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Synergistic antibacterial effects of aloe vera doped with Chitosan against Porphyromonas gingivalis as a natural periodontal inflammatory remedy

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Abstract

With the rise of antibiotic resistance, there is growing interest in developing herbal treatments for periodontal inflammation. Aloe vera has attracted scientific interest for its therapeutic potential, although formulating effective herbal medicines remains a challenge. Chitosan-based nanoparticles offer a promising strategy to enhance the delivery of herbal compounds. This study investigates the combination of Aloe vera extract and chitosan nanoparticles to create a potent antibacterial agent targeting *Porphyromonas gingivalis*, while also assessing its toxicity on BHK-21 fibroblast cells. The antibacterial efficacy was evaluated using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods, employing 80% Aloe vera and 25% chitosan nanoparticles. The combination of 80% Aloe vera extract with varying chitosan nanoparticle concentrations resulted in a greater inhibition zone than either component alone. While both agents demonstrated antibacterial activity, their effects were additive rather than synergistic. Furthermore, MTT Assay results showed that Aloe vera extract at 100% and 80% concentrations, combined with different chitosan nanoparticle formulations, was non-toxic to BHK-21 fibroblast cells.

Keywords: Antibacterial activity, chitosan nanoparticles, Porphyromonas gingivalis, viability of fibroblast cells.

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1. Introduction

Porphyromonas gingivalis, an anaerobic gram-negative bacterium, is widely regarded as a primary causative agent of periodontal disease [1, 2]. This microorganism produces various extracellular proteases and virulence factors such as lipopolysaccharides, fimbriae, gingipains, and a capsule, all of which contribute to dysbiosis and disease progression [2]. Gingipain, in particular, is considered the most significant virulence factor and can be found either attached to the bacterial surface or secreted into the surrounding environment [2]. The primary approach to both the prevention and treatment of periodontal disease involves the routine mechanical removal of dental plaque. However, to enhance treatment outcomes, especially in challenging areas like deep periodontal pockets and furcation sites, antibiotics are often used alongside scaling and root planning [3, 4]. Research has indicated that this combo therapy can greatly increase the efficacy of treatment [3].

Given the growing problem of antibiotic resistance due to misuse and overuse of conventional antimicrobials, alternative treatment options must be found immediately [5]. One promising approach involves the use of herbal medicines with antimicrobial properties to manage periodontal inflammation [6, 7]. Natural remedies, such as herbal formulations, are typically more cost-effective and are less likely to cause adverse consequences than manufactured medications [7, 8]. Aloe vera, one of the oldest known medicinal plants, has shown considerable therapeutic potential due to its biological activities. It thrives in tropical regions and contains bioactive compounds with antioxidant, antifungal, anti-inflammatory, antibacterial, and wound-healing properties. Traditionally, aloe vera has been used to manage periodontitis and is commonly incorporated into mouthwashes and topical gels [7, 8]. Despite its benefits, challenges remain in the formulation and standardization of herbal medicines [4].

The use of herbal medicines is frequently restricted by formulation issues, such as the active compounds' large molecular size that prevents them from crossing lipid membranes, their unpredictable toxicity, poor bioavailability, low solubility, limited oral absorption, and instability in acidic environments [9]. To overcome these barriers and enhance both the absorption and targeted delivery of herbal compounds, nanoparticle-based drug delivery systems are being investigated [10].

Chitosan, a natural polymer, has emerged as a promising carrier for nanoparticle-mediated drug delivery. It possesses lysozyme and amino polysaccharides with antibacterial properties that can inhibit microbial growth [10, 11]. Chitosan is particularly effective against bacteria such as E. coli and E. faecalis, with its antimicrobial activity linked to potassium ion release that disrupts bacterial function [10, 12]. Nanoparticles made from chitosan, typically ranging from 10 to 1000 nm, have being investigated more and more for their capacity to pass across cell membranes, pass through narrow capillaries, and reach target tissues quickly, thereby accelerating therapeutic action. Additionally, chitosan nanoparticles offer enhanced stability and may reduce adverse drug reactions [13-15].

Aloe vera has also been recognized for its antibacterial potential and is considered a valuable therapeutic agent in dentistry, especially in the management of periodontal disease, as supported by numerous in vivo and in vitro studies. When combined, aloe vera and chitosan nanoparticles have shown effectiveness in inhibiting anaerobic bacteria such as Candida albicans, Streptococcus mutans, and Enterococcus faecalis. Previous research has examined the antibacterial effects of calcium hydroxide and chitosan nanoparticles in root canal treatments [14, 15]. However, few studies have looked specifically at the antibacterial efficacy of chitosan nanoparticles and aloe vera extract against periodontal infections. The purpose of this research is to create a local antimicrobial gel with chitosan and aloe vera nanoparticles and assess how well it inhibits *Porphyromonas gingivalis bacteria*.

2. Method

2.1. Grouping

The Faculty of Dentistry at Universitas Indonesia's Research Ethics Commission granted ethical approval for this work under ethical clearance number 04/Ethical/FKGUI/III/2022. The guiding principles of the Declaration of Helsinki were followed when conducting the study. An experimental design was utilized, with sample groups categorized as follows:

- Group 1: Determination of the Minimum Inhibitory Concentration (MIC) of *Aloe vera* extract at concentrations of 20%, 40%, 60%, 80%, and 100%.
- Group 2: MIC testing using chitosan nanoparticles at concentrations of 12.5%, 25%, 50%, and 100%.
- Group 3: MIC evaluation of a combined formulation of *Aloe vera* extract and chitosan nanoparticles at varying concentrations.

Dimethyl sulfoxide (DMSO) was used as a negative control, while metronidazole served as the positive control. Each group was tested in triplicate.

2.2. Aloe Vera Extraction

As a member of the Asphodelaceae family, Aloe vera (Aloe vera (L) Burm.f) was taxonomically confirmed by the Botany Research Centre for Biology - LIPI Bogor Herbarium. A maceration procedure employing 96% ethanol (Merck, Germany) in a 1:10 ratio produced the gel [16]. After combining the Aloe vera gel with ethanol and stirring it for six hours, it was left to incubate for twenty-four hours without stirring.

A rotary evaporator was employed to eliminate the solvent at 40–50°C, resulting in a thick, gel-like Aloe vera extract. This extract was subsequently standardized and readied for phytochemical analysis. To obtain varying concentrations (20%, 40%, 60%, 80%, and 100%), the extract was dissolved in DMSO (Merck Chemicals, Germany), with each solution prepared in a total volume of 10 ml.

2.3. Synthesis of Chitosan Nanoparticles

Chitosan nanoparticles were synthesized using the ionic gelation technique, which relies on the electrostatic interaction between positively charged chitosan and negatively charged tripolyphosphate (TPP) [17]. The process began by dissolving 350 mg of low molecular weight chitosan powder (Sigma Aldrich, Germany) in a 1% acetic acid (CH_3COOH) solution (Merck, Germany). This chitosan solution was then added dropwise at a controlled rate of 1 ml per minute to a sodium tripolyphosphate solution (Merck, Germany), while stirring continuously with a magnetic stirrer (Thermo Fisher Scientific, USA) for 24 hours. The resulting mixture was then sonicated in an ultrasonic bath (Branson, USA) for 60 minutes. Sodium chloride (NaCl) was added to improve the stability of the nanoparticles. To prepare varying concentrations (100%, 50%, 25%, and 12.5%), the stock solution was diluted with 1% acetic acid, and each dilution was adjusted to a final volume of 10 ml.

2.4. Preparation of Bacterial Sample and Antimicrobial Measurement

Porphyromonas gingivalis cultures were sourced from the Laboratory of Oral Biology at Universitas Indonesia. The bacteria were grown in nutrient broth (HiMedia, USA) on petri dishes and incubated at 37°C for 48 hours. After incubation, the bacterial suspension was homogenized and adjusted to a 0.5 McFarland standard (approximately 10^8 CFU/mL) using spectrophotometric analysis [17]. The minimum inhibitory concentration (MIC) was assessed using the broth microdilution technique in 96-well microplates [18, 19]. Each well received 100 µL of BHI broth (HiMedia, USA), 5 µL of P. gingivalis culture, and 5 µL of either Aloe vera extract (at concentrations of 100%, 80%, 60%, 40%, or 20%) or chitosan nanoparticles (at 100%, 50%, 25%, or 12.5%). For combination treatments, 5 µL of Aloe vera extract and 5 µL of chitosan nanoparticles were mixed, followed by the addition of 5 µL of triphenyl tetrazolium chloride (TTC) reagent (HiMedia, USA). The plates were then incubated at 37°C for 24 hours. MIC was identified as the lowest concentration at which no visible bacterial growth was observed, indicated by a clear well. To determine the minimum bactericidal concentration (MBC), aliquots from wells showing no visible growth were cultured on agar plates. The MBC was defined as the lowest concentration where no bacterial colonies developed, signifying total inhibition of P. gingivalis [17]. All tests were carried out in triplicate.

2.5. Viability Assay and Data Analysis

To assess cytotoxicity through the MTT assay, BHK-21 fibroblast cells were seeded into 96-well microplates at a density of 1×10^5 cells per well and incubated for 24 hours in a CO_2 incubator to allow cell attachment. After the initial incubation, different concentrations of Aloe vera extract and chitosan nanoparticles were introduced into the wells, followed by a second 24-hour incubation period. Post-treatment, the wells were washed three times with Phosphate Buffered Saline (PBS) (Gibco, Thermo Fisher Scientific, USA) to remove any residual media components. Subsequently, 10 µL of MTT reagent was added to each well, and the plate was returned to the CO_2 incubator for 3 hours to enable formazan crystal formation. After incubation, the MTT solution was discarded, and 200 µL of HCl-isopropanol solution was added to each well to halt the reaction and dissolve the formazan crystals. The plate was gently agitated for 5–10 minutes to ensure thorough solubilization. Cell viability was then determined by measuring absorbance at 620 nm using an ELISA microplate reader. The percentage of viable cells was calculated using the following formula (Equation 1).

$$CV = \frac{\left(OD_T - OD_M\right)}{\left(OD_C - OD_M\right)} \times 100\% \quad (1)$$

Where CV represents cell viability (%), and ODT, ODM, and ODC are the optical density values of the treatment, medium, and control groups, respectively.

2.6. Statistical Analysis

In this study, the presentation of results for both the inhibition zone diameter and the cell viability assay involved the reporting of the mean value along with its corresponding standard deviation (SD). A one-way analysis of variance (ANOVA) was initially employed as the primary analysis method to meticulously assess the acquired data, providing a comprehensive overview of variations among the different data sets. Subsequently, a post hoc test was conducted for a more in-depth examination and comparisons, allowing for a nuanced understanding of specific group differences. The statistical analysis for this research was conducted using IBM SPSS version 21.0, ensuring the rigor and reliability of the data evaluation process.

3. Result

3.1. Characterization of Aloe vera extract

This study involved a comprehensive evaluation process, beginning with the phytochemical screening and standardization of Aloe vera extract. The extract was assessed for quality using both general and specific criteria, ensuring compliance with the standards set by the *Farmakope Herbal Indonesia* (FHI) for Aloe vera, as presented in Table 1 [20]. Antibacterial activity was then investigated through inhibition zone analysis, along with the determination of MIC and MBC. In addition, in vitro cytotoxicity testing was performed on fibroblast cells to assess cell viability. Phytochemical analysis revealed the presence of key bioactive constituents in the extract, including flavonoids, tannins, saponins, alkaloids, and glycosides as outlined in Table 2.

Table 1.
Standardized Aloe vera extract parameters.

Standardized parameters	Prepared extract	Standardized FHI guidebook
Nonspecific parameters		
1. Value weight	0.9751 g/cm^3	Close to value of 1 g/cm^3
2. Moisture level	0.983%	Under than 12.5%
3. Ash level	4.7%	Under than 4.9%
Specific parameters		
1. Identity	Biological name: Aloe vera	Biological name: Aloe vera
	Plant part used: Aloe vera leaf flesh	Plant part used: Aloe vera leaf flesh
2. Organoleptic	Form: viscous liquid	Form: Viscous liquid
	Flavor: rather bitter	Flavor: rather bitter

Table 2.
Compound identifications of the prepared Aloe vera extract.

Aloe vera extract	**Flavonoid**	**Tannin**	**Saponin**	**Alkaloid**	**Glycoside**	**Steroid**
Identifications	Yellow solution	Greenish-black solution	Stable foam	Brownish-red solution	Brick red precipitate	No color change
Results	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Negative (-)

3.2. Characterization of Chitosan Nanoparticles

Figure 1 shows pictures from scanning electron microscopy (SEM) at various magnifications that contrast raw and nano-formulated chitosan. The nano-chitosan particles display a spherical morphology and significantly reduced sizes compared to the raw form. These observations are consistent with the particle size analysis (PSA) results shown in Figure 2. As illustrated in Figure 2, raw chitosan exhibits a broad particle size distribution ranging from 90 to 1000 µm, whereas the synthesized nano-chitosan demonstrates a much narrower and more uniform distribution, with particle sizes between 10 and 100 nm [21, 22]. This confirms the substantial downscaling in particle size achieved through the nanoparticle synthesis process.

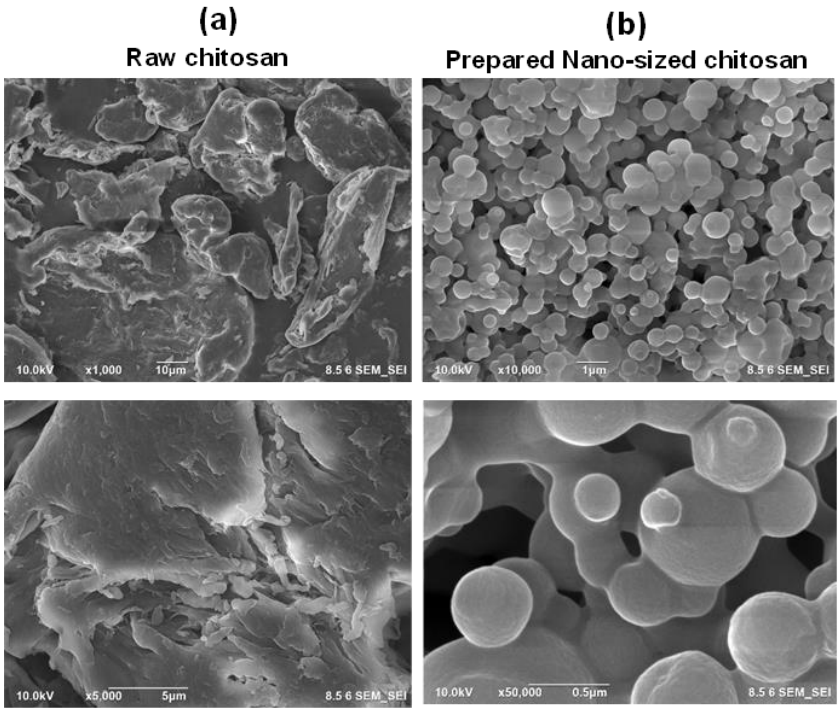


Figure 1.
SEM images of: (a) raw chitosan and (b) prepared nano-sized chitosan.

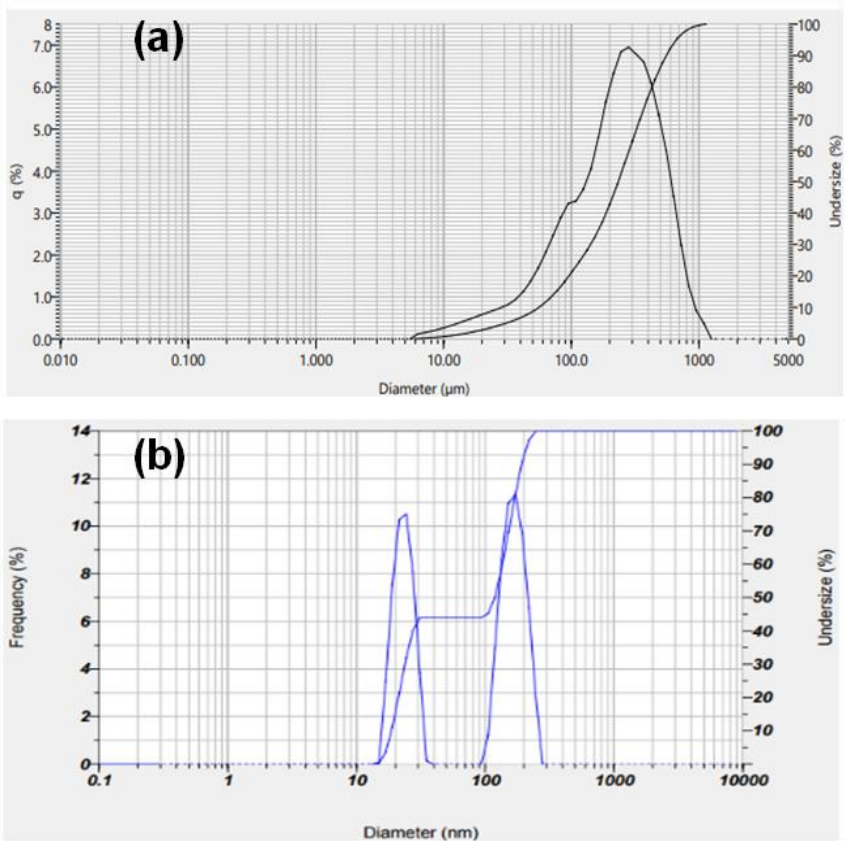
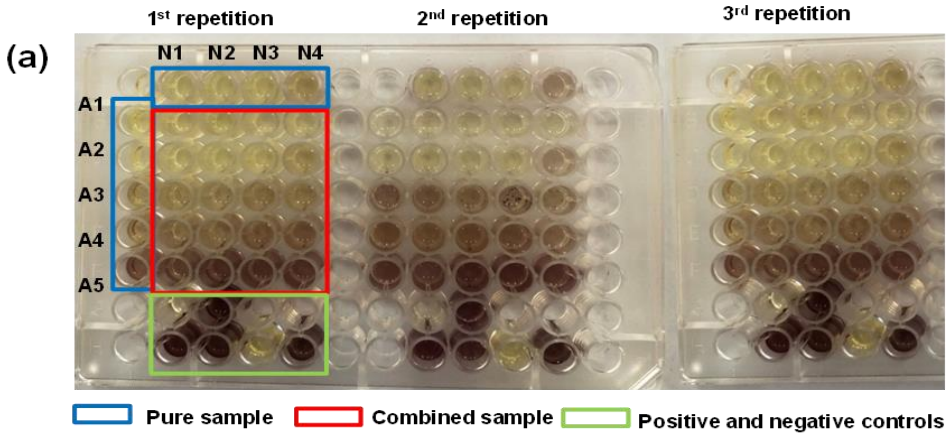


Figure 2.
Particle size distribution charts of PSA analysis of: (a) raw chitosan and (b) prepared nano-sized chitosan.

3.3. MIC and MBC Assessments

The MIC results indicated that the combination of *Aloe vera* extract and chitosan nanoparticles effectively inhibited the growth of *Porphyromonas gingivalis*, particularly in well 4C, which contained 80% *Aloe vera* and 25% chitosan nanoparticles. The outcomes of the MIC test, conducted using 96-well plates, are illustrated in Figure 3. To establish the MBC, mixtures with concentrations above and below that in well 4C were cultured on BHI agar in separate petri dishes. The MBC findings were consistent with the MIC results: no bacterial growth was observed in well 4C (80% *Aloe vera* - 25% chitosan nanoparticles). Similarly, well 3C (80% *Aloe vera* - 50% chitosan nanoparticles) also showed no bacterial presence, whereas bacterial growth was still evident in well 5C (80% *Aloe vera* - 12.5% chitosan nanoparticles). The MBC outcomes are depicted in Figure 4.



(b)

Aloe Vera Extract		Chitosan nanoparticle concentration (wt %)						Chitosan nanoparticle concentration (wt %)						Chitosan nanoparticle concentration (wt %)					
			100 %	50 %	25 %	12.5 %			100 %	50 %	25 %	12.5 %			100 %	50 %	25 %	12.5 %	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	A		−	−	−	+			−	−	−	+			−	−	−	+	
100 %	B	−	−	−	−	−		−	−	−	−	−		−	−	−	−	−	
80%	C	−	−	−	−	+		−	−	−	−	+		−	−	−	−	+	
60 %	D	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
40 %	E	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
20 %	F	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	
	G		−	+					−	+					−	+			
	H		+	+	−	+			+	+	−	+			+	+	−	+	

Figure 3.
The MIC test results on 96-well plates: (a) actual results and (b) interpretations of the data.

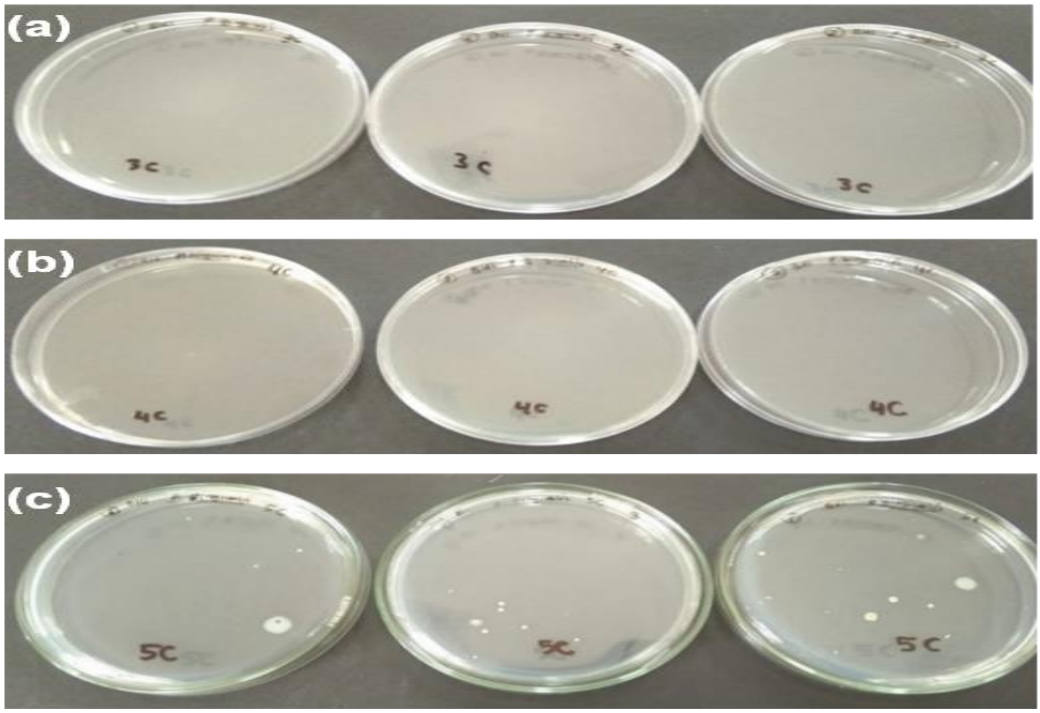


Figure 4.
The MBC test results showed that (a) 80% aloe vera and 50% chitosan nanoparticle, (b) 80% aloe vera and 25% chitosan nanoparticle, and (c) 80% aloe vera and 12.5% chitosan nanoparticle were combined.

3.4. Evaluation of Inhibition Performance Against P. gingivalis.

Figure 5 illustrates the mean diameters of inhibition zones formed by various concentrations of Aloe vera extract and chitosan nanoparticles against P. gingivalis. A clear increase in the inhibition zone size was observed with higher concentrations of Aloe vera extract, and a similar pattern emerged with increasing chitosan nanoparticle concentrations. The most significant inhibition zone for Aloe vera extract was recorded at 100% concentration, averaging 23.79 mm in diameter. Likewise, chitosan nanoparticles at 100% concentration produced an inhibition zone with an average diameter of 15.73 mm. Table 3 presents the statistical analysis of the antibacterial effects of both Aloe vera extract and chitosan nanoparticles on P. gingivalis. According to the data, the inhibition zone produced by metronidazole (average diameter 25.71 mm) did not differ

significantly from those of Aloe vera at 80% and 100% concentrations, which had average diameters of 22.11 mm and 23.79 mm, respectively (p=0.283 and p=0.826, p>0.05). Similarly, the inhibition zones for chitosan nanoparticles at 50% and 100% concentrations were statistically comparable to metronidazole (p=0.1 and p=0.411, p>0.05). Additional statistical results concerning fibroblast cell viability are presented in Table 4.

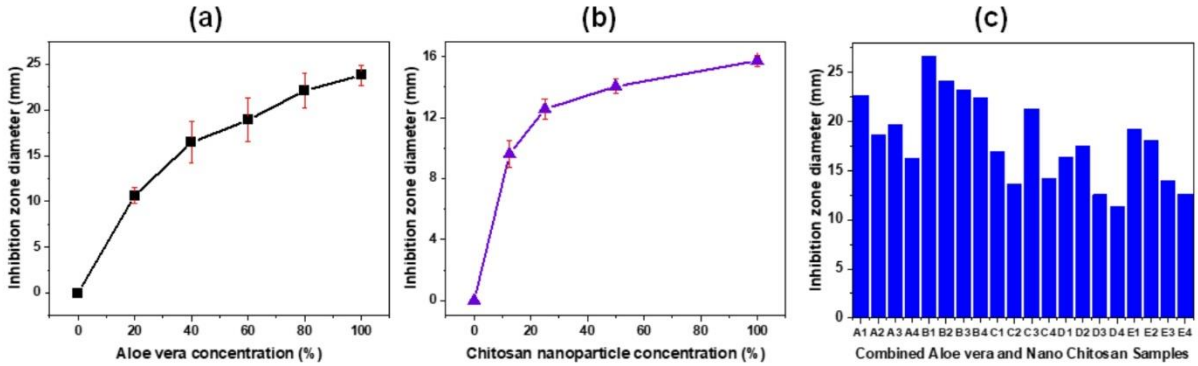


Figure 5.
(a) Mean inhibition zone of single Aloe vera extract against *P. gingivalis,* (b) Mean inhibition zone of single chitosan nanoparticle against *P. gingivalis,* (c) Mean inhibition zone combination of Aloe vera extract and chitosan nanoparticle against *P. gingivalis.*

Table 3.
Statistical evaluation of P. gingivalis inhibitory zone measurement following exposure to a combined concentration of chitosan nanoparticles and aloe vera extract over a 24-hour period.

Group	Inhibition zone diameter (mm)	Standard deviation	p-value compared to control cell	p-value compared to control (+) metronidazole
Control (+) metronidazole	25.71	2.59	0.003*	---
Aloe vera extract				
100%	23.79	1.08	0.000*	0.826
80%	22.11	1.84	0.000*	0.283
60%	18.88	2.40	0.000*	0.011*
40%	16.43	2.28	0.000*	0.001*
20%	10.65	0.91	0.000*	0.000*
Chitosan nanoparticles				
100%	15.73	0.35	0.000*	0.411
50%	14.04	0.49	0.000*	0.100
25%	12.55	0.67	0.000*	0.014*
12.5%	9.60	0.90	0.000*	0.001*
Combination				
(A1) A - N 100% - 100%	22.66	1.25	0.000*	0.593
(A2) A - N 100% - 50%	18.67	1.45	0.000*	0.102
(A3) A - N 100% - 25%	19.70	0.30	0.000*	0.167
(A4) A - N 100% - 12.5%	16.22	4.90	0.000*	0.024*
(B1) A - N 80% - 100%	26.58	1.98	0.000*	0.894
(B2) A - N 80% - 50%	24.12	1.85	0.000*	0.806
(B3) A - N 80% - 25%	23.19	0.95	0.000*	0.656
(B4) A - N 80% - 12.5%	22.42	4.74	0.000*	0.462
(C1) A - N 60% - 100%	16.95	4.00	0.000*	0.038*
(C2) A - N 60% - 50%	13.61	1.58	0.000*	0.004*
(C3) A - N 60% - 25%	21.25	1.35	0.000*	0.361
(C4) A - N 60% - 12.5%	14.19	3.21	0.000*	0.006*
(D1) A - N 40% - 100%	16.33	2.59	0.000*	0.021*
(D2) A - N 40% - 50%	17.49	1.85	0.000*	0.040*
(D3) A - N 40% - 25%	12.53	3.33	0.000*	0.002*
(D4) A - N 40% - 12.5%	11.32	1.22	0.000*	0.000*
(E1) A - N 20% - 100%	19.21	2.96	0.000*	0.161
(E2) A - N 20% - 50%	18.02	2.95	0.000*	0.069
(E3) A - N 20% - 25%	13.98	1.11	0.000*	0.004*
(E4) A - N 20% - 12.5%	12.58	1.46	0.000*	0.002*

Note: one-way ANOVA analysis: *p-value < 0.05 (significant effect).

Table 4.
Results of the statistical analysis show the standard deviation, viability, and significance of BHK-21 fibroblast cells following a 24-hour exposure to different combinations of concentrations of chitosan nanoparticles and aloe vera extract.

Group	Mean value of absorbance (SD)	Cell viability (%)	p-value compared to control cell	p-value compared to control (+) metronidazole
Control cell	0.879 (0.115)	100	--	1.000
Control (+) metronidazole	0.900 (0.129)	102.7	1.000	--
Aloe vera extract				
100%	1.027 (0.183)	118,7	0.711	0.829
80%	0.915 (0.079)	104.5	1.000	1.000
60%	0.613 (0.026)	66.5	0.147	0.101
40%	0.371 (0.026)	36,0	0.002*	0.001*
20%	0.178 (0.155)	11.6	0.000*	0.000*
Chitosan nanoparticles				
100%	0.742 (0.037)	82.8	0.745	0.623
50%	0.710 (0.185)	78.7	0.562	0.443
25%	0.655 (0.056)	71.8	0.288	0.211
12.5%	0.608 (0.143)	65.8	0.143	0.101
Combined samples				
A - N 100% - 100%	0.937 (0.135)	107.5	0.815	0.782
A - N 100% - 50%	0.516 (0.382)	54.2	0.349	0.372
A - N 100% - 25%	0.884 (0.207)	100.6	0.790	0.823
A - N 100% - 12.5%	0.743 (0.167)	82.9	0.431	0.457
A - N 80% - 100%	0.770 (0.042)	86.3	0.530	0.559
A - N 80% - 50%	0.782 (0.048)	87.8	0.552	0.580
A - N 80% - 25%	0.853 (0.048)	96.8	0.983	0.983
A - N 80% - 12.5%	0.761 (0.092)	85.2	0.552	0.580
A - N 60% - 100%	0.604 (0.083)	65.3	0.131	0.142
A - N 60% - 50%	0.552 (0.026)	58.8	0.078	0.085
A - N 60% - 25%	0.402 (0.265)	39.8	0.033*	0.037*
A - N 60% - 12.5%	0.472 (0.377)	48.7	0.180	0.195
A - N 40% - 100%	0.306 (0.151)	27.8	0.022*	0.024*
A - N 40% - 50%	0.266 (0.150)	22.8	0.014*	0.016*
A - N 40% - 25%	0.227 (0.143)	17.9	0.006*	0.007*
A - N 40% - 12.5%	0.323 (0.028)	30.0	0.013*	0.014*
A - N 20% - 100%	0.131 (0.028)	5.8	0.009*	0.009*
A - N 20% - 50%	0.068 (0.003)	-2.3	0.001*	0.002*
A - N 20% - 25%	0.076 (0.010)	-1.2	0.002*	0.002*
A - N 20% - 12.5%	0.075 (0.020)	-1.3	0.001*	0.002*

Note: one-way ANOVA analysis: *p-value < 0.05 (significant effect).

4. Discussion

Aloe vera extract at concentrations of 40% and 20% resulted in statistically significant reductions in cell viability compared to both the control group and the metronidazole-treated group, with p-values of 0.002 and 0.000, respectively ($p < 0.05$). In contrast, all tested concentrations of chitosan nanoparticles did not produce any significant changes in cell viability when compared to the control or metronidazole groups ($p > 0.05$). When Aloe vera extract was combined with chitosan nanoparticles, cell viability remained unaffected at 100% and 80% extract concentrations across all nanoparticle levels, showing no significant difference from the control and metronidazole groups. However, combinations containing 60%, 40%, and 20% Aloe vera extract with various nanoparticle concentrations did lead to significant reductions in cell viability ($p < 0.05$). Despite these reductions, all chitosan nanoparticle concentrations maintained cell viability above 50%, indicating they were non-toxic. Similarly, combinations of 100% and 80% Aloe vera extract with chitosan nanoparticles also preserved cell viability above 50%, confirming their safety for use.

This study utilized the plant *Aloe vera* (L.) Burm.f., commonly known as *Aloe barbadensis* [23]. Specifically, the *Aloe vera* gel was used as the active component. The *Aloe vera* plant consists of three parts: the outer green rind, the latex layer, and the inner gel, each with distinct medicinal uses for both topical and oral applications. Among these, the gel is typically preferred due to its higher antibacterial potential [24]. The middle latex layer, which is yellow and bitter, contains anthraquinones and glycosides responsible for laxative effects. In contrast, the gel is richer in antibacterial compounds, enhancing its effectiveness against microbes [25]. To ensure product quality and consistency, standardization of herbal extracts is vital [26]. As shown in Table 1, the extract met all required parameters such as weight, moisture, ash content, identity, and sensory attributes aligned with the standards established in the Farmakope Herbal Indonesia (FHI). Fulfilling these requirements is necessary to guarantee the extract's safety and therapeutic efficacy.

Accurately identifying the active chemicals in the Aloe vera plant employed in this investigation was the primary objective of the phytochemical analysis [27]. In this qualitative test, particular chemical reagents were added, and color changes were observed. Because different species of Aloe vera can have biological differences that affect the amounts of active chemicals they contain, it is essential to do a first phytochemical investigation [28]. For example, the elements of aloe vera grown in one region (known as region X) may differ from those of aloe vera grown in another region (known as region Y). The findings of the phytochemical test revealed a number of significant chemicals in the aloe vera extract, such as flavonoids, tannins, saponins, alkaloids, and glycosides, as indicated in Table 2.

Through their distinct modes of action, flavonoids modify bacterial metabolism and interfere with the functionality of bacterial cell membranes [29]. Flavonoids might potentially harm bacterial cell membranes by disrupting membrane function and forming complex molecules with external proteins through hydrogen bonding. By deactivating bacterial adhesins, inhibiting the activity of enzymes, and impeding the passage of proteins across bacterial membranes, tannins function. Bacterial cell death results from saponins' impact on the permeability of bacterial cell membranes, which allows essential elements, including proteins, nucleic acids, and nucleotides, to escape [30, 31]. Proteins inside bacterial cells become denatured as a result of the alkaloids' disruption of biological functions. Inhibiting bacterial action is how glycosides work [32].

Porphyromonas gingivalis (P. gingivalis), the main bacteria that causes periodontitis, was chosen for testing in this investigation. Because of its complex peptidoglycan layer, P. gingivalis is an obligatory anaerobic Gram-negative bacterium with a more complex cell wall than Gram-positive bacteria [33, 34]. Because of its proven efficacy against anaerobic bacteria such as P. gingivalis, metronidazole, a popular antibiotic used to treat periodontitis, was utilized as a positive control in this investigation [35]. As shown in Figure 4, higher concentrations of Aloe vera extract resulted in larger inhibition zones when exposed to P. gingivalis, signifying reduced bacterial growth [27]. The antimicrobial effectiveness of Aloe vera extract correlates with its active compounds. Increased concentrations of the extract lead to a higher presence of antibacterial agents such as flavonoids, tannins, saponins, and glycosides. These substances have bactericidal action by altering the permeability of the bacterial cell membrane, which damages the cell and releases essential components such as proteins, nucleic acids, and nucleotides, which finally cause the bacterial cells to be destroyed [34, 36]. Chitosan nanoparticles showed a similar trend, with greater inhibition zones against P. gingivalis resulting from higher concentrations. Chitosan nanoparticles' antibacterial activity is directly related to ion release, which happens when the positively charged nanoparticles and the negatively charged bacterial cell membrane interact electrostatically. The permeability of the cell wall is broken by this contact [37].

Aloe vera extract at 100% and 80% concentrations combined with different chitosan nanoparticles produced inhibitory zones that were either identical to or only marginally different from those observed with metronidazole [38], as illustrated in Figure 5. Interestingly, compared to samples that included solely Aloe vera or individual chitosan nanoparticles, the combination of 80% Aloe vera extract with various kinds of chitosan nanoparticles produced a bigger inhibitory zone. Chitosan nanoparticles' function as a carrier, which aids in the delivery and release of the active ingredients from the aloe vera extract and increases their antimicrobial efficacy is responsible for this improved antibacterial activity [30, 39].

Large surface area, reduced particle size, and improved stability are some of the special properties of chitosan nanoparticles that enable them to efficiently pass through bacterial cell membranes [19, 28]. Additionally, because of their superior absorption qualities, chitosan nanoparticles can quickly target particular organs and offer efficient illness treatment. It's also crucial to remember that chitosan nanoparticles and aloe vera extract use distinct antibacterial processes to combat P. gingivalis, and these mechanisms complement one another without compromising their antibacterial efficacy [19]. The antimicrobial effect of Aloe vera extract is bactericidal, while chitosan nanoparticles are bacteriostatic. Chitosan nanoparticles inhibit bacterial growth by binding to intracellular components, blocking mRNA, disrupting protein synthesis, and reducing cellular metabolism [20].

The purpose of the cytotoxicity test is to evaluate a material's or substance's biocompatibility, making sure that it doesn't result in irritation, allergies, or hazardous effects. Primary and secondary cell cultures, often known as cell lines, can be used for initial in vitro cytotoxicity assessments [24]. The BHK-21 fibroblast cell line was used in this investigation. Derived from hamster kidney fibroblasts, these cells are structurally comparable to human fibroblast cells that are found in gums, periodontal ligaments, and tooth pulp. Furthermore, BHK-21 fibroblast cells are a popular model for evaluating the cytotoxicity of medications and dental products due to their ease of culture and subculture [40].

5. Conclusion

The results of the MIC, MBC, and inhibition zone tests demonstrate the effectiveness of the combination of chitosan nanoparticles and aloe vera extract against P. gingivalis. According to the MIC and MBC results, a mixture of 25% chitosan nanoparticles and 80% aloe vera extract works well. Larger inhibition zones were formed in the inhibition zone test when 80% aloe vera extract and different types of chitosan nanoparticles were combined, as opposed to samples that included just aloe vera or chitosan nanoparticles. These substances each help to eradicate bacteria on their own, even though they do not have a synergistic antibacterial action. Furthermore, the MTT experiment showed that BHK-21 fibroblast cells were not harmed by the combination of 100% and 80% aloe vera extract with different chitosan nanoparticles.

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