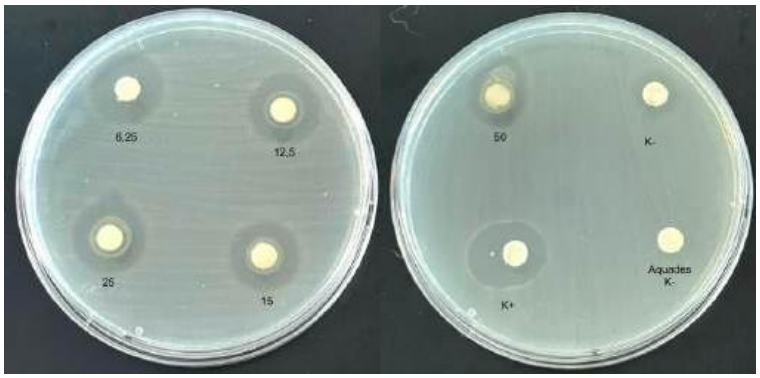


Figure 2. AgCh nanocomposite antibacterial activity test results against *P. aeruginosa* at 48 hours

The negative control group showed the smallest zone of inhibition when compared to the other groups. The positive control group showed no significant difference (P > 0.05) in the AgChCc 6.25 mg/mL and 25 mg/mL groups, but significantly different (P < 0.05) with the negative control, AgChCc 12.5, 15, and 50 mg/mL groups. The 6.25 mg/mL AgChCc group demonstrated the largest inhibition zone, followed by the AgChCc 25, 12.5, 15, and 50 mg/mL groups. The AgChCc 50 mg/mL group demonstrated the smallest inhibition zone among all AgChCc-treated groups. The results of the zone of inhibition at 48 hours observation can be seen in **Table 3**.

Table 3. Results of zone of inhibition of *P. aeruginosa* at 48 hours observation

Group	Number of samples	Zone of Inhibition (mm) ± SD	P Value
Acetic acid negative control	3	5.20± 0.99^{d}	P< 0.05
Positive control *chlorhexidine*	3	12.18± 0.75a	P< 0.05
Composite 6.25 mg/mL	3	11.11± 0.11^{ab}	P< 0.05
12.5 mg/mL Composite	3	10.50± 0.35^{b}	P< 0.05
Composite 15 mg/mL	3	9.59± 0.47^{bc}	P< 0.05
25 mg/mL Composite	3	10.59± 0.62^{ab}	P< 0.05
50 mg/mL Composite	3	8.62± 0.51^{c}	P< 0.05

a-d in different columns show significant differences

Zone observation results at 72 hours

The observation of the inhibition zone at 72 hours showed significant differences (P< 0.05) in the study group. The results of the observation of the inhibition zone at 72 hours can be seen in **Figure 3**.

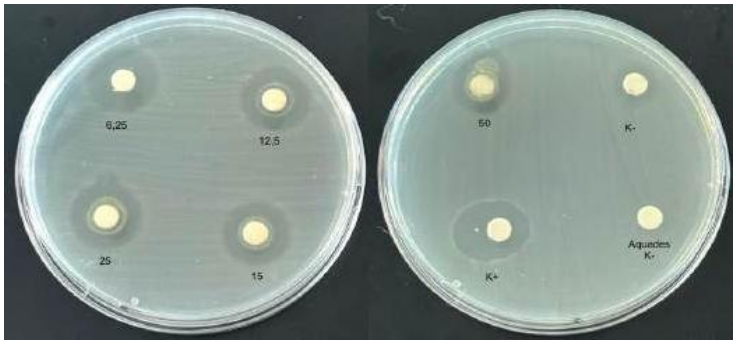


Figure 3. AgCh nanocomposite antibacterial activity test results against *P. aeruginosa* at 72 hours

The negative control group showed significant differences (P < 0.05) when compared to the other groups, by forming the smallest zone of inhibition. The positive control group, showed significant differences (P < 0.05) in the negative control group, AgChCc 12.5, 15, 25, and 50 mg/mL. While the AgChCc 6.25 mg/mL group did not show significant differences (P>0.05) in the positive control group. The positive control group showed the formation of the largest inhibition zone then the AgChCc 6.25, 25, 12.5 15 and 50 mg/mL groups. The results of the inhibition zone at 72 hours observation can be seen in **Table 4**.

Table 4. Results of zone of inhibition of *P. aeruginosa* at 72 hours observation.

Group	Number of samples	Zone of Inhibition (mm)± SD	P Value
Acetic acid negative control	3	5.695± 0.51^{e}	P< 0.05
Positive control *chlorhexidine*	3	12.20± 0.74^{a}	P< 0.05
Composite 6.25 mg/mL	3	11.09± 0.14^{ab}	P< 0.05
12.5 mg/mL Composite	3	10.48± 0.32^{bc}	P< 0.05
Composite 15 mg/mL	3	9.56± 0.48^{cd}	P< 0.05
25 mg/mL Composite	3	10.67± 0.61^{bc}	P< 0.05
50 mg/mL Composite	3	8.61± 0.56^{d}	P< 0.05

a-d in different columns show significant differences

Antibacterial activity showed that AgChCc treatments at concentrations of 6.25 mg/mL, 12.5 mg/mL, and 25 mg/mL formed inhibition zones that were not significantly different from the chlorhexidine positive control group, but the inhibition zone formed by chlorhexidine was slightly larger than the AgChCc 6.25 mg/mL, 12.5 mg/mL, and 25 mg/mL groups. In addition, the 15 mg/mL AgChCc group and the 50 mg/mL group did not show significant differences, forming the smallest zone of inhibition compared to the 6.25 mg/mL, 12.5 mg/mL, and 25 mg/mL AgChCc groups. The negative control group administered with acetic acid formed the smallest zone of inhibition compared to the other groups.

The AgChCc group showed the ability to form inhibition zones at all concentrations. The ability of AgChCc to inhibit the growth of *P. aeruginosa* is influenced by the materials that make up the nanocomposite, the three constituent materials are able to synergise well so as to increase its activity as an antibacterial. AgChCc is the result of the synthesis of silver nanoparticles (AgNPs) that are reduced through chemical reduction methods, and involves three main components, namely metal precursors, reductants, and capping agents. The precursor used was silver salt ($AgNO_3$), while *C. citratus* extract served as the reductant and chitosan as the stabilising agent and capping agent. Chitosan has amine (-NH2) and hydroxyl (-OH) groups that can interact with transition metal cations, making it effective in stabilising AgNPs [16]. The large inhibition zone formed by the AgChCc nanocomposite is because the nanocomposite has pores so that silver nanoparticles can be well immobilised. Silver nanoparticles are nanoparticle materials that have good antibacterial properties the presence of silver nanoparticles allows for an increase in the inhibition zone. The results of the study are in line with the results of previous research using AgNP nanocomposites synthesised with macassar fruit extract and capped using chitosan, showing the inhibition zone of nanocomposites is better than the positive control [17].

In the field of health, silver nanoparticles have been widely used because they can function as antibacterials. Silver nanoparticles can interact directly with bacterial membranes and cause membrane damage and bacterial death. Silver nanoparticles will first adhere to the surface of the bacterial membrane and penetrate inside which eventually changes the permeability of the bacterial membrane. The change in permeability causes damage to the membrane. The antibacterial activity of silver nanoparticles depends on the size, shape, and surface which determine the success in damaging the bacterial membrane. Silver nanoparticles with a small size can interact

with the protective lignin layer on bacteria better [17].

Based on the results of the antibacterial activity test of AgChCc nanocomposites against the growth of *P. aeruginosa* bacteria, showing the formation of inhibition zones at 24 hours, 48 hours and 72 hours of observation, the inhibition zones remained consistent over prolonged incubation, indicating stable antibacterial activity.

The results of this study are in line with previous results showing antibacterial and antibiofilm activity of ZnO-Ag nanocomposites with clove oil against *P. aeruginosa* with the Green One Pot Synthesis method showing the ability to inhibit the growth of *P. aeruginosa* bacteria, by preventing the attachment of *P. aeruginosa* biofilm for 72 hours [18]. Another study on AgCh nanocomposites synthesised with Allium sp. had good antibacterial activity against *P. aeruginosa*, by forming a clear zone of inhibition [9]. In addition, this study is also in line with previous research that showed AgCh nanocomposites synthesised with Officinale rhizome indicated the inhibition of *P. aeruginosa* growth [19].

A decrease in the effectiveness of nanocomposites at high concentrations can occur, which is influenced by many factors such as the interaction of active compounds to form aggregates that reduce the availability to react with AgNO3. The next possibility is the saturation effect that occurs when the target is met, so that excess material becomes ineffective, in addition it can also produce large amounts of by-products at high concentrations so that, it can inhibit the reaction. The last possibility could also be structural damage or toxic effects interfering with the interaction of the material with the target [18].

This study has several methodological and technical limitations that need to be considered in the interpretation of results and planning of further studies. One of the main obstacles is the potential for human error during the experimental process, such as in the weighing of materials, homogenisation of nanocomposite suspensions, and measurement of inhibition zones, which is subjective, which can affect the accuracy and reproducibility of data. In addition, in-depth characterisation of the physicochemical properties of the $AgNO_3$-*Cymbopogon citratus*-Kitosan (AgChCc) nanocomposite has not been conducted, such as analysis of particle size and distribution, morphological shape through electron microscopy (SEM/TEM), and zeta potential which plays an important role in the stability and bioactivity of nanoparticles. The absence of this data makes it difficult to understand the relationship between nanoparticle structure and their mechanism of action as antibacterial agents. This study was limited to a single Gram-negative bacterial strain, *Pseudomonas aeruginosa*, so it does not reflect the scope of antibacterial activity against Gram-positive bacteria or multiresistant strains. In addition, the method used in the test, namely the disc diffusion method, is semi-quantitative and has not been equipped with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests which are more representative to measure antibacterial potential quantitatively. Toxicological studies on nanocomposites have also not been conducted, either in vitro or in vivo, so it cannot be concluded how safe these materials are to be used in pharmaceutical or biomedical applications. Thus, the results of this study still need to be further studied through a multidisciplinary approach involving material characterisation, advanced activity tests, and toxicity analysis to produce more comprehensive and applicable data.

4. Conclusion

The antibacterial activity test results showed that the $AgNO_3$-*Cymbopogon citratus*-Kitosan nanocomposite was effective in inhibiting the growth of *Pseudomonas aeruginosa*, with the best inhibition zone achieved at a concentration of 6.25 mg/mL with an average diameter of 11 mm. This effectiveness is close to the positive control group (chlorhexidine), although it has not been able to surpass it statistically. The next order of effectiveness was shown by concentrations of 25 mg/mL, 12.5 mg/mL, 15 mg/mL, and 50 mg/mL. Although the effectiveness has not surpassed chlorhexidine, this nanocomposite shows great potential as an alternative antibacterial candidate based on environmentally friendly natural materials, which opens up application opportunities in antimicrobial formulations in the pharmaceutical and biomedical fields. To strengthen

these findings, further research that includes in-depth physicochemical characterisation of the nanoparticles (size, morphology, and stability), testing against various other types of bacteria, as well as in vitro and in vivo toxicity evaluation to comprehensively assess their safety and clinical effectiveness, including testing quantitative parameters such as MIC and MBC, is recommended.

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Conflicts of Interest:
The authors declare no conflicts of interest.

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