Antibacterial activity of *Aloe vera* extract and chitosan nanoparticles against *Porphyromonas gingivalis* and fibroblast viability assay analysis

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The use of herbal medicine as an alternative therapy to reduce periodontal inflammation is then being developed to resolve antibiotic resistance. Aloe vera is receiving much scientific attention, but herbal drug formulation has limitations. The efficiency of herbal medicines can improve with a drug delivery system utilizing chitosan nanoparticles. This research aims to develop a potential antibiotic combining Aloe vera extract and chitosan nanoparticles. Evaluate their antibacterial activity against *P.gingivalis* and toxicity on BHK-21 fibroblast cells. Determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values used the microdilution method in 96 well plates. ELISA did a viability assay. The MIC and MBC were obtained in concentrations of 80% Aloe vera – 25% chitosan nanoparticles. A combination of 80% Aloe vera extract and various chitosan nanoparticle formulations had a greater inhibitory zone than a single sample used. Combining these two materials showed potent antibacterial activity and was not toxic.

Keywords: Antibacterial activity, Aloe vera extract, chitosan nanoparticles, *Porphyromonas gingivalis*, fibroblast cell viability

INTRODUCTION

Periodontitis is a multifactorial disease caused by an imbalance in the interaction between the subgingival biofilm and the host immune response, which destroys the periodontal tissue[1,2]. *Porphyromonas gingivalis* is an anaerobic Gram-negative bacterium, often associated with the pathogenesis of periodontitis[3,4]. The best way to treat periodontitis is through mechanical debridement of the pockets, done with scaling and root planning. Antibiotics are frequently used to improve the effectiveness of treatment, particularly for deep pockets with complex anatomical forms and furcation areas that are challenging to clean mechanically[5,6]. It has been demonstrated that using antibiotics in addition to scaling and root planning will enhance periodontitis treatment[6].

In recent decades, the Inappropriate use of antibiotics has accelerated antibiotic resistance[7]. It is crucial to find new antibacterial substances[7]. The use of herbal medicine as an alternative therapy to reduce periodontal inflammation is then being developed to resolve this issue[8,9]. Utilizing natural biomaterials as herbal medicines have been shown to have fewer adverse effects and be more cost-effective than chemical pharmaceuticals[10]. Aloe vera is one of the oldest medical plants with health benefits and biological properties as an antibacterial agent Aloe vera[9,10].

Aloe vera has simple plants to cultivate in tropical climates, like Indonesia[9]. The bioactive components in Aloe vera have been reported to have antioxidants, antifungal, anti-inflammatory, antibacterial, and accelerate wound healing. Aloe vera has been commonly used in mouthwash and topical gel as a herbal component in treating periodontitis[9,10]. Although it has been reported to have many health benefits, the herbal ingredient has limitation[s].

The limitation of formulation herbal drugs such as large molecular sizes of active compounds so that difficult to penetrate the lipid membrane cell, poor bioavailability, poor solubility, low oral absorption, instability in highly acidic pH conditions, limited dose administration to target site, and unpredictable toxicity of herbal medicines limit their use[11]. Therefore, a drug delivery system utilizing nanoparticles, a different approach to improve the efficiency of absorption and targeted action of herbal medicines, is currently being developed[12].

Chitosan is one of the natural polymers that can use as nanoparticle carriers for drug delivery[11,12]. Chitosan consists of the enzyme lysozyme and amino polysaccharide that can inhibit bacterial growth[13]. The use of chitosan is increasingly being developed into nanoparticle sizes ranging from 10-1000 nm[14]. Chitosan nanoparticles, which are very small in size, can quickly enter the membrane cell, penetrate smaller capillaries, and penetrate cells and tissue to reach the target so that the onset of therapeutic action is faster and more effective[14]. Additionally, chitosan nanoparticles have better stability and can reduce drug side effects[13,14].

Research on the ability of Aloe vera as an antibacterial has been carried out. The results of other studies comparing the antibacterial properties of Aloe vera chitosan nanoparticles and calcium hydroxide as intracanal medications in root canal treatment show that the combination of Aloe vera chitosan nanoparticles is effective in preventing the growth of anaerobes bacteria like Enterococcus faecalis, Streptococcus mutans, and Candida albicans[15]. Therefore, it is necessary to study Aloe vera extract and chitosan nanoparticle as an antibiotic made from herbs that can be used as an alternative therapy for periodontal disease to inhibit Gram-negative bacteria like *Porphyromonas gingivalis.*

MATERIALS AND METHODS

This research has obtained an ethical research permit from the Research Ethics Commission, Faculty of Dentistry, University of Indonesia, with No. *04 /Ethical/FKGUI/III/2022.* This research method is a pure experimental method with a post-test only with a control group design divided into five groups. Group 1 were given treatment with a single Aloe vera extract with concentrations of 20%, 40%, 60%, 80%, and 100%; Group 2 were given treatment with a single chitosan nanoparticle with 12,5%, 25%, 50%, and 100%: Group 3 were given treatment with combination Aloe vera extract and chitosan nanoparticle. Dimethyl sulfoxide (DMSO) was in the negative control, and metronidazole was in a positive control. Each treatment group was three times repetitions.

Preparations of Aloe vera extracts

Aloe vera (L) Burm.f is included in *Asphodelaceae*. Identification of Aloe vera species made by Herbarium Botany Research Centre for Biology – LIPI Bogor. The extraction procedure of Aloe vera gel using maceration method with ethanol solvent of 96% (Merck, Germany) and comparison 1:10. Aloe vera gel stirrer in ethanol solvent of 96% for 6 hours and keep quiet for 24 hours. A rotary evaporator with a heating temperature of 40-50˚C removes the solvent in the extract to attain a semi-solid Aloe vera extract. Then, Aloe vera extract gel will prepare for phytochemical tests and standardization of the extracted test. Dimethyl sulfoxide (DMSO) (Merck, Germany) was used to dilute Aloe vera extract into five concentrations: 100%, 80%, 60%, 40%, and 20%. Each dilution concentration was made up to 10 ml.[16]

Preparations of Chitosan Nanoparticle

The preparation of chitosan nanoparticles using the ionic gelation method involves polyelectrolyte complexation between chitosan being charged with positive electricity and tripolyphosphate being charged with negative electricity[14]. The procedure was carried out with 350 mg chitosan powder with low molecular weight from shrimp shell (Sigma, Aldrich, USA), dissolved into an acetic acid solution of 1% (Merck, Germany). Then, mix chitosan solution into sodium tripolyphosphate solution (Merck, Germany) using a burette as much as 1 ml/minute, stir with a magnetic stirrer (Thermo Fisher Scientific, USA) for 24 hours, sonification solution using an ultrasonic bath (Branson, USA) for 60 minutes, and add stabilizer NaCl (Merck, Germany). An acetic acid solution of 1%

was added to the chitosan nanoparticle solution to create dilution solutions at four different concentrations: 100%, 50%, 25%, and 12.5%. Each dilution concentration was made up to 10 ml.

Bacterial Preparation

Porphyromonas gingivalis were maintained at Laboratory Oral Biologic, University of Indonesia. The bacteria were cultured into the broth of nutrients (HiMedia, USA) in a petri dish and incubation for 48 hours at 37°C. The deferral of the bacteria cell was homogenized. Then, the 0.5 McFarland standards (10^8 CFU/mL) were adjusted using spectrophotometry[17].

Determining the Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs)

The determination of MIC values was carried out by utilizing the method of broth microdilution in 96 well plates[18,19]. Design of antibacterial test in 96 well plates in Fig 1. In single sample well consist of 100 µL BHI broth medium (HiMedia, USA), 5 µL *Porphyromonas gingivalis* bacterial culture, 5 µL Aloe vera extract concentration of 100%, 80%, 60%, 40%, 20% or 5 µL chitosan nanoparticles 100%, 50 %, 25%, 12.5%, and add 5 µL of triphenyl tetrazolium chloride reagent (HiMedia, USA). In combination sample well, mixed approximately 5 µl of Aloe vera extract concentration and 5 µl chitosan nanoparticles concentration, then add 5 µL of triphenyl tetrazolium chloride reagent. It was then incubated at 37°C for 24 hours. The MIC value is the lowest material concentration necessary to prevent bacteria from growing in the medium[18]. The clearer well indicates the absence of bacterial growth. The MBC value is calculated by observing the growth of bacteria on agar media. The MBC was determined from clear plate agar media, indicating no bacterial growth, so the antibacterial test solution had an effective 100% inhibition value[18]. All of the experiments were conducted in 3 times repetition.

		Chitosan nanoparticle						Chitosan nanoparticle					
			100 %	50 %	25 %	12,5 %			100 %	50 %	25 %	12,5 %	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aloe Vera Extract	**A**	x	N1	N2	N3	N4		x	N1	N2	N3	N4	
100 %	**B**	A1	2B	3B	4B	5B		A1	2B	3B	4B	5B	
80%	**C**	A2	2C	3C	4C	5C		A2	2C	3C	4C	5C	
60 %	**D**	A3	2D	3D	4D	5D		A3	2D	3D	4D	5D	
40 %	**E**	A4	2E	3E	4E	5E		A4	2E	3E	4E	5E	
20 %	**F**	A5	2F	3F	4F	5F		A5	2F	3F	4F	5F	
	G		K (+)	K (-)					K (+)	K (-)			
	H		K(-)	K (-)	K(-)	K (-)			K(-)	K (-)	K (-)	K (-)	

Single sample
Combination sample
Positive control
Negative control

Fig. 1 Antibacterial Test Design on 96 well plates

Antibacterial Inhibition Zone Growth

The antibacterial assay of a combination of Aloe vera extract and chitosan nanoparticle was conducted using the diffusion method. A sterilized 15 ml Brain Heart Infusion Agar (HiMedia, USA) was added to the sterile petri dishes. Add 100 µl of bacterial test suspension with 10^8CFU/mL poured to the base plate surface of petri dishes. Then, put a paper disk with a 6 mm diameter. In single sample petri dishes, a micropipette was added to approximately 5 µl of Aloe vera extract concentration or 5 µl nanoparticle chitosan concentrations. In combination sample, mixed 2,5 µl Aloe vera extract concentration and 2,5 µl chitosan nanoparticle concentration. The negative control was filled with 5 µl DMSO, the positive control used metronidazole 50 mg, and the petri dish was incubated at

37°C for 24 hours. After the incubation, the measurement for inhibition zones of growth using a digital calliper was performed by three observers and taken the average. All of the experiments were conducted in 3 times repetition[17].

Preparation of BHK-21 Fibroblast Cell

Baby Hamster Kidney (BHK-21) Fibroblast cells were maintained in liquid nitrogen at Laboratory Oral Biologic, University of Indonesia. BHK-21 fibroblast cell culture was carried out in a flask bottle by inserting alpha mem medium (Gibco, Thermo Fisher Scientific, USA), 10% *Fetal Bovine Serum (*FBS) (Gibco, Thermo Fisher Scientific, USA), and *antibiotic antimycotic solution (AA*) (Gibco, Thermo Fisher Scientific, USA), which was incubated at 37°C. Every two days, the cell growth medium was changed until 70 to 80 per cent of the cells were confluent and ready to be harvested. The fibroblast cells were then transferred to a 96-well microplate for toxicity testing[20)].

Viability Assay

BHK-21 fibroblast cells ($1x10^5$) on a 96-well microplate were incubated with a CO2 incubator for 24 hours, for toxicity testing using MTT assay method. Application Aloe vera extract concentration and chitosan nanoparticle concentration suitable with design 96 well microplate and incubate for 24 hours. The microplate was washed thrice using Phosphate Buffer Saline (PBS) (Gibco, Thermo Fisher Scientific, USA), to purge the remaining serum from the microplate. 10 µl of MTT reagent was added to each well and incubated in a CO2 incubator for 3 hours. The MTT reagent was discarded, and an isopropanol HCl 200 µl/*well* stopper was added to each well to stop the reaction between MTT and cells. The microplate was shaken for 5-10 minutes and then inserted into an ELISA reader with a wavelength of 620 nm to read the viability of BHK-21 fibroblast cells after treatment[17,20)].

The reading was successfully carried out by calculating the cell viability value using the following formula:

$$\%\text{Cell Viability} = \frac{(\text{OD treatment} - \text{OD medium})}{(\text{OD cell control} - \text{OD medium})} \times 100\%$$

Statistical Analysis

Inhibition zone diameter and cell viability assay data were presented as the mean ± standard deviation (SD), and one-way ANOVA was used to analyse the obtained results, followed by a post hoc test.

RESULTS

This study is an in vitro laboratory experiment using *Porphyromonas gingivalis* bacteria with a post-test control group design. This research group was made up of a single Aloe vera group with concentrations of 100%, 80%, 60%, 40%, and 20%, a single nanoparticle chitosan group with concentrations of 100%, 50%, 25%, and 12.5%, a combined group of Aloe vera and chitosan nanoparticle, DMSO as a negative control, and metronidazole as a positive control.

This study carried out phytochemical tests and standardization tests in the Aloe vera extract, followed by antibacterial activity tests to obtain inhibition zone growth, minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC). Viability cell tests were carried out in vitro on fibroblast cells.

Standardization Aloe vera extract

The extract standardization test aims to evaluate the quality of the aloe vera extract using nonspecific and specific test criteria. The findings of the analysis of the standardized Aloe vera extract parameters are shown in Table 1. The results follow the Aloe vera extract guidelines in the Farmakope Herbal Indonesia (FHI) guidebook[16].

Table 1 Result of standardization Aloe vera extract

Standardized parameters		Value extract	Standardized FHI guidebook
a.	Nonspecific parameters		
	1. Value weight	0,9751 g/cm^3	Close to value 1 g/cm^3
	2. Moisture level	0,983%	Under than 12,5%
	3. Ash level	4,7%	Under than 4,9%
b.	Specific parameters		
	1. Identity	Biologic name: Aloe vera Plant part used: Aloe vera leaf flesh	Biologic name: Aloe vera Plant part used: Aloe vera leaf flesh
	2. Organoleptic	Form: viscous liquid Colour: brown Flavour: rather bitter	Form: viscous liquid Colour: brown Flavour: rather bitter

Phytochemical test

According to the chemical compounds of the phytochemical analysis test, the Aloe vera extract used in this study contained flavonoid, tannin, saponin, alkaloid, and glycoside, as shown in Fig 2 and Table 2.

Fig 2 Result of Phytochemical test

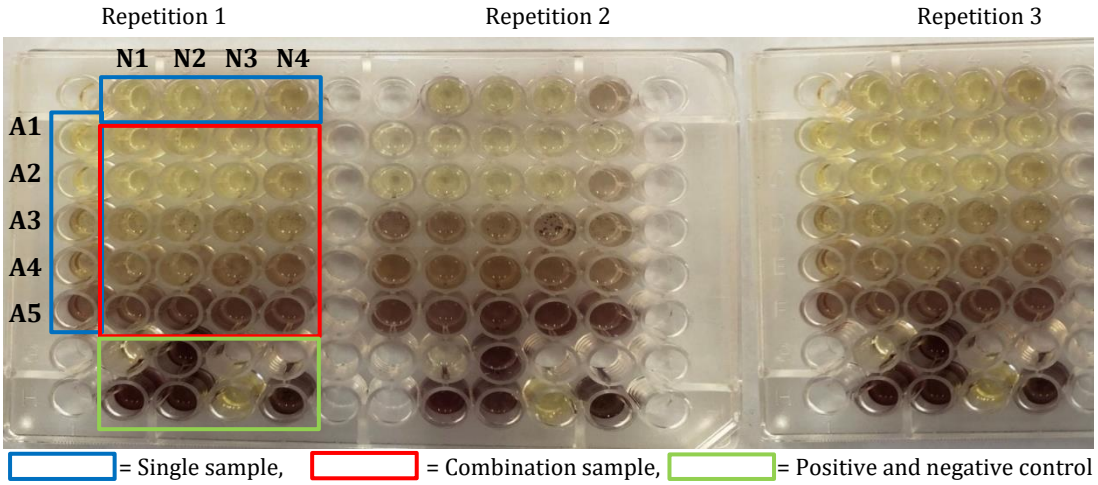
Table 2 Chemical compounds of Aloe vera extract

Aloe vera extract	Flavonoid	Tannin	Saponin	Alkaloid	Glycoside	Steroid
Identification result	Yellow solution	Greenish black solution	Stable foam	Brownish red solution	Brick red precipitate	No colour change
Conclusion	+	+	+	+	+	-

+ = Positive; - = Negative

Minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC)

The MIC of a single Aloe vera extract was obtained at a concentration of 80%, which means the MIC concentration of 80% can inhibit the growth of *Porphyromonas gingivalis*. The results MIC of a single chitosan nanoparticle was obtained at a concentration of 25%, which means the MIC concentration of 25% can inhibit the growth of *Porphyromonas gingivalis.* The results MIC of Aloe vera extract and chitosan nanoparticles that can inhibit *Porphyromonas gingivalis* were obtained in wells with code 4C (80% Aloe vera – 25% chitosan nanoparticles). The results of the MIC test on 96 well plates can be seen in Fig 3.



Repetition 1

Aloe Vera Extract		Chitosan nanoparticle											
			100 %	50 %	25 %	12,5 %			100 %	50 %	25 %	12,5 %	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	A		–	–	–	+			–	–	–	+	
100 %	**B**	–	–	–	–	–		–	–	–	–	–	
80%	**C**	–	–	–	–	+		–	–	–	–	+	
60 %	**D**	+	+	+	+	+		+	+	+	+	+	
40 %	**E**	+	+	+	+	+		+	+	+	+	+	
20 %	**F**	+	+	+	+	+		+	+	+	+	+	
	G		–	+					–	+			
	H		+	+	–	+			+	+	–	+	

(+): with growth *P.gingivalis*; (-) : without growth *P.gingivalis*

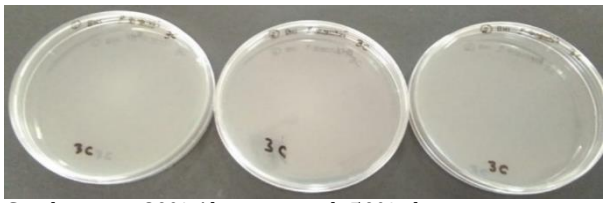
Repetition 3

Aloe Vera Extract		Chitosan nanoparticle					
			100 %	50 %	25 %	12,5 %	
		1	**2**	**3**	**4**	**5**	**6**
	A		–	–	–	+	
100 %	**B**	–	–	–	–	–	
80%	**C**	–	–	–	–	+	
60 %	**D**	+	+	+	+	+	
40 %	**E**	+	+	+	+	+	
20 %	**F**	+	+	+	+	+	
	G		–	+			
	H		+	+	–	+	

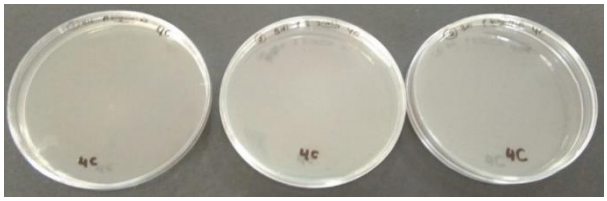
(+): with growth *P.gingivalis*; (-) : without growth *P.gingivalis*

Fig 3. The results of the MIC test on 96 well plates

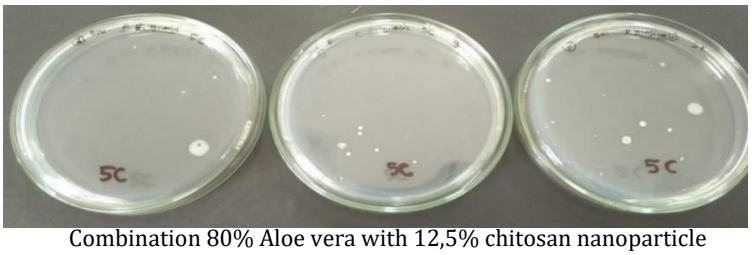
Upper and lower concentration of MIC combination Aloe vera and chitosan nanoparticle solution was poured on BHI agar media in a petri dish to be tested for MBC. The result of the MBC test was obtained the same as the MIC results in wells with code 4C (80% Aloe vera – 25% chitosan nanoparticle). In wells with code 3C (80% Aloe vera – 50% chitosan nanoparticle was no bacterial growth, while in wells with code5C (80% Aloe vera – 12,5% chitosan nanoparticle) still had bacterial growth. The results of MBC test can be seen in Fig 4.



Combination 80% Aloe vera with 50% chitosan nanoparticle



Combination 80% Aloe vera with 25% chitosan nanoparticle



Combination 80% Aloe vera with 12,5% chitosan nanoparticle

Fig 4. The Results of the MBC Test

Inhibition zone growth against P.gingivalis.

Results of measuring the average diameter of the inhibition zone from various concentrations of Aloe extract vera and chitosan nanoparticles against *Porphyromonas gingivalis*, and the significance value of the results of the one-way ANOVA test can be seen in Fig 5.

The inhibitory zone will be significantly wider due to the rising concentration of Aloe vera extract exposed to *P.gingivalis.* A similar phenomenon occurred with exposure to chitosan nanoparticles; when the concentration of chitosan nanoparticles was increased, a wider inhibitory zone developed. The widest inhibition zone diameter of Aloe vera extract was found at a concentration of 100%, with an average inhibition zone was 23.79 mm. The widest inhibition zone diameter of chitosan nanoparticles was found at a concentration of 100%, with an average inhibition zone was 15,73 mm.

The mean diameter of the metronidazole inhibition zone was 25,71 mm, which was similar or not statistically different with aloe vera extract concentration of 80% and 100% of 22,11 mm and 23,79 mm ($p=0,283$ and $p=0,826$, $p>0,05$). The mean diameter of chitosan nanoparticles was similar or not statistically different from metronidazole, found in concentrations of 50% and 100% ($p=0,1$ and $p=0,411$, $p>0,05$). Except for the concentration of 100% Aloe vera - 12.5% chitosan nanoparticles ($p = 0.024$), all variations of the inhibitory zone of the combination of Aloe vera extract and chitosan nanoparticles were similar to the inhibition zone of metronidazole. A combination of 80% Aloe vera extract and various chitosan nanoparticle formulations had a greater inhibitory zone than a single Aloe vera or single chitosan nanoparticles used. Mean Inhibition zone growth against *P.gingivalis.* can be seen in Fig 6.

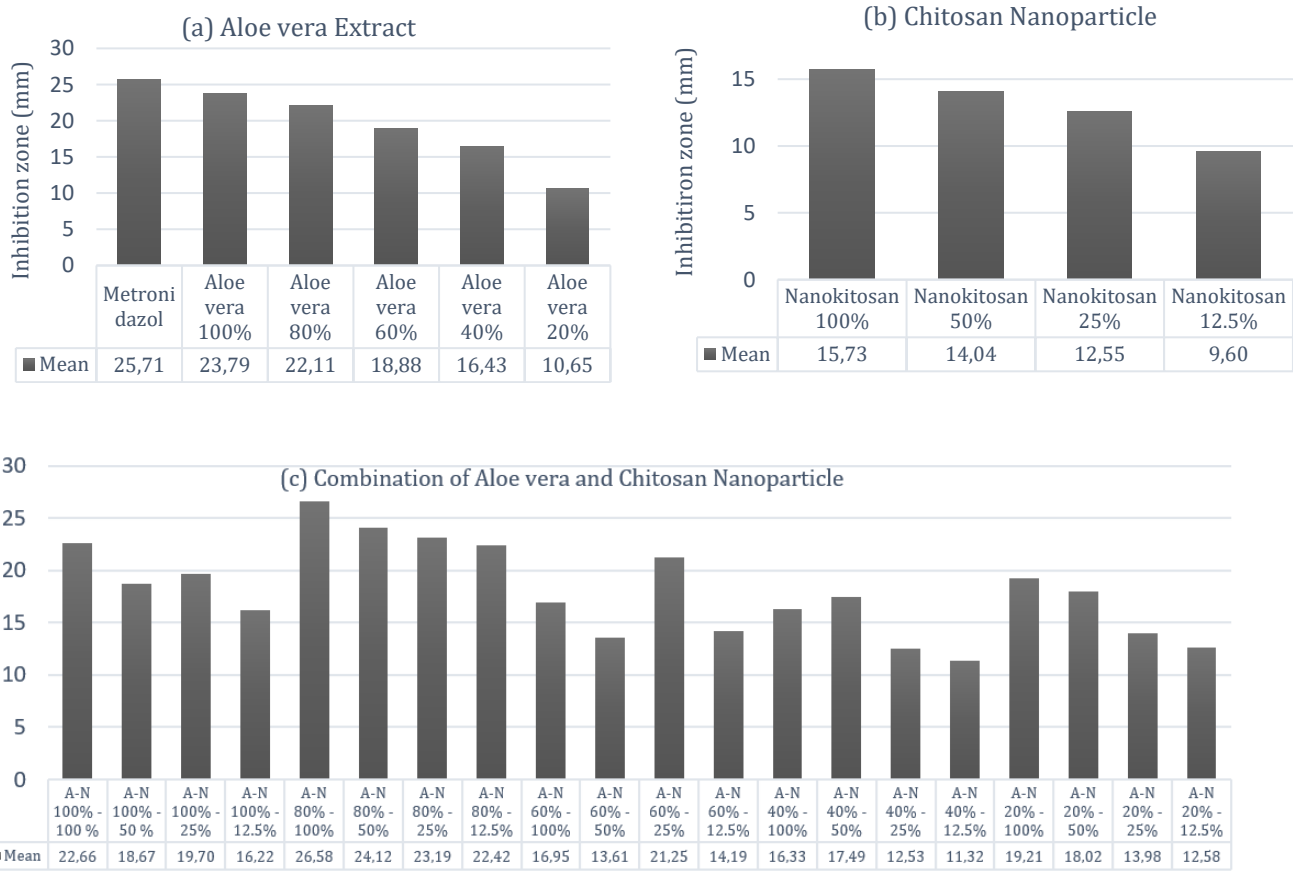


Fig 5 a. Mean inhibition zone of single Aloe vera extract against *P.gingivalis,* b. Mean inhibition zone of single chitosan nanoparticle against *P.gingivalis*, c. Mean inhibition zone combination of Aloe vera extract and chitosan nanoparticle against *P.gingivalis.*

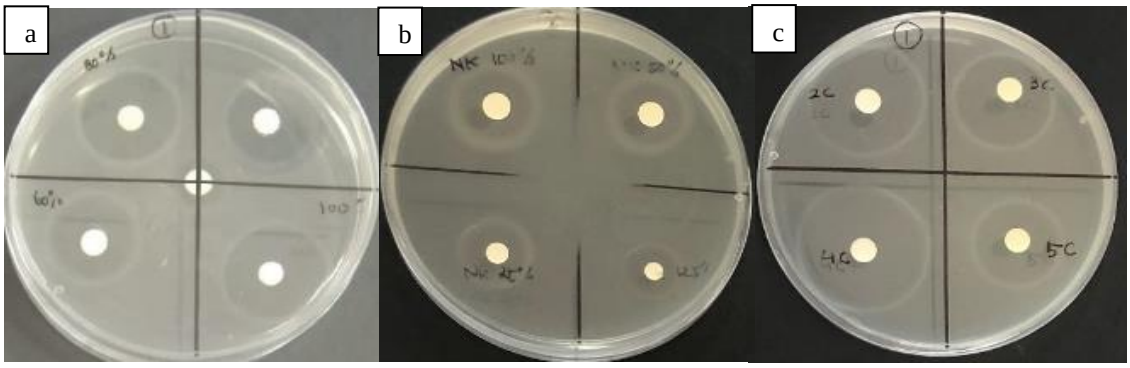


Fig 6 Inhibition zone in petri dish. a. Aloe vera extract, b. Chitosan nanoparticle, c. Combination of Aloe vera and chitosan nanoparticle

Viability cell of BHK-21 fibroblast cell

Based on the absorbance value of BHK-21 fibroblast cells, the cytotoxicity test of Aloe vera extract and chitosan nanoparticles was evaluated. The absorbance value was calculated and expressed as a percentage of viable cells compared to control cells. The absorbance and viability values of fibroblast cells can be seen in Fig 7 and Fig 8.

Viability cells at 40% and 20% Aloe vera extract concentrations compared to control cells and metronidazole showed significantly different ($p = 0.002$ and $p = 0.000$, $p > 0.05$). In contrast, there was no significant difference in cell

viability in all variations of chitosan nanoparticle concentration compared to control cells and metronidazole. Viability cells in the combination of Aloe vera extract and chitosan nanoparticles showed no significant difference at 100% and 80% concentrations of Aloe vera extract combined with various nanoparticle concentrations compared to control cells and metronidazole ($p > 0.05$). The majority of cell viability at concentrations of 60%, 40%, and 20% Aloe vera extract combined with variations in nanoparticle concentrations had significant differences compared to control cells and metronidazole ($p > 0.05$).

According to the IC50 value, a material was considered toxic if it results in less than 50% of living cells being viable after exposure. Concentrations of 100%, 80%, and 60% Aloe vera extract have cell viability values of >50%, which means they are not toxic. All variations of chitosan nanoparticles have a cell viability value of > 50%, which means they are not toxic. The combination of 100% and 80% Aloe vera extract combined with variations of chitosan nanoparticles was not toxic, with a cell viability value of > 50%.

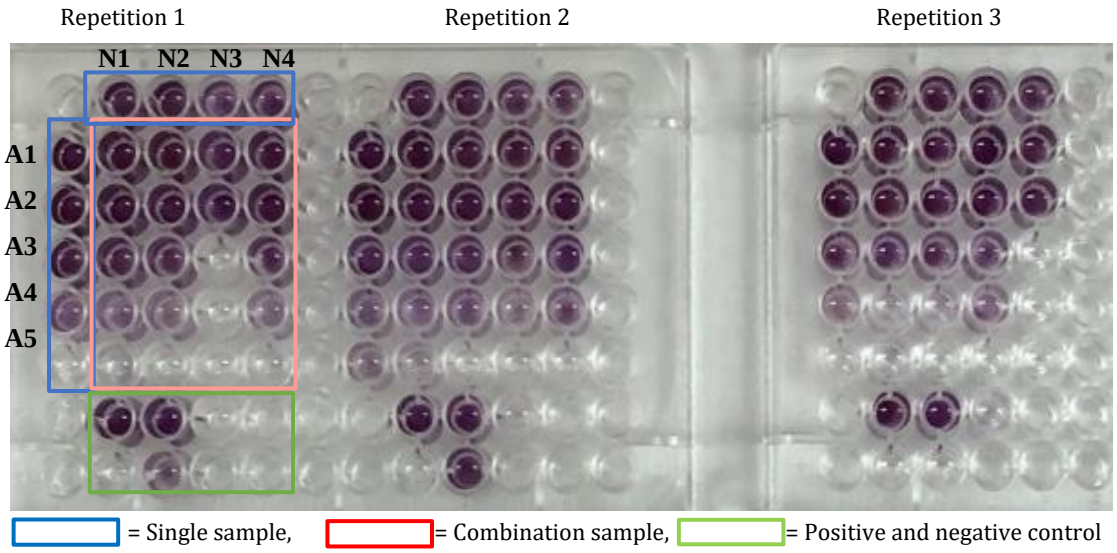


Fig 7. absorbance value with MTT Assay method

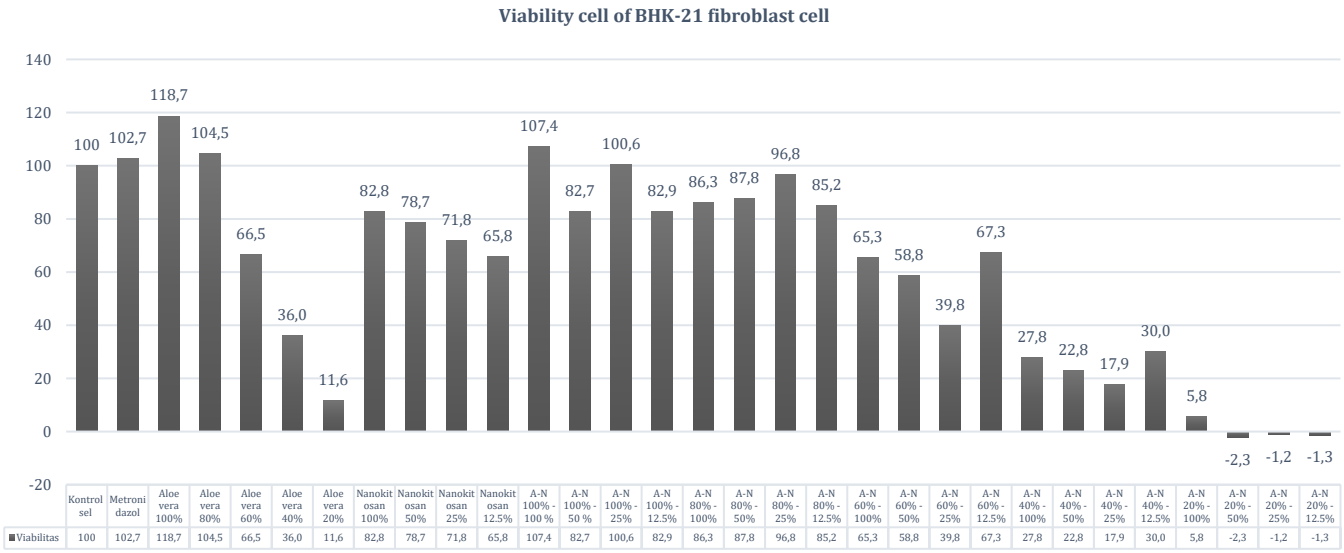


Fig 8. Viability cell of BHK-21 fibroblast cells after exposure variant combination concentration of Aloe vera extract and chitosan nanoparticle during 24 hours

DISSCUSION

The Aloe vera (L) Burm.f., also known as Aloe barbandesis Miller, was used in this study[21]. The part of the Aloe vera plant is used as Aloe vera gel. There were three components of aloe vera—the green aloe vera cuticle, Aloe vera latex, and Aloe vera gel- suitable for topical and oral medicinal medication use. Compared to other parts, aloe vera gel is the safest to use[22,8]. The middle layer comprises latex, a bitter yellow sap containing anthraquinones and glycosides. Molecules from this layer give Aloe its laxative effects. Aloe vera gel contains more antibacterial compounds than aloe cuticle and aloe latex, which means using aloe vera gel has a stronger antibacterial impact[23].

Standardization of extracts is a technique that must be followed to achieve the quality standards of herbs to have effective and secure effects[24]. Based on Table 1, the results of standard extract parameters such as value weight, moisture level, ash level, identity, and organoleptic complied with the quality requirements given in the Farmakope Herbal Indonesia (FHI) guidebook. An extract must adhere to these standards for quality to be regarded as a high grade[16].

The phytochemical test aims to determine with certainty the active compound content of the Aloe vera plant used in this study[25]. The phytochemical test was conducted qualitatively by observing the colour change after adding specific chemical reagents[25]. A preliminary phytochemical test is necessary because each species of Aloe vera has biological variations, which will affect the number of active compounds[26]. Aloe vera planted in region X might contain different ingredients than aloe vera grown in region Y. Based on Table 2, the phytochemical test findings revealed that the Aloe vera extract included beneficial substances such as flavonoids, tannins, saponins, alkaloids, and glycosides.

The mechanism of action of flavonoids is by inhibiting the function of membrane cells and the metabolism of bacteria[27]. When flavonoids hinder the function of membrane cells, they create complicated compounds with

extracellular proteins through hydrogen bonds that can harm membrane cell bacteria. The mechanism action of tannin is that it can deactivate bacterial adhesin, inhibit enzyme action and inhibit protein transport in a membrane cell[8]. The mechanism of action of saponin is to interfere with membrane cell bacterial permeability and the release of numerous vital components from the bacterial cell, including proteins, nucleic acids, and nucleotides[8]. Bacterial cells will be destroyed as a result. The mechanism of action of alkaloids is to interfere with the activity of bacterial cells, causing the denaturation of bacterial cell proteins. The mechanism action of glycosides can inhibit bacterial activity[27].

As the predominant bacterium causing periodontitis, *P.gingivalis* was selected as the test bacteria in this study. *P.gingivalis* is an obligate anaerobic Gram-negative bacterium. The structure of the Gram-negative cell wall is formed from a more complex peptidoglycan layer than Gram-positive[28]. Metronidazole, a bactericidal antibiotic frequently used in the treatment of periodontitis, was also employed in this study as a positive control because it has been demonstrated to be effective against anaerobic bacteria like *P.gingivalis*[28].

According to Fig 5, The higher the concentration of Aloe vera extracts exposed to *P.gingivalis. T*he greater the inhibition zone produced. The greater the inhibition zone formed, the fewer bacteria grew[29]. The antibacterial activity of Aloe vera extract is directly correlated to the number of active compounds; the greater the concentration of Aloe vera extract, the more active compounds there are[29]. The content of active antibacterial compounds in Aloe vera extract, such as flavonoids, tannins, saponins, and glycosides, will inhibit bacterial growth with a bactericidal mechanism, namely disrupting the permeability of the bacterial cell membrane, which results in damage to the permeability of the cell membrane and causes the release of various essential components from inside the bacterial cell, proteins, nucleic acid, and nucleotides. This condition eventually causes the bacterial cells to undergo lysis[29,30]. A similar outcome was seen when chitosan nanoparticles were exposed to *P.gingivalis.* The higher the concentration of chitosan nanoparticles exposed to *P.gingivalis,* the greater the inhibition zone produced[31]. The antibacterial activity of nanoparticles is directly correlated to the release of ions through electrostatic interactions between the positive charges of chitosan nanoparticles and the negative charges on the bacterial membrane cell, which changes the permeability of the cell wall[31,32].

According to Fig 6, Aloe vera extract at 100% and 80% concentrations combined with various chitosan nanoparticles had an inhibitory zone equivalent to or not significantly different from that of metronidazole. The inhibition zone in the mixture of 80% Aloe vera extract and various chitosan nanoparticle variations is greater than in a single Aloe vera or single chitosan nanoparticles sample. This is due to the function of chitosan nanoparticles as a matrix material that transports and delivers active chemicals from extracts increasing their antibacterial activity[33]. Chitosan nanoparticles can easily penetrate the bacterial membrane cell because of their specific surface area, reduced size, and increased stability[14,13,32]. In addition, chitosan nanoparticles have a better absorption capacity to quickly reach the intended organs and treat illnesses efficiently and promptly[14,13].

Aloe vera extract and chitosan nanoparticles have different antibacterial mechanisms against *P.gingivalis*, which have different effects and do not mutually influence the antibacterial activity of one another[34]. The antibacterial action of Aloe vera extract is bactericidal, whereas the antibacterial action of chitosan nanoparticles is bacteriostatic[35]. Chitosan nanoparticles will carry out intracellular binding, block mRNA, inhibit protein synthesis, and reduce cell metabolism to suppress bacterial growth[35] .

Cytotoxicity test to viability cell assay

The cytotoxicity test aims to ensure that a substance or material is biocompatible, does not have toxic potential, and does not cause allergies and irritation[36]. The first-level cytotoxicity test can be carried out in vitro using primary and secondary cell cultures (cell lines)[37]. In this study, the BHK-21 fibroblast cell line was used, derived from hamster kidney fibroblasts, which have structural similarities to human fibroblast cells found in the pulp, periodontal ligament, and gingiva. Furthermore, BHK-21 fibroblast cells may be easily grown and subcultured, making them a popular choice for testing the cytotoxicity of dental materials and medications[37,37].

The cytotoxicity test in this study used the MTT Assay method. The principle of the MTT Assay is a colourimetric (enzymatic) assay using the ability to live cells to reduce tetrazolium salts into insoluble purple formazan crystals[38]. A microplate reader was used to detect absorbance and calculate the number of alive cells. There is a direct relationship between the number of live cells and the intensity of the purple colour that results[20]. The absorbance value increases as the intensity of the resulting purple colour increases. This suggests that many cells are active and react with tetrazolium salt (MTT) to produce additional formazan[38]. According to Fig 7, the intensity of the formed purple colour is increasingly visible as the concentration of Aloe vera extract and chitosan nanoparticles increases.

There is a direct correlation between the intensity of the purple colour formed and the absorbance value[38,39]. Based on Fig 8, an increased Aloe vera extract and chitosan nanoparticles concentration directly correlates with increased absorbance value. The absorbance value increased with increasing concentration[38]. As a result of exposure to Aloe vera extract and chitosan nanoparticles, it was shown that there was no appreciable drop in the number of live cells. A substance is considered toxic if less than 50% of exposed cells survive following exposure[17].

At a concentration of 100%, Aloe vera extract has a cell viability percentage of 118,7%. A concentration of 80% has a cell viability percentage of 104,5%, and a concentration of 60% has a cell viability percentage of 66,5%. Due to the percentage of cell viability greater than 50%, these findings suggest that Aloe vera extract is biocompatible or non-toxic[17]. This is because Aloe vera extract contains active compounds like flavonoids, tannins, saponins, and glycosides that are safe and acceptable to fibroblast cells[40].

The percentage of cell viability was greater than 50% at four different concentrations of chitosan nanoparticles. This demonstrates that chitosan nanoparticles are not toxic to fibroblast cells; a concentration of 12.5% chitosan nanoparticles can be used as the minimum concentration. Chitosan is produced by extracting animal skin from

crustacean species such as crabs and shrimp, which are biocompatible, non-toxic, and have high molecular weights[35,41]. Chitosan that has been reduced in size to nanoparticles will penetrate cells more easily and effectively, increasing the therapeutic action potential[13]).

The percentage of cell viability is greater than 50% in a combination of 100% and 80% Aloe vera extract with various chitosan nanoparticles. This demonstrates that the combination of 100% and 80% Aloe vera extract and chitosan nanoparticles is not toxic, safe, or compatible for use as a treatment. The use of herbal medicines supported by nano-sized drug delivery systems has a promising future because it can increase the biological activity of herbs, reduce toxicity, and overcome the limitations of chemically synthesized drugs[12]). This herbal nanomedicine innovation must be further developed and evaluated in terms of efficacy and safety before it can be used more broadly in dentistry to prevent and treat various oral cavity diseases[12,32]).

CONCLUSION

The combination of Aloe vera extract and chitosan nanoparticles has antibacterial activity against *P.gingivalis* based on the minimal inhibitory concentration (MIC), minimal bactericidal concentration (KBM) and inhibition zone tests. The results of the MIC and KBM obtained were at a combined concentration of 80% Aloe vera – 25% chitosan nanoparticles. The inhibition zone in the combination of 80% Aloe vera extract with various variations of chitosan nanoparticles has a greater inhibition zone than in a single Aloe vera or single chitosan nanoparticles sample. Both of these materials do not have a synergistic antibacterial effect but have a different effect on killing bacteria. According to the MTT Assay, combining 100% and 80% concentrations of Aloe vera extract with various chitosan nanoparticles was not toxic to BHK-21 fibroblast cells.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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