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Formulation and Evaluation of Bioactive Composite Hydrogel Nanochitosan from Siwalan Fruit Shell (Borassus flabellifer) against Enterococcus faecalis

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A B S T R A C T

Pulp capping is one of the leading treatment methods for reversible pulpitis to maintain pulp vitality. Common pulp capping failures, such as CaOH (calcium hydroxide) and ZOE (zinc oxide eugenol), often result from contamination by microleakage and *Enterococcus faecalis* bacteria. Siwalan (*Borassus flabellifer*) shell, containing chitosan, has antibacterial properties and supports tissue regeneration. This research aims to create a bioactive composite hydrogel with nanochitosan extract from a siwalan shell and evaluate its quality and antibacterial effectiveness against *Enterococcus faecalis*. The nanochitosan extract was prepared through deproteination, demineralization, and deacetylation, followed by FTIR and SEM analysis. The final formulation was evaluated to match the quality standard of the hydrogel. The study used an experimental post-test-only group design with three treatment groups (0.5%, 1%, and 1.5% siwalan shell nanochitosan extract) and two control groups. The antibacterial test was conducted using the tube dilution method, and data were analyzed with One-way ANOVA and Tukey HSD tests. The hydrogel's Minimum Bactericidal Concentration (MBC) was 0.5%. The results of statistical tests show that the calculated F value (37.185) is greater than the F table (2.8661) with a sig value of 0.000 (0.000 < 0.05). Thus, the bioactive composite hydrogel nanochitosan extract from the siwalan shell effectively inhibits the growth *of Enterococcus faecalis* and meets the quality parameters for pulp capping material.

Keywords: *Borassus flabellifer* Shell, Nanochitosan, Bioactive Composite Hydrogel, *Enterococcus faecalis*

INTRODUCTION

The oral cavity is a complex and dynamic environment comprising various surfaces and conditions that foster microbial growth. These conditions, including mucosal and dental surfaces, warm temperatures, high humidity, and nutrient-rich surroundings, contribute to the onset of oral diseases such as dental caries. Dental caries is a bacterial infection that affects the hard tissues of teeth through a slow, degenerative process. According to the 2018 Basic Health Research Data (RISKESDAS), around 88.8% of the Indonesian population is affected by caries, making it one of the top six health issues in the country and signaling a significant public health concern (1). Several factors, such as poor dietary habits, inadequate oral hygiene, and limited access to dental care services, drive the high incidence of caries. Bacteria, particularly *Enterococcus faecalis*, play a central role in the development of caries. This bacterium is not only a pivotal contributor to caries but is also resistant to antibiotics, complicating treatment and potentially leading to further infections if left unchecked. While a combination of antimicrobial and remineralizing agents and antibiotics can help protect and heal pulp tissue, antibiotics present challenges. Over-reliance on antibiotics in caries treatment may fail (2).

Indonesia is recognized as a global biodiversity hotspot, offering substantial potential for various fields, including dental caries management. One plant with considerable potential is the siwalan (*Borassus flabellifer*), a palm species widely found in coastal and tropical areas. Known for its versatility, nearly every part of the siwalan plant is valuable. A notable feature is the siwalan shell, often considered waste, yet it contains 33.34% chitosan (3). Chitosan is well-known for its antibacterial properties and role in promoting tissue regeneration. In its nano form, chitosan exhibits enhanced antimicrobial effects, excellent biocompatibility, and increased efficiency as a drug delivery system. Utilizing siwalan shells for nanochitosan production converts waste into valuable material, significantly amplifying chitosan's bioactivity. This increase in bioactivity makes nanochitosan more effective in promoting the proliferation of odontoblast-like cells and facilitates

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the binding of transforming growth factor-beta 1 (TGF-β1), accelerating the formation of reparative dentin. Consequently, this contributes to faster tooth repair and regeneration (4).

Bioactive composite hydrogels are advanced materials designed to replicate the structure and function of natural tissues. These hydrogels consist of tissue fibbers combined with macromolecules, allowing them to mimic the properties of biological tissues. When formulated with nanochitosan, bioactive composite hydrogels can demonstrate ideal characteristics for use as pulp-capping agents in dental treatments. Their bactericidal or bacteriostatic properties and ability to stimulate reparative dentin formation make them particularly effective for treating dental caries. Developing a bioactive composite hydrogel using nanochitosan derived from siwalan shells offers a promising strategy for dental caries management. This formulation has shown potential as an antimicrobial agent effective against *Enterococcus faecalis*, a primary bacterium involved in caries, thus providing a novel solution for preventing and treating this dental condition (2).

The primary aim of this study is to formulate and evaluate the effectiveness of a bioactive composite hydrogel that contains nanochitosan extracted from siwalan shells against *Enterococcus faecalis*. The effectiveness of the hydrogel was evaluated through antimicrobial tests, exploring its mechanism of action and material stability to ensure its reliability and efficacy. This study seeks to validate the bioactive composite hydrogel's effectiveness in treating dental caries and contributes to developing nanotechnology-based solutions in dental care. The research promotes sustainability by utilizing waste materials such as siwalan shells while offering an alternative, innovative solution to the high prevalence of dental caries in Indonesia.

MATERIALS AND METHODS

The research was conducted at the Pharmacy and Biochemistry Laboratory of the Faculty of Medicine, as well as the Dentistry Faculty Laboratory, Brawijaya University from May to August 2024.

Materials

Siwalan fruit from Tuban Regency, Indonesia is used as raw material for making chitosan extract, The materials used for nanochitosan preparation consist of NaOH 2N, HCI 2N, NaOH 60%, STPP, acetone, and NaOCI, Furthermore, the hydrogel product was made with three concentrations.

Tabel 1. Formulation of Composite Hydrogel Nanochitosan

Materials	0.5 % (g/100 ml) Concentration	1 % (g/100 ml) Concentration	1.5 % (g/100 ml) Concentration
Nanochitosan	0.5	1.0	1.5
HA	-	1.0	1.5
Acetic acid 1%	0.5	1.0	1.5
Carbopol	1.0	1.0	1.5
Propylene Glycol	10.0	10.0	10.0
Glycerin	5.0	5.0	5.0
Methyl Paraben	0.18	0.18	0.18
Propyl Paraben	0.02	0.02	0.02
TEA	1.5	1.5	2.25
Aquadest	Add 100	Add 100	Add 100

Methods

Preparation and Isolation of Siwalan Shell

The preparation was started by selecting the fresh siwalan fruit shell and performing wet sortation. The shell fruit is chopped and dried in direct sunlight for 3 hours. The dried shell was ground using a blender. The isolation process involves several stages. The first stage is deproteinization, which starts by weighing the powder of the siwalan fruit shell and mixing it with 2N NaOH at a ratio of 1:5 (w/v). The dilution was heated at 70°C for two hours, stirred and cooled for ± 30 minutes, and then filtered. The resulting residue was washed using distilled water and dried at 60°C for four hours (6). The demineralization stage begins by mixing the deproteinization result with 2N HCl at a ratio of 1:5 w/v.

The mixture was left for two days at room temperature. The sample was heated at 75°C for one hour while stirred and filtered. The residue was washed and dried as before for two hours. The deacetylation stage begins by mixing the demineralization result with 60% NaOH at a ratio of 1:1 w/v. Then, the sample was heated at 90°C for 3 hours while stirred. The mixture was filtered, washed, and dried (5, 6). The depigmentation stage is carried out by adding acetone and NaOCl to the deacetylation residue at a ratio of 1:2 (w/v), then leaving for one day and drying for three hours (6). Chitosan characterization was carried out by determining the functional groups of the material extracted through the last stage of isolation, namely deacetylation, using Fourier Transform Infrared (FTIR) spectroscopy analysis. In the FTIR Spectroscopy analysis, the chitosan sample was placed on an FTIR plate and compared with the FTIR results of pure chitosan standards and library match results (8).

Nanoparticles Preparations

The chitosan extract was dissolved in 300 mL of 1% acetic acid. Then, 60 mL of 0.84% tripolyphosphate solution was dripped while stirring at a speed of 1200 rpm. The suspension was lyophilized (7). Chitosan nanoparticles were analyzed using Scanning Electron Microscopy (SEM). SEM determines the shape and size of chitosan particles.

Composite Hydrogel Nanochitosan Preparations

The process begins with dissolving the nanoparticles in 1% acetic acid. Prepare 70 grams of hot aquadest at 98-100°C, then add methylparaben and propylparaben while stirring at 500 rpm. Propylene glycol was added, the temperature was decreased to 38-42°C, and the dissolved extract and HA were added alternately. Add glycerin and stir at 1000 rpm. Disperse carbopol 940, then add triethanolamine until a gel with a watery consistency is formed (19). Evaluation of finished products includes organoleptic, homogeneity, pH, viscosity, spreading, and adhesion tests.

Hydrogel Quality Evaluation

The evaluation of hydrogel preparation consists of several stages. First, organoleptic observations were carried out on the product's color, shape, and odor. Furthermore, the hydrogel should be semisolid, watery, homogeneous in color, and have a characteristic extract odor. Second, a homogeneity test was performed by placing the hydrogel between two glass slides and checking for coarse particles. Third, pH was measured using a calibrated pH meter, aiming for an alkaline pH suitable for reparative dentinogenesis. Fourth, viscosity was measured using a Rion viscometer with spindle number 2. Fifth, spreadability was evaluated by measuring the diameter of the spread with varying weights. Sixth, adhesion was tested by measuring the time the hydrogel remained stuck between two glass slides under pressure (9).

Antibacterial Activity Test

The study used three treatment groups, each of which was a concentration of bioactive composite hydrogel nanochitosan extract of siwalan shell of 0.5%, 1%, 1.5%, a positive control group (CaOH paste), and a negative control group (aquadest) with each repetition five times. The method used was tube dilution. *Enterococcus faecalis* was incubated for 24 hours at 37°C. Incubation was conducted in an anaerobic test tube; then, the colony was inserted into BHI-B (Brain Heart Infusion Broth) until the amount obtained was comparable to Brown III solution with a concentration of 10^8 CFU/ml. The solution was diluted again until it reached 10^6 CFU/ml.

Five sterile test tubes were prepared. Each tube was filled with distilled water, CaOH, bioactive composite hydrogel nanochitosan formulation of 0.5%, 1%, and 1.5% siwalan shell extract as much as 1 ml, then added 1 ml of each bacterial suspension. All tubes were incubated in anaerobic tubes for 24 hours at 37°C. The antibacterial power of the test material was determined by measuring the turbidity of the tube using white paper with black lines. In contrast, the control tube was used as a comparison. After that, each tube was given a level based on its clarity.

After determining the antibacterial power, each material was taken using an ose, scratched on BHI-A (Brain Heart Infusion Agar), and incubated in anaerobic tubes for 24 hours at 37°C. The bacterial colonies that grew were counted using a colony counter. Antibacterial activity was determined by calculating the OI (original inoculum), which is bacteria with a concentration of 10^6CFU/ml cultured in agar media before incubation (21).

Statistical Test

The bacterial colony data were analyzed using SPSS 25. If a normal statistical distribution and the same or homogeneous data variance ($p > 0.05$) were obtained, then one ANOVA analysis was continued to determine the significance of the influence of the results of each treatment. A Post Hoc Tukey HSD test determined the treatment group with the most significant difference in reducing *Enterococcus faecalis*.

RESULTS AND DISCUSSION

Isolation of Chitosan from Siwalan Shell

The first steps of raw material preparation include sorting, washing, drying, and grinding. This process aims to extract the necessary part of the siwalan fruit, specifically the shell, and remove impurities that may affect the isolation results. Next is the deproteinization process, which involves removing the protein from the siwalan shell. Demineralization aims to eliminate the minerals from the siwalan shell, resulting in a chitin product. During deacetylation, the acetyl groups in chitin are degraded and produce chitosan, identified as the primary process of chitosan isolation. The isolation process is carried out to obtain purer chitosan. The depigmentation or color separation stage is conducted to remove pigments from the isolated chitosan product (6). The yield results obtained step by step in this experiment are summarized in **Table 2.**

Table 2. Table of Chitosan Isolation Results

Stage	Dry weigh	Yield (w/w)	Color
Initial mass: 35 gram			
Deproteinization	34	97.1%	Brown
Demineralization	32	94.1%	Brown
Deacetylation	25	78.1%	Brown
Depigmentation	20	80%	Brown
Used 6 gram			
Nanoparticle Synthesis	5	83.3%	Brown

The table shows that the highest yield is obtained during deproteinization, while the lowest occurs during deacetylation. The yield decreases as the processing stages advance, producing chitosan with 80% acetyl removal and brown coloration. This

brown color, produced during deacetylation, does not meet the desired white chitosan specification, indicating a successful depigmentation stage. The incomplete depigmentation process accounts for this discrepancy. According to the quality standards set by Protan Laboratories Inc. (1987), chitosan should be white. The suboptimal soaking time during the depigmentation stage likely contributed to the color issue. A proper depigmentation process, which produces white chitosan powder, typically involves acetone and additional bleaching agents such as sodium hypochlorite (NaOCl) (6). In this case, using acetone alone was insufficient to remove all pigments, and adding NaOCl would be necessary to achieve the desired whiteness.

A comparison with previous research on chitosan isolation from green mussel shells involved a deproteinization step using a 3.5% (w/v) NaOH solution at a ratio of 1:10 (w/v) (5). The demineralization phase was performed using 1N HCl with a 1:10 (w/v) ratio followed by deacetylation using 40% (w/v) NaOH at 1:15 (w/v). Another study focused on chitosan isolation from vannamei shrimp shells, the depigmentation stage was conducted by adding acetone at a 1:2 (w/v) ratio (6). The results from studies on chitosan isolation from green mussel shells are presented in **Table 3**

Table 3. Table of Chitosan Isolation Result from Green Mussel Shells

Stage	Yield (w/w)	Color
Deproteinization	92.452%	Cream
Demineralization	46.477%	Grey
Deacetylation	81.33%	White
Depigmentation	76.038%	Brownish White

FTIR Analysis

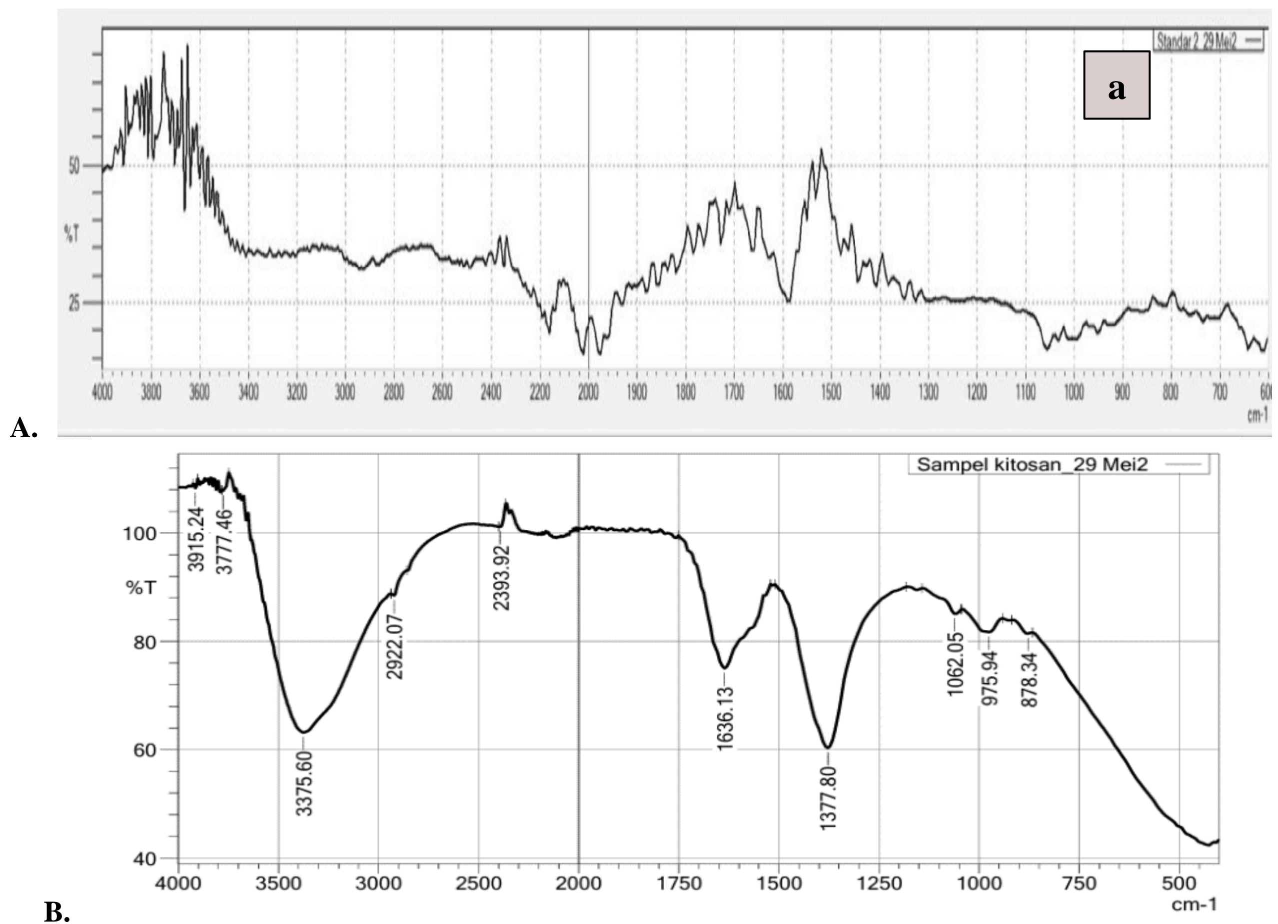


Figure 1. (A) FTIR spectrum of standard chitosan; (B) FTIR spectrum of isolated chitosan sample of siwalan fruit shell

Chitosan characterization was conducted using FTIR instrumentation by analyzing the functional groups of the material isolated from the siwalan shell, comparing it with the FTIR spectrum of standard chitosan, and matching the sample spectrum with a library. Based on the FTIR spectrum in the figure above, the produced chitosan sample meets the specifications regarding the functional groups present in chitosan (8). Compared with the standard and library, the functional groups of the chitosan sample show a high degree of similarity. This is evidenced by comparing functional groups at specific wavelengths we conducted on the FTIR chromatogram results, as shown in **Table 4.**

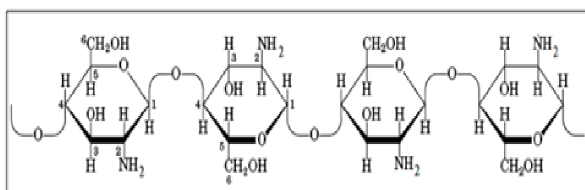


Figure 2. Molecular structure of chitosan

The functional groups in the molecular structure of chitosan, as shown in **Figure 2**, include amine groups ($-NH_2$) and hydroxyl groups (-OH). The amine group is primarily found on the second carbon atom (C-2) within the D-glucosamine units of chitosan, while the hydroxyl groups are located at the C-3 and C-6 positions. From the FTIR spectrum data above, absorption bands can be observed, indicating vibrations of the functional groups present in chitosan. The characterization results of the functional groups found in chitosan can be seen in **Table 4 below**.

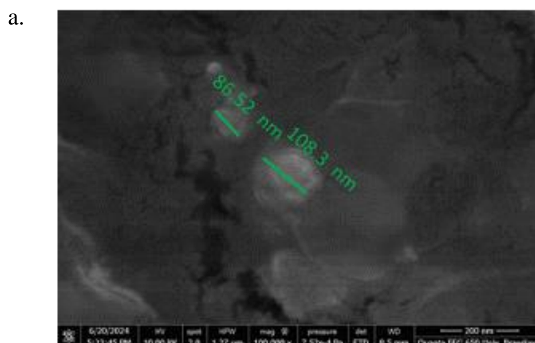
Table 4. Functional Groups of Chitosan

Functional Groups	Compound	Vibration Type	Wave Number (cm-1)		Range of Wave Number (cm-1) (Arsyi *et al.*, 2018)
			Chitosan Sample	Chitosan Standard	
C-H	Saturated Alkane	*bending*	3777.46	3811.90	3000-3850
O-H and -NH	Phenol and Amine	*stretching*	3375.60	3639.68	3650-3200
C – H aliphatic	Alkane	*stretching*	2922.07	2801.50	2800-2925
C-H	Aromatic Ring	*stretching*	2393.92	2013.58	3300-2700
C = O	Carbonyl	*bending*	1636.13	1666.30	1740-1560
C – H	Aliphatic	*bending*	1377.80	1382.13	1465-1370
C-N	Amine	*stretching*	1062.05	1045.42	1350-1000

SEM Analysis

Dentin consists of microscopic dentinal tubules with a diameter of approximately 2-4 µm. The size and reactivity of nanoparticles allow the material to penetrate further into the dentinal tubules, with the potential for decontamination, remineralization, and sensitivity reduction (12). SEM observations showed chitosan nanoparticles with spherical morphology (nanospheres) ranging in size from 39.0 nm to 108.3 nm, enabling good penetration into the dentinal tubules, in comparison to previous research, which utilized nano-hydroxyapatite material for in vitro study on human third molars. The same testing method was used, namely SEM particle size analysis, which indicated a size of 50 nm (17). The result indicates that the result of the chitosan isolation nanoparticle sample is appropriate for its intended application, specifically for use in dentin.

a.



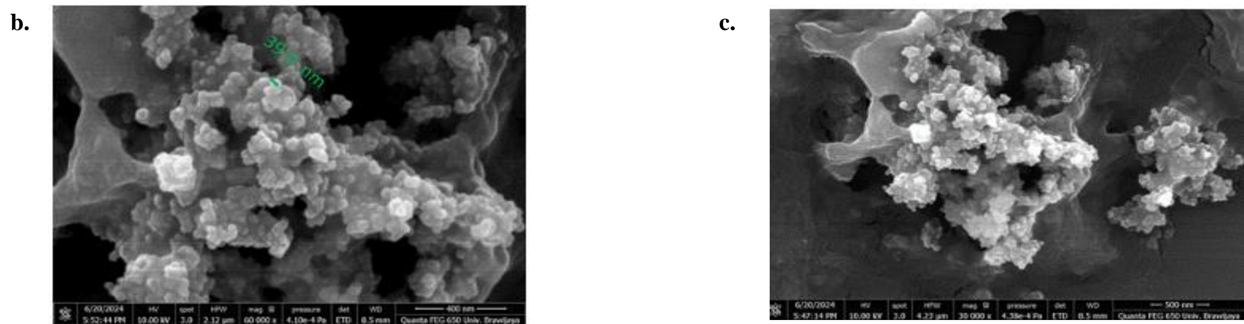


Figure 3. (a) Chitosan nanoparticles at 100,000x magnification with particle size of 86.52 nm and 108.3 nm; (b) Chitosan nanoparticles at 60,000x magnification with particle size of 39 nm; (c) Chitosan nanoparticles at 30,000x magnification

Evaluation Results of the Preparation Organoleptic Test

Table 5. Organoleptic Test Result

Formula	Odor	Color	Form
K 0.5%	Distinctive smell	Clear dark brown	Semi-solid, watery
K 1%	Distinctive smell	Turbid light brown	Semi-solid, watery
K 1.5%	Distinctive smell	Clear dark brown	Semi-solid, watery

Observations of organoleptic characteristics conducted visually revealed that the preparations exhibited identical odor, color, and form among all concentrations, except for the color at 0.5%. Therefore, each preparation is similar to the criteria due to the absence of a strong odor (13) characterized as good specification.

Homogeneity Test

Table 6. Homogeneity Test Result

Formula	Homogeneity
K 0.5%	✓
K 1%	✓
K 1.5%	✓

The formulas have a homogenous structure and soft texture, which indicates that the mixing process was successful. It can be concluded that the preparation meets good specifications as it is free from clumped particles or coarse grains (22).

pH Test

Table 7. pH Test Result

Formula	pH
K 0.5%	8.10
K 1%	8.14
K 1.5%	8.12

Alkaline conditions might promote the release of bonds between the dentin collagen and growth factors, leading to the release of TGF-ß1 and other bioactive molecules initially trapped within the dentin. The alkalinity in pulp capping materials and the presence of TGF-ß1 have been proven beneficial in reparative dentinogenesis (10). Based on previous research examining the chemical and physical properties of pulp capping agent formulations, the alkaline pH observed ranged between 8 and 12.4. Thus, the pH of the prepared hydrogel formulation falls within the optimal pH range.

Viscosity Test

Table 8. Viscosity Test Result

Formula	Viscosity (dPa.s)
K 0.5%	120
K 1%	140
K 1.5%	120

The viscosity results fulfill the specification range, confirming its suitability for safe and effective use. Hydrogels' optimal viscosity range is between 50 dPa.s and 400 dPa.s. Compared to

previous research, four hydrogel formulations containing the active ingredient sodium diclofenac were reported to have viscosity values ranging from 260 to 380 dPa.s (20).

Spreadability Test

Table 9. Spreadabilty Test Result

Formula	Weight of 50 g (cm)	Weight of 100 g (cm)	Weight of 200 g (cm)	Weight of 500 g (cm)
K 0.5%	4.5	4.7	5.1	6.1
K 1%	4	4.3	4.7	5.3
K 1.5%	4.6	4.9	5.2	6

The spreadability results rise as the weight of weights increases and meets the specified range of 5-7 cm. Therefore, it can be concluded that the preparation can be effectively used (16). The evaluation of spreading ability in hydrogel formulations is crucial for determining the extent to which the formulation can disperse over the exposed dentine surface. Optimal spreading assures that the formulation is distributed and effective in delivering therapeutic benefits, such as improved absorption of active ingredients and enhanced local concentration at the target site, namely dentinal tubules. Furthermore, optimal spreading contributes to user comfort and satisfaction, as it can influence the ease of application and the sensory properties of the hydrogel (14).

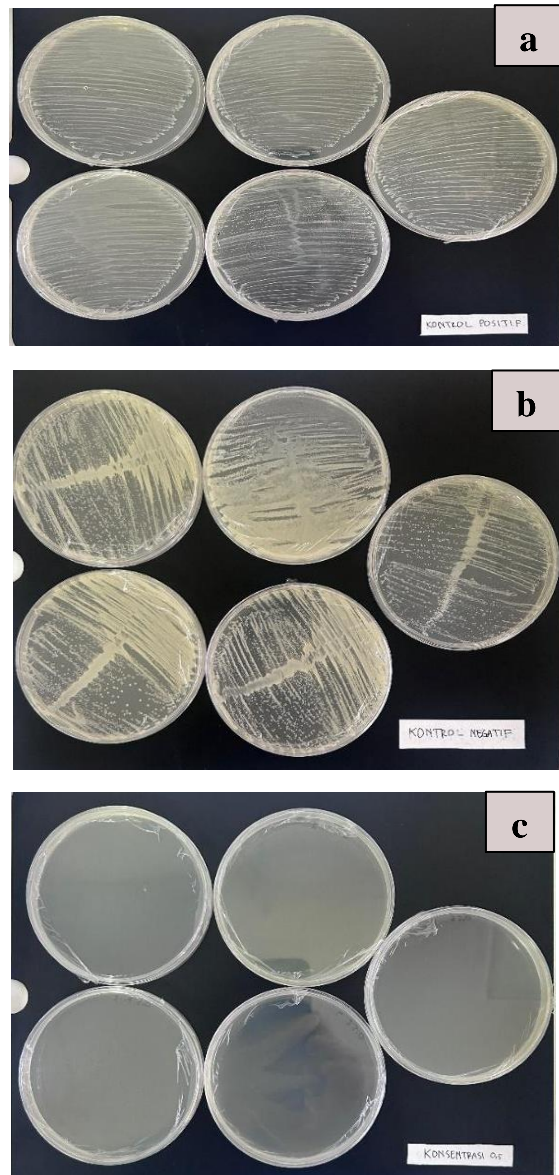
Adhesive Strength Test

Table 10. Adhesive Strength Test Result

Formula	Time (seconds)
K 0.5%	7.13
K 1%	6.42
K 1.5%	4.69

The adhesive strength test results indicate that as the concentration of the preparation increases, its adhesive strength decreases. However, the results still fall within the range of good adhesive strength for hydrogel preparations. The requirement for adhesive strength in gel formulations is at least 4 seconds (14).

Result of Antibacterial Activity Test



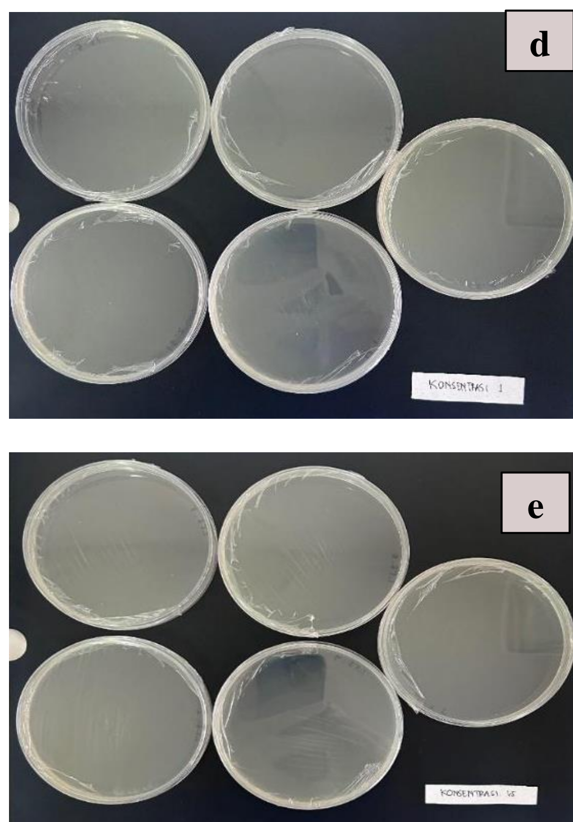


Figure 4. (a) Positive control antibacterial test; (b) Negative control antibacterial test; (c) Antibacterial test of concentration 0.5; (d) Antibacterial test of concentration 1; (e) Antibacterial test of concentration 1.5

The antibacterial activity test was determined based on the turbidity of tubes before and after incubation, followed by turbidity level assessment. The research findings indicate that there was no change in turbidity of the bioactive composite hydrogel tubes containing nanochitosan extract from siwalan fruit shell at concentrations of 0.5%, 1%, and 1.5% after incubation. From the test results, it was found that the bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell at concentrations of 0.5%, 1%, and 1.5% could inhibit the activity of *Enterococcus faecalis* bacteria.

The minimum bactericidal concentration (MBC) of the bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell against *Enterococcus faecalis* was determined when colony growth was 0 or less than 0.1% of the initial inoculum (OI) (21). It was found that there was no bacterial colony growth in the test material at a concentration of 0.5%. According to the criteria,

The bioactive composite hydrogel containing nanochitosan extract from the siwalan fruit shell at a concentration of 0.5% represents the MBC. This finding is consistent with previous research, which reported that the ethanol extract of siwalan fruit shell showed activity against *Streptococcus mutans* in MIC and MBC tests at a concentration of 7.813 mg/mL (21). Another study mentioned that chitosan isolated from siwalan fruit shell showed MIC activity against *E. coli* at concentrations below 1% (3).

Table 11. Colonies Growth and Turbidity Level of Each Formulation

Treatment	Number of Colonies					Average	Turbidity
	I	II	III	IV	V		
Control (-)	636	1452	872	1208	844	1002.4	Turbid (++)
Control (+)	648	960	452	472	652	636.8	Turbid (++)
K 0.5%	0	0	0	0	0	0	Clear (-)
K 1%	0	0	0	0	0	0	Clear (-)
K 1.5%	0	0	0	0	0	0	Clear (-)

Based on the one-way ANOVA analysis, the research data shows that the calculated F value exceeds the tabulated F value. The calculated F value in this study is 37.185, with a tabulated F value of 2.8661 and a significance value of 0.000 ($0.000 < 0.05$), indicating rejection of the null hypothesis (H0) and acceptance of the alternative hypothesis (H1). In other words, the treatment

of bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell significantly influences the antibacterial effectiveness against *Enterococcus faecalis* growth. This significant antibacterial capability is attributed to the active substance reaction from siwalan shell extract, specifically optimized into nanochitosan. The antimicrobial mechanism of chitosan against bacteria can be explained through two theories. The first theory states that the amino functional groups on chitosan can bind to bacterial cell walls, causing leakage of intracellular constituents and resulting in bacterial lysis. The second theory suggests that chitosan begins its action by damaging bacterial cell walls, subsequently binding to intracellular components, blocking mRNA, and inhibiting protein synthesis (17).

Based on Pos Hoc Tukey HSD analysis, it is shown that the first treatment using Formula 1 (0.5%), positive control (CaOH 1.0 g), and negative control (distilled water) have significantly different levels with values of 0.00 CFU/mL, 636.80 CFU/mL, and 1002.40 CFU/mL. The second treatment using Formula 2 (1.0%), positive control (CaOH 1.0 g), and negative control (distilled water) also show significantly different levels with values of 0.00 CFU/mL, 636.80 CFU/mL, and 1002.40 CFU/mL. The third treatment using Formula 3 (1.5%), positive control (CaOH 1.0 g), and negative control (distilled water) similarly exhibit significantly different levels with values of 0.00 CFU/mL, 636.80 CFU/mL, and 1002.40 CFU/mL. Therefore, based on these research findings, it can be explained that the concentrations in Formula 1, Formula 2, and Formula 3 have equal antibacterial effectiveness against *Enterococcus faecalis* growth, which is significantly better compared to the antibacterial effectiveness of the standard pulp capping material CaOH.

CONCLUSION

The bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell has been proven to have nanoparticle sizes ranging from 39.0 nm to 108.3 nm. It has met all quality standards for hydrogel formulations, as confirmed by organoleptic, homogeneity, pH, viscosity, spreadability, and adhesiveness evaluations, thus making it suitable for use as a pulp capping agent. The bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell can be formulated as a hydrogel preparation with concentrations of 0.5%, 1%, and 1.5%. The Minimum Bactericidal Concentration (MBC) of the bioactive composite hydrogel containing nanochitosan extract from siwalan fruit shell against *Enterococcus faecalis* is found at a concentration of 0.5%. The antibacterial activity of all concentrations against *Enterococcus faecalis* growth is the same, which is much better than the antibacterial activity of the standard pulp capping material.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest to disclose regarding this research.

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