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Inhibitory test of turmeric leaves extract (*Curcuma Longa. L*) against the growth of *Streptococcus mutans* bacterial growth *in vitro*

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ABSTRACT. *Streptococcus mutans* is a bacterium that plays an essential role in forming dental caries. Odontogenic infection is an oral cavity infection that can develop from dental caries if not appropriately treated. Caries treatment can be done with the use of antimicrobial agents. The increasing resistance to antimicrobial drugs has made herbal medicines the choice because they are more effective and less harmful. The community uses turmeric leaves as a cooking spice and treatment for stomach aches in children. Turmeric leaf extract contains flavonoids, tannin, and phenolic antimicrobial active compounds. This research aims to find out if turmeric leaf extract inhibits the growth of the *Streptococcus mutans*. This study uses a post-test-only control condition approach in a laboratory setting. The concentration of turmeric leaf extract tested was 10%, 15%, and 20% with the positive control (Chlorhexidine 0,2% mouthwash) and negative control (DMSO) with five repetitions. Antibacterial effectiveness test using Kirby-Bauer disc diffusion method. The data analysis using ANOVA showed a p-value of 0.00 on inhibition, which indicates that turmeric leaf extract can suppress the development of *Streptococcus mutans*. The average inhibition zone obtained was 8.7 mm at a concentration of 10%, at a concentration of 15% at 9.5 mm, and at a concentration of 20% at 8.54 mm. This research concludes that turmeric leaf extract can inhibit the proliferation of *Streptococcus mutans* bacteria

KEYWORDS: Turmeric Leaf, *Streptococcus mutans*, Antibacterial

INTRODUCTION

Dental caries is a multifactorial disease that occurs due to the production of acid by bacterial fermentation of food debris on the tooth surface, causing damage to the hard tissues and demineralizing the teeth. The factors that cause caries are bacteria, time, host, and substrate.[1] *Streptococcus mutans* is the bacteria that contributes **the** most to tooth decay. *Streptococcus mutans* bacteria are Gram-positive bacteria with a cocci shape and are found in the flora normal of the oral cavity.[2]

Odontogenic infections can also occur due to the bacterium *Streptococcus mutans*. Odontogenic infection may be the initiation or continuation of pulp, periodontal disease, pericoronal disease, trauma, or postoperative infection. One of the leading causes of odontogenic infections is dental caries. However, odontogenic infections can be caused by exodontia, periodontal pockets, or pericoronitis. Odontogenic infections can usually start from dental or periodontal tissues and expand to complex anatomical structures in the oral cavity. When bacteria reach the tooth's pulp, necrosis will occur, which will induce abscess formation. The infection begins in the periapical tissue and continues across the periosteum of the cortical bone and over the pathway of least resistance.[3]

Streptococcus mutans bacteria can be found in the flora normal of the oral cavity, odontogenic infections, and dental caries. Bacteria found in the flora normal of the oral cavity can turn into pathogenic bacteria, causing odontogenic infections. *Streptococcus mutans* have a significant function in the formation of caries due to the carbohydrate fermentation process resulting in acid production and lowering the pH of the oral cavity, which causes tooth demineralization and infection. The infection can occur through several routes in the oral cavity, such as the pulpo periapical pathway. Bacteria can enter this pathway through the enamel, dentin, and pulp chambers to the apical area of the tooth. Infection in the pulpo periapical tissue begins with

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dental caries resulting from the invasion of *Streptococcus mutans* bacteria. Bacterial involvement in the periapical tissue can initiate an inflammatory response to the infected tissue. An abscess can form if the host conditions are not satisfactory and the virulence of bacteria is high enough in the infected areas.[4]

Various methods are used to prevent caries, odontogenic infection, and abscess formation by inhibiting the growth of caries-causing bacteria using herbal medicines. Herbal medicines are the choice because they increase efficiency and suppress dangerous traits, show low toxic effects on mammals, and are generally considered safe. Medicinal herbs such as turmeric leaf (*curcuma longa. l*) comes from the Zingiberaceae family and originates from South Asia with well-known records of use as medicine commodities. In addition, turmeric leaves are also known to be utilized in conventional cooking methods to add flavor and function as a food preservative.[5]

Secondary metabolites that act as antibacterial ingredients in turmeric leaves are flavonoids, tannins, and phenolic compounds. Flavonoid compounds and tannins can prevent spoilage, also **known as** antibacterial.[6] The flavonoid bioactive compounds found in turmeric leaves have antibacterial properties through complex compounds that form to attack extracellular proteins and degrade the membrane cells' integrity by dissolving the membrane, thus damaging the cell membrane and releasing its intracellular components—followed by cell death. The active compounds of tannin also show antibacterial properties utilizing protein deposition because it is suspected that tannins have the same effect as phenolic compounds. Tannins can cause bacterial lysis by forming complexes with polypeptide proteins in the bacterial cell wall, causing disruption of the cell wall. In addition to harming cell membranes, phenolic substances can modify the permeation walls, preventing or speeding up cellular proliferation.[7]

MATERIALS AND METHODS

This study was carried out in vitro in an experimental laboratory setting at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, and at the Research and Development Laboratory of Medicinal Plants ASPETRI (Association of Indonesian Traditional Herbs Medicine). The research period was approximately two months, from February to April 2022.

The samples used in this research were turmeric leaves grown at the Tasbi Complex 2 block VI no. 57, which were studied as fresh leaves. Extraction of manufacture began with the manufacturing of Simplicia. Turmeric leaves were cut and dried in the drying cabinet, then mashed to obtain Simplicia powder.

Extracts were made by the maceration method. Simplicia was then immersed in 96% ethanol solvent for 24 hours, then filtered to obtain the first macerate. Then re-immersed with 96% ethanol and filtered to obtain a second macerate. The two macerates were combined and put into a rotary evaporator to obtain a thick extract of turmeric leaves, followed by the manufacture of turmeric leaf extract with various concentrations of 10%, 15%, and 20%.

Streptococcus mutans bacteria were planted in nutrient agar media and incubated at 37°C for 24 hours. Then, the bacterial suspension was taken in streaks on MHA media using a sterile cotton swab. An inhibition test was performed using the Kirby-Bauer method using disc paper with 10%, 15%, and 20% turmeric leaf extract, positive control (chlorhexidine 0.2%), and negative control (DMSO) on the media. Each concentration was repeated five times. Incubation was carried out at 37°C for 24 hours. The inhibition zone observed was measured with a caliper.

RESULTS

The study findings showed that turmeric leaf extract concentrations of 10%, 15%, and 20% and positive control (0.2% chlorhexidine) indicated the formation of an inhibitory zone. In contrast, the negative control (DMSO) did not show an inhibition zone. The inhibition zones formed at concentrations of turmeric leaf extract 10%, 15%, 20%, and 0.2% chlorhexidine against *Streptococcus mutans* were 8.7±0.45 mm; 9.5±0.58mm; 8.54±0.42mm; 14.42±1.40mm. In contrast, the negative control (DMSO) did not form a diameter of the inhibition zone.

The observation of the diameter of the inhibition zone showed the strength of the inhibition against *Streptococcus mutans* bacteria categorized according to Davis & Stout (1976), shown in Table 1. The power of the diameter of the inhibition zone at concentrations of 10%, 15%, and 20% was in the moderate category. In comparison, the strength of the diameter of the 0.2% chlorhexidine inhibition

zone as a positive control was classified as strong. Table 2. Shows the inhibition of *Streptococcus mutans*

Table 1. Inhibitory Zone Diameter Category[8]

Antibacterial Activity	Inhibition zone diameter (mm)
Weak	<5
Average	5-10
Strong	10-20
Very Strong	>20

Table.2 Diameter of inhibition zone of turmeric leaf extract (*Curcuma Longa.l*) against Streptococcus mutans

Groups	Inhibition zone diameter (mm)	Antibacterial strength
10% Turmeric leaf Extract (*Curcuma Longa. L*)	8.7	Average
15% Turmeric leaf Extract (*Curcuma Longa. L*)	9.5	Average
20% Turmeric leaf Extract (*Curcuma Longa. L*)	8.54	Average
Control positive (chlorhexidine 0,2%)	14.42	Strong
Control negative (DMSO)	0	Weak

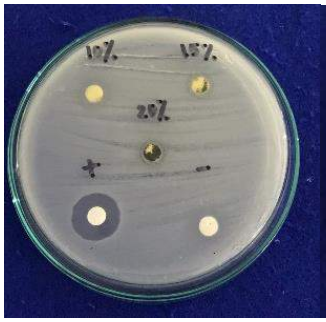

Figure 1. Repetition 1 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

Figure 2. Repetition 2 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

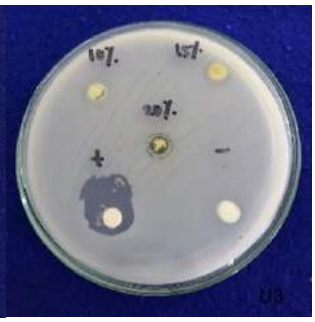

Figure 3. Repetition 3 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

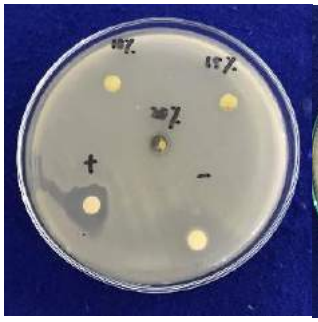

Figure 4. Repetition 4 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

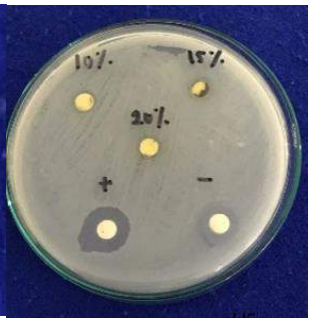

Figure 5. Repetition 5 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

DISCUSSION

The best treatment results in the inhibition test of turmeric leaf extract against *Streptococcus mutans* bacteria was at a concentration of 15% with an average inhibitory diameter of 9.5 mm. The 0.2% chlorhexidine mouthwash formula was used as a positive control with an average diameter of inhibition of 14.42 mm. When compared with the positive control treatment, the results showed the best treatment was turmeric leaf extract with a concentration of 15%. Still, it could not exceed the antibacterial activity of the 0.2% chlorhexidine mouthwash formula.

The existence of the inhibitory ability of turmeric leaf extract on the growth of *Streptococcus mutans* bacteria in vitro proves that there are compounds that function as bacteriostatic agents in the extracts that play a role in inhibiting the growth of the bacteria tested. The active substance of phenol in turmeric leaf extract has bacteriostatic properties. Phenol is an antimicrobial agent that denatures proteins and damages cell membranes. Antibacterial compounds can interact with the cell walls of bacteria, causing a decrease in the permeability of bacterial cells, which diffuses into the cells causing inhibition of bacterial growth. It shows the bacteriostatic properties of turmeric leaf extract.[9]

Streptococcus mutans is a gram-positive bacterium. There are differences between the structure of the cell walls of gram-positive and gram-negative bacteria that can affect the sensitivity towards antibacterial agents, thus showing different results. In gram-positive bacteria, a thicker cell wall is constructed of approximately 40 layers of peptidoglycan. The peptidoglycan reaches 70% of the cell wall's dry mass, causing the cell wall to become thick and stiff. Meanwhile, in gram-negative bacteria, the wall thickness is thinner, about 10% of the dry mass of the cell wall. The lipid content in gram-negative bacteria is high. Thus, it has a protein porin that acts as a channel for the entry of active substances into bacterial cells. The permeability of the active substance increases with high levels of lipids in the cells. It allows the active substance to enter the cell, causing cell damage which impairs enzymes' activity in the cell.[10]

The temperature is a factor that can affect the quality of the extract. Extract storage temperature can affect the content of active compounds in the extract. The extraction storage at higher temperatures decreases the range of active compounds in the extract. Meanwhile, storage of the extract at a low temperature, between 2-5^{o}C, can maintain the content of chemical compounds in the extract from decreasing.[11] In conclusion, the long storage time of the extract can affect its strength. Storage of sections for more than seven days can cause an increase in the activity of microorganisms that will lead to spoilage of the extract..

The purpose of this study is to continue research by making turmeric leaf extract as a mouthwash, asepsis, or antibiotics. It is because turmeric leaf extract has compounds that have the potential as antibacterial ingredients. Secondary metabolites such as saponins, tannins, terpenoids, triterpenoids, and flavonoids are polar so that they can inhibit bacterial growth by denaturing proteins in bacterial cells. Turmeric leaf extract is an antibacterial material suitable for aseptic ingredients such as mouthwash, asepsis ingredients, and antibiotics. It is proven according to previous research, which showed the antibacterial properties of turmeric leaves after being used as a hand sanitizer and food preservative.

This study proves that turmeric leaf extract has an antibacterial effect on the growth of *Streptococcus mutans* bacteria in the oral cavity in vitro. It is proven by the presence of an inhibition zone (clear zone) around the paper disc. This result is the first step in the possibility of using turmeric leaf as an alternative antibacterial ingredient in dentistry. Turmeric leaves are good antibacterial ingredients because turmeric leaves can help accelerate wound healing. In addition, turmeric leaf can also be used as a food preservative due to its strong antibacterial properties. In daily life, turmeric leaves are usually used as a cooking spice and medicine for stomach aches in children. The results of this study indicate that turmeric leaves have moderate antibacterial power. There is antibacterial potential against *Streptococcus mutans* because turmeric leaves have flavonoid, tannin, and active phenolic compounds that have antibacterial activity so they can be used as a basis for research. It shows turmeric leaves' antibacterial properties, which is suitable for further study as an antiseptic ingredient such as mouthwash.

CONCLUSION

Turmeric leaf extract (*Curcuma Longa. L*) can restrict the development of *Streptococcus mutans* bacteria in vitro. At a concentration of 15%, turmeric leaf extract (*Curcuma Longa. L*) had the largest inhibition zone in restricting the development of *Streptococcus mutans* bacteria in vitro, with an average inhibition diameter of 9.5 mm.

Recommendation

1. Further research is needed on the antibacterial effect of turmeric leaf extract (*Curcuma Longa. L*) in dosage form so that it can be used in treating dental and oral diseases.
2. Further research is needed on the antibacterial effect of turmeric leaf extract (*Curcuma Longa. L*) in dosage form so that it can be used in treating dental and oral diseases.
3. Further research is needed for antibacterial tests with other methods so that the values of the Minimum Inhibitory Concentration (MIC) and the minimum bactericidal concentration (MBC) of turmeric leaf extract (*Curcuma Longa. L*) can be calculated on the growth of *Streptococcus mutans* bacteria.
4. It is necessary to carry out an antibacterial test with other extraction methods against other gram-positive and gram-negative bacteria.
5. It is necessary to do an antibacterial test of turmeric leaves using another formula for determining sample size to prevent bias.
6. Further research is needed on turmeric (*Curcuma Longa. L*) leaves as asepsis, mouthwash, and antibiotics.

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Antibacterial potential ethanol extract of beluntas leaves (*Pluchea indica L*) to *Streptococcus sanguinis*

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ABSTRACT. Recurrent aphthous stomatitis (RAS) is one of the most common oral diseases in the community, with a prevalence of 5-66%, with one of the predisposing factors being *Streptococcus sanguinis*. Treatment for RAS has been symptomatic and supportive, including antiseptic mouthwash such as chlorhexidine gluconate 0.2% or topical corticosteroids (triamcinolone acetonide 0.1% in Orabase). However, these drugs have some side effects. Treating herbal ingredients such as Beluntas leaves low prices and minimal side effects. The active compounds in Beluntas leaves are phenols, tannins, flavonoids, saponins, triterpenoids, essential oils, terpenoids, and many compounds known to have antibacterial activity. Methods: This study aimed to determine the minimum inhibitory level (MIC), and minimum killing rate (MBC) of 96% ethanol extract of Beluntas leaves on the growth of *Streptococcus sanguinis*. MIC was measured by broth microdilution technique with DMSO solvent 10% and eight concentrations of beluntas extract. Chlorhexidine gluconate 0.2% was used as a positive control for the comparison compound. Furthermore, the MBC test was carried out using the total plate count method for treatments that gave the MIC value. One Way Anova analysis with Post Hoc Tukey was used to determine the significant difference between treatments. Results: The ethanol extract of Beluntas leaves (*Pluchea indica L*) has a Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the growth of *Streptococcus sanguinis* with a minimum inhibitory concentration of 3.95 g/mL and a minimum concentration of 7.8 g/mL. Conclusion: The ethanol extract of Beluntas leaves (*Pluchea indica L*) has the potential as an antibacterial against *Streptococcus sanguinis*.

KEYWORDS: Recurrent aphthous stomatitis, *Streptococcus sanguinis*, ethanol extract of beluntas leaves, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a problem that often arises in dentistry and is one of the most common oral diseases in the community, with a prevalence of 66%. RAS is characterized by recurrent ulcers on the patient's oral mucosa and can be painful. The prevalence of oral ulceration worldwide is as much as 4%, where RAS is the disease with the greatest prevalence of 25% and is experienced by most women in the second and third decades.[1,2]

The clinical manifestations of RAS are ulcers, single or multiple, shallow, oval, and pain.[3] Prodromal symptoms appear before the onset of RAS, including discomfort and redness for 1-3 days.[4] The exact etiology of RAS has not been determined so the research focused on the predisposing factors for RAS. Several factors are considered predisposing factors for RAS, including immunological abnormalities, genetics, systemic factors, endocrine system, stress, smoking cessation, allergic factors, and microorganisms. The main microorganisms associated with the formation of RAS are pleomorphic transitional L α-hemolytic Streptococcus and *Streptococcus sanguinis*.[5]

One of the efforts to manage RAS is by symptomatic and supportive treatment because, until now, the etiology of RAS is still unknown. Therefore, treatment is only aimed at curing complaints. The goal of symptomatic treatment is to reduce symptoms and the number and size of ulcers. Currently used drugs include: antiseptic mouthwash (chlorhexidine gluconate 0.2%) to reduce the

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duration and discomfort of RAS or topical corticosteroids (triamcinolone acetonide 0.1% in Orabase).[6] Chlorhexidine gluconate 0.2% is the gold standard, effective against gram-negative and positive and facultative aerobes and anaerobes. However, its use in more than two weeks has side effects, including causing burning of the oral mucosa, impaired taste, tooth staining, erosion of the oral surface, and dryness of the oral cavity.[7]

Therefore, it is necessary to develop an alternative therapy by utilizing herbal plants in curing ulcers in RAS. It is intended so that patients can recover without side effects at a relatively low cost.[8] The development of a back-to-nature lifestyle has made herbal plants increasingly widespread by the community as alternative materials for treating or preventing certain diseases. The community considers that herbal ingredients are easier to obtain, the price is more affordable, and the side effects are minimal, so it is safer for consumption.[9] One of the natural ingredients that can be used in traditional medicine is the beluntas plant.[10] The leaves, flowers, and roots of beluntas can be used for therapy, but the beluntas leaves are the part that has the highest biologically active component.[11]

Beluntas (*Pluchea indica L*) is a wild plant in dry areas on hard and rocky soils or grown as a hedge.[12] This plant has a distinctive aromatic odor and bitter taste. The part used from this plant is the leaves and roots, which are efficacious for eliminating body odor and bad breath, increasing appetite, overcoming digestive disorders in children, relieving pain in rheumatism, and so on. The active compounds in beluntas leaves are flavonoids, triterpenoids, phenols, and essential oil derivatives.[13] Beluntas leaves have antibacterial properties due to the content of phenolics, triterpenoids, and tannins that can cause bacterial cell death.[14,15]

Based on several studies that have been carried out, the ethanol extract of beluntas leaves (*Pluchea indica L*) has been shown to inhibit bacterial growth. These bacteria include Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis, and *Pseudomonas aeruginos*a.

Based on the explanation above and there is no research on *Streptococcus sanguinis* bacteria, so the authors are interested in research to determine the antibacterial effect of beluntas leaves through testing the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanol extract of the beluntas leaves on the growth of *Streptococcus sanguinis* which is one of the bacteria that causes recurrent aphthous stomatitis.

MATERIALS AND METHODS

Determination and Ethanol Extract of Beluntas Leaves

Beluntas leaves were obtained from the Subang Tropical Balitbu plantation, which was then determined at the Biology Laboratory of Padjadjaran University. Extract beluntas leaves with 96% ethanol and let stand for three days, then the extract is filtered using filter paper to obtain the filtrate. Then the filtrate was evaporated using a rotary evaporator at a temperature of 70°C until the consistency was like a paste.

The ethanol extract of beluntas leaves was put into a 15 mL tube with as much as 0.5 grams and dissolved in 10% DMSO to reach a concentration of 500 g/mL. The extract was diluted using a multichannel Micropipette so that the final concentrations were 250g/ml, 125g/ml, 62.5g/ml, 31.3g/ml, 15.6g/ml, 7.8g/ml, 3.95g/ml, and 1.95g/ml.

Preparation of *Streptococcus sanguinis* inoculum

The bacteria used in this study is *Streptococcus sanguinis* ATCC 10556. Take two oses and suspension in BHI-B, incubated for 24 hours. Then standardized with 0.5 McFarland solution, the total density of bacteria used is 1.5 x 10^8 CFU/ml. Check using a spectrophotometer with a wavelength of 600nm, then dilute to 10^5.

Minimum Inhibitory Level (MIC) and Minimum Killing Rate (KBM)

The method used in this study followed the method developed by CLSI (Clinical Laboratory Standard Institute) with a slight modification, namely adding 2% sucrose to BHI-A and BH-B media.

This study consisted of ten treatments, namely eight treatments of ethanol extract of beluntas leaves with concentrations of 250g/ml; 125g/ml; 62.5g/ml; 31.3g/ml; 15.6g/ml; 7,8g/ml; 3.95g/ml, and

1.95g/ml; positive control (chlorhexidine gluconate 0.2%); and negative control (DMSO).

200 L/mL of BHI-Broth media was added to all 96 well plates and added 200 l of ethanol extract of beluntas leaves of each concentration, and 10 l of bacterial suspension, with the format of media+sample (negative control), media+solvent (solvent control), media+sample+bacteria (test sample), media+solvent+bacteria (positive control). Incubate at 37°C for 16-20 hours. Insert the well plate into a spectrophotometer with a wavelength of 600 nm to see the minimum inhibitory concentration using the absorbance method.

Take 200 L of 0.2% Chlorhexidine gluconate and put it into the well plate with 3 repetitions, 10 l of bacterial suspension 10^5 for two repetitions, and one repetition plate without bacteria as a positive control. Incubate for 24 hours at 37°C with 5% CO_2.

96-well plates indicated as MIC and KBM were inoculated into sterile BHI-Agar medium in Petri dishes and incubated at 37°C with 5% CO_2 for 24 hours, calculating the number of colonies in each petri dish from each treatment with various concentrations of extract using a colony counter.

The number of colonies contained in BHI-Agar media was used to determine MIC, and MBC was calculated using a Colony counter. The calculation of the number of colonies formed is called the total plate count (TPC), with colony forming units/mL (CFU/mL) calculated by the formula:

$$\text{TPC value (CFU/ml)} = \frac{\text{Number of colonies formed x Dilution factor}}{\text{The volume of bacterial colonies that is included in the criteria}}$$

The formula measures the calculation of the percentage of bactericidal power:

$$\text{\% Kill Efficiency} = \frac{\text{TPC bacteria control (CFU/ml)} - \text{TPC treatment (CFU/ml)} \times 100\%}{\text{Mean bacterial colony control}}$$

RESULTS

The Beluntas plants' determination results at the Biology Laboratory of Padjadjaran University, Jatinangor, and West Java showed that the sample used was Beluntas (*Pluchea indica L*). Phytochemical tests were carried out at the Central Laboratory of Padjadjaran University, Jatinangor, West Java. Table 1 shows the results of qualitative phytochemical tests taken from the ethanol extract of beluntas leaves.

Table 1. Qualitative Phytochemical Test Results

No	Secondary Metabolite	Methods	Results
1	Phenolic	Reagent $FeCL_3$ 5%	++
2	Tanin	Reagent $FeCL_3$ 1%	++
3	Flavonoid	a. Reagent HCL concentrated + Mg	-
		b. Reagent H_2SO_4 2N	-
		c. Reagent NaOH 10	+
4	Saponin	Heated	+
5	Triterpenoid	Reagent H_2SO_4 concentrated + CH_3COOH	++
	dan Steroid	anhydrous	-
6	Alkaloid	Reagent Dragendorff	+

Description: +: Little; ++: Medium; +++: Much ; - : None

Minimum Inhibitory Level (MIC)

The results of the MIC test observed the growth of *Streptococcus sanguinis*, which was indicated by turbidity at the bottom of the well in several treatments compared to negative controls. The results of visual observations can be seen in the good extract of beluntas at a concentration of 1.95g/mL. There is turbidity at the bottom which is a culture of *Streptococcus sanguinis*. There was slight

turbidity at well 3.9g/mL, while at wells 7.8 to 250, visually, no difference in turbidity was observed. Solvent control and clear positive control showed no contamination in the experiment.

Figure 1. The plate of minimum inhibitory content test results

The average results of the absorbance of the effect of the ethanolic extract of beluntas leaves on the number of colonies of *Streptococcus sanguinis* using spectrophotometry with a wavelength of 600nm, resulting in quantitative data that confirmed that the MIC was right at a concentration of 3.9 g/mL. (**Table 2**)

Table 2. The absorbance of ethanol extract of beluntas leaves against *Streptococcus sanguinis*

Extract Concentration	Media control	Average Absorbance Bacteria+ Sample	Difference	Note(s)
250µg/mL	2,039	2,018	0,021	
125µg/mL	1,4203	1,3964	0,023	
62.5µg/mL	0,8977	0,8568	0,04	
31.25µg/mL	0,5208	0,4938	0,27	
15.62µg/ mL	0,312	0,2874	0,25	
7.8µg/mL	0,1873	0,1789	0,84	
3.9µg/mL	0,1245	0,114	0,1	MIC
1.95µg/mL	0,0795	0,0833	-0,0035	

The results of the positive control test (chlorhexidine gluconate 0.2%) obtained the average number of colonies of *Streptococcus sanguinis* bacteria with three repetitions after 24 hours of incubation was 0 CFU/mL with an average bactericidal power of 100%.

Minimum Bactericidal Concentration (MBC)

The results of the MBC test showed that at a concentration of 7.8g/mL, the ethanol extract of the tested beluntas leaves was not observed for the growth of *Streptococcus sanguinis*. To count the number of bacteria at each test concentration, the number of bacteria was calculated using the total plate count technique with a colony counter, as shown in **Figure 2** and **Table 3**.

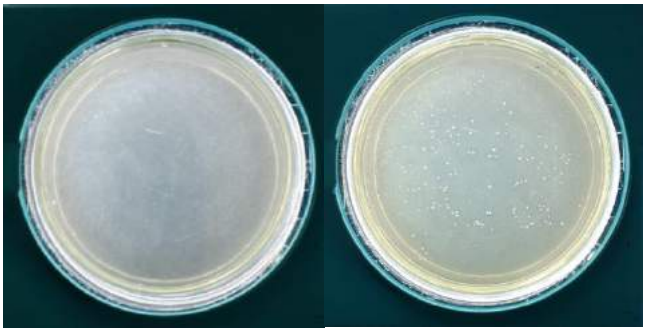

Figure 2. The results of the calculation of the number of bacteria using the Colony Counter
Notes: (A) Number of *Streptococcus sanguinis* Bacteria Colonies at the Concentration of Ethanol Extract of Beluntas Leaves 7.8g/mL (KBM). (B) Number of *Streptococcus sanguinis* Bacteria Colonies at Concentration of Ethanol Extract of Beluntas Leaves 3.9g/mL (MIC)

Table 3. Number of *Streptococcus sanguinis* Bacteria Colonies After 24 hours of incubation at various concentrations

Extract Concentration	Mean Colony (CFU/mL)	Mean kill Efficiency	Note(s)
Group A (250 µg/mL)	0	100%	
Group B (125 µg/mL)	0	100%	
Group C (62.5 µg/mL)	0	100%	
Group D (31.25 µg/mL)	0	100%	
Group E (15.62 µg/ mL)	0	100%	
Group F (7.8 µg/mL)	0	100%	MBC
Group G (3.9 µg/mL)	$12{,}9 \times 10^{1}$	99%(0,9999612)	MIC
Group H (1.95 µg/mL)	$27{,}3 \times 10^{5}$	99%(0,9999178)	

Colony Number Normality Test Results

The normality test results using Shapiro-Wilk showed that two variables were tested, namely concentrations of 3.95g/mL and 1.95g/mL. It is known that the significance value is 0.147 and 0.114, where the value is more significant than with a value of 0.05. Thus, it can be concluded that the data is normally distributed.

One-Way ANOVA Analyses

The results of the One Way ANOVA test to determine whether the data has an average difference or cannot be known from the significance value of the number of colonies then compared with the significance value, with a test decision, if the value (Sig) > 0.05, then there is no difference in average if value (Sig) < 0.05 then there is an average difference. The results of the ANOVA test above show the F value for the number of colonies of 22.882 and the significance (p-value) of 0.000 is less than 0.05, so it can be concluded that at concentrations of 3.95 and 1.95, there was a different effect on the number of colonies.

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Tukey's Post-Hoc Test Results

Based on the results of the Tukey Post Hoc test in appendix 3, the number of colonies at a concentration of 250 was the same as a concentration of 125, 62.5, 31.3, 15.6, 7.8, and 1.95 because they were in the same subset. Meanwhile, the number of colonies at a concentration of 3.95 was not the same as that of 125, 62.5, 31.3, 15.6, 7.8, and 1.95 because they were indifferent subsets. The comparison results can be seen through the mean difference column, obtained at a concentration of 3.95 significantly different ($p < 0.05$).

DISCUSSION

Based on the research results presented in **Table 2**, it was found that the ethanol extract of beluntas leaves had an inhibitory and killing effect on the growth of *Streptococcus sanguinis* bacteria and there was MIC at a concentration of 3.9g/mL with 99% killing power and MBC was found at a concentration of 7.8g/mL. mL with 100% killing power. The positive control group of chlorhexidine gluconate at a concentration of 0.2% had an inhibitory and killing effect on *Streptococcus sanguinis* bacteria with an average killing power of 100%. Therefore, it can be said that the ethanol extract of beluntas leaves at a concentration of 7.8g/mL had the same antibacterial effect as 0.2% chlorhexidine gluconate. The statistical test value obtained a P-value of 0.00, which states that the MIC and MBC values significantly affect the growth of *Streptococcus sanguinis* bacteria.

These results follow previous research, which stated that the ethanol extract of beluntas leaves (*Pluchea indica L*) could inhibit the growth of bacteria, *Staphylococcus aureus*, *Bacillus subtilis,* and *Pseudomonas aeruginosa*.[16,17] Based on the results of qualitative phytochemical tests, active compounds in beluntas leaves, such as phenols, tannins, flavonoids, saponins, triterpenoids, and alkaloids. Phenol compounds have antibacterial properties due to the presence of hydroxyl and carbonyl groups that can interact with bacterial cells through hydrogen bonds. It will cause the coagulation of proteins and bacterial cell membranes, which will cause bacteria to lyse.[18] Phenol can also cause hyperpolarization of the cytoplasmic membrane and increase the instability membrane. It causes membrane dysfunction and bacterial cell death.[14,15]

Tannins are one type of compound that belongs to the polyphenol group and has soluble properties in water and organic solvents. Tannins can be used as antibacterial because they have an antiseptic phenol group, so they can be used as an antibacterial component through hydrogen bonds which will form complex compounds with proteins and will cause bacterial cell proteins to be denatured so that bacterial metabolism is disrupted. [14,15,19]

Flavonoids also have a role as antibacterial compounds by inhibiting cell membrane function, nucleic acid synthesis, and inhibiting energy metabolism. The position in inhibiting the synthesis of nucleic acids occurs through the accumulation of nucleic acid bases, thereby inhibiting the formation of DNA and RNA because rings A and B of flavonoid compounds play an important role in the process of intercalation or hydrogen bonding. The result of flavonoid interaction will also cause damage to the permeability of the bacterial cell wall. Flavonoids will also form complex extracellular compounds and soluble proteins that cause damage to cell membranes and the release of intracellular compounds, thus causing cell function to be inhibited. In addition, energy metabolism will also be hampered due to the inhibition of oxygen used by bacteria. It can occur by preventing the formation of energy in the cytoplasmic membrane and inhibiting the motility of bacteria which play a role in antimicrobial activity and extracellular proteins.[20,21]

Other components, namely saponins, have broad biological activity and act as antibacterial and antifungal agents. Saponins can cause leakage of bacterial cell membranes, resulting in damage to membrane permeability which can interfere with the survival of bacteria. When the stability of the bacterial cell membrane is disturbed, it will cause the cytoplasm to come out of the cell. It occurs when saponins bind to the cytoplasmic membrane through the outer membrane, which will cause the stability of the bacterial cell membrane to be disturbed and result in bacterial cell lysis and then death. [21,22]

Triterpenoid compounds are also reported to have antibacterial activity. Triterpenoids will react with porins (transmembrane proteins) on the outer wall of the bacterial cell membrane causing the formation of strong polymer bonds. This condition will cause damage to the porin, which is the entrance and exit of the compound. Furthermore, this causes the permeability of the bacterial cell wall to decrease, the bacterial cell to lysis, and the death of the bacterial cell. [14,15,23,24]

Alkaloids also have antibacterial properties that cause bacterial cell death because the cell wall layer is not completely formed by interfering with the peptidoglycan constituent components of bacterial cells by interfering with the formation of cross-bridges of peptidoglycan constituent components in bacterial cells. Alkaloids can also inhibit enzymes that play a role in the DNA replication process, inhibiting bacterial growth because bacteria cannot divide by inhibiting DNA replication.[25,26]

Because beluntas leaves have an active biological content that acts as an antibacterial, the ethanol extract of beluntas leaves can be developed and used as natural antibacterial ingredient because there is a significant value at a concentration of 7.8g/mL with the number of colonies of *Streptococcus sanguinis* bacteria 0 CFU/mL.

CONCLUSION

The ethanol extract of beluntas leaves (*Pluchea indica L*) has the potential as an antibacterial against *Streptococcus sanguinis*.

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Original Research

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Effectiveness of ethanol extract of kirinyuh leaf (*Chromolaena odorata* L) on the increase the fibroblasts and angiogenesis in nose mucosa of rabbit (*lepus curpaneums*)

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ABSTRACT. Wounds are the loss or damage to a part of the body's tissue that disrupts the tissue's continuity. Kirinyuh plants have been used as a traditional wound treatment in various cultures worldwide. Purpose: To prove the effectiveness of topical ethanol extract of kirinyuh leaf ointment on increasing angiogenesis and fibroblasts in healing rabbit nasal mucosal incisions. Methods: This research is an experimental study with a posttest-only control group design. The research subjects consisted of 25 rabbits divided into five groups; one positive control group, one negative control group, and three treatment groups. The positive control group was given betadine ointment, the negative control group was made by incision of the nasal mucosa, and the three treatment groups were given 5%, 10%, and 15% concentrations of kirinyuh leaf ethanol extract ointment. Results: The results showed that administering kirinyuh leaf ethanol extract ointment with concentrations of 5%, 10%, and 15% could improve the healing of rabbit nasal mucosal incisions (Lepus Curpaneums), which were characterized by an increase in the number of fibroblasts and angiogenesis. In general, the increase in fibroblast cells between the concentrations of the ethanol extract of kirinyuh leaf ointment was significantly different ($p<0.05$) with a negative correlation ($r= -0.538$). Meanwhile, there was no significant difference in the amount of angiogenesis between concentrations ($p>0.05$) with a positive relationship ($r=0.099$).Conclusion: the ethanol extract of kirinyuh leaf ointment applied topically improves the healing of rabbit nasal mucosal incisions

KEYWORDS: ethanol extract of kirinyuh leaf ointment, wound healing, fibroblast, angiogenesis

INTRODUCTION

Wounds are loss or damage to somebody's tissues caused by sharp or blunt trauma, changes in temperature, chemicals, explosions, electric shocks, or animal bites. Injuries to the nasal mucosa can be caused by trauma, radiotherapy, chronic infections such as sinusitis, and rhinoplasty.[1,2,3]

The wound healing process consists of various complex processes to restore tissue integrity. Blood clots, acute and chronic inflammatory responses, neovascularization, cell proliferation, and apoptosis occur during this process. Wound healing consists of several stages and is influenced by many factors, both internal and external, starting with inflammation and followed by the process of fibroplasia, then tissue remodeling and scar tissue formation[4,5]

Kirinyuh or siam weed (*Chromolaena odorata (L)*) is a medicinal plant used by the community as a medicine for wounds, treating infections, headaches, and diarrhea, as an astringent, antispasmodic, antihypertensive, anti-inflammatory, and diuretic.[6]

Ayyanar et al., 2009, determined that extracts from *Chromolaena odorata (L)* help treat wounds. In traditional use, the leaves are ground into a paste and applied to the affected area to heal the wound. Thang et al. (1998 and 2001) reported that Chromolaena odorata(L) extract stimulated the proliferation of keratinocytes in human epidermal keratinocytes at low concentrations (0.1–5 µg/mL).[8]

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This study was conducted to evaluate the role of *Chromolaena odorata (L)*) ethanol extract ointment in increasing the acceleration of wound healing in the nasal mucosa of rabbits in increasing fibroblast cells and angiogenesis so that it can be used as a wound medicine candidate.

MATERIALS AND METHODS

This research was an experimental study with a posttest-only control group design, and the research subjects were male rabbits (*Lepus curpaeums*), body weights 2000-2500 grams, and the ethanol extract of kirinyuh leaf (*Chromolaena odorata (L)*) obtained from the Darussalam area.

The experimental group was divided into five different groups:

1. Treatment group P1, the experimental animal group, by gave 5% topical ethanol extract of kirinyuh leaves ointment
2. Treatment group P2, the experimental animal group, offers 10% topical kirinyuh leaf ethanol extract ointment
3. P3 treatment group, experimental animal group by giving 15% topical kirinyuh leaf ethanol extract ointment
4. KN Negative Control Group, an experimental group of animals with nasal mucosal incisions.
5. KP Positive Control Group, an experimental group of animals by administering betadine ointment to incisions of the nasal mucosa

RESULTS

Figure 1 shows that the quantity of fibroblast cells increased in all treatment groups. These results indicate that the concentration does not affect the development of fibroblast cells. It means that the percentage distribution of fibroblast cells in the treatment group did not follow the concentration of the ethanol extract of kirinyuh leaf ointment as a test material for wound healing of nasal mucosal incisions. In this study, it was also shown that the ability to induce the development of fibroblast cells in the ethanol extract of kirinyuh leaf ointment was better than positive and negative controls.

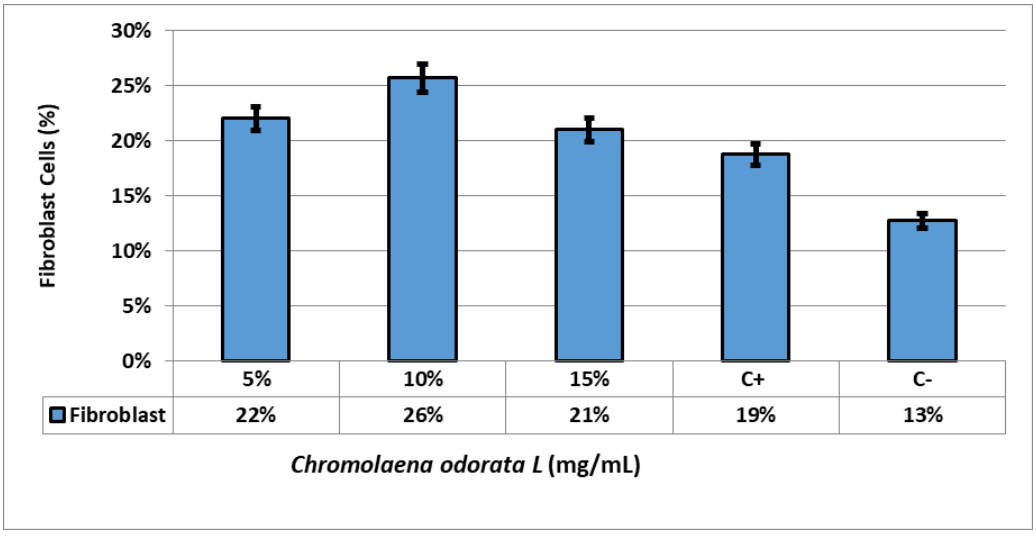

Figure 1. Increase in the number of Fibroblast cells

Figure 2 shows that the highest angiogenesis occurred in the positive control group, while the treatment group had the same amount of angiogenesis as the negative control group.

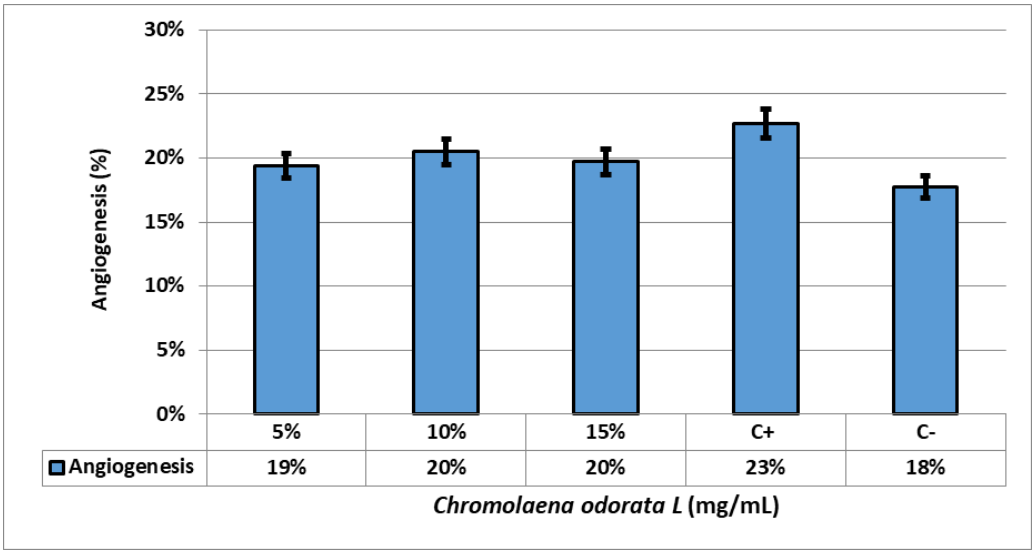

Figure 2. Increased Number of Angiogenesis

Figure 3 shows a graph of the relationship between fibroblast cell variables and angiogenesis. The 15% kirinyuh leaf ointment concentration had a better relationship than the other treatments. It means that in wound healing, this concentration has an excellent response to the host immune system involved in wound healing. The presence of fibroblast cells simultaneously increases angiogenesis in the wound area.

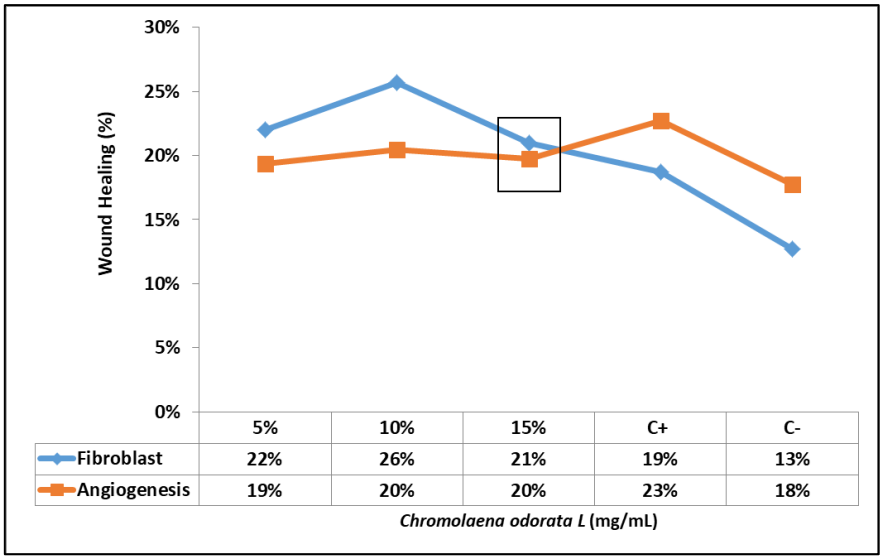

Figure 3. Relationship of Increased Angiogenesis with Fibroblast Cells

Table 1 shows the distribution and frequency of involvement of fibroblast cells and angiogenesis in wound healing. In general, of all concentrations, the increase in fibroblasts reached 69% while the increase in angiogenesis reached 60%. It means that the activity of fibroblast cells determines the development of angiogenesis.

Table 1. Distribution and frequency of development of fibroblast cells and angiogenesis in rabbit nasal mucosal incisions after being given kirinyuh leaf ethanol extract ointment Kirinyuh

Concentration	N	Fibroblast				P	Angiogenesis				p
		Cell	SDV	Freq (%)	Scale		Blood Vessel	SDV	Freq (%)	Scale	
5%	5	8.6	1.19	22%	Moderate	p<0.05 (0,04)	2.8	0.40	19%	Low	p >0.05 (0.685)
10%	5	10.0	1.23	26%	High		3.0	0.62	20%	Moderate	
15%	5	8.2	0.74	21%	Moderate		2.9	0.49	20%	Moderate	
C+	5	7.3	0.50	19%	Moderate		3.3	0.33	23%	High	
C-	5	5.0	0.81	13%	Low		2.6	0.25	18%	Low	

DISCUSSION

This study evaluates the role of kirinyuh leaf ethanol extract ointment in improving the healing of incisions on the nasal mucosa of rabbits. Specifically, it estimates the increase in fibroblast cells and angiogenesis as a reference for wound healing.

Gonzales (2016) reported that fibroblasts are the primary cells in wound healing. These cells are cellular elements commonly found in connective tissue that proliferate and actively synthesize matrix components in wound healing and repair of damaged tissue.[9] Fibroblasts are the primary material for forming scar tissue and collagen, providing tensile strength in wound healing of soft tissue. When the tissue is inflamed, the primary cells that migrate toward the wound are fibroblasts.[10] In addition, the assessment of angiogenesis in this study relates to forming new blood vessels. It is also involved in healing and developing new tissue after damage. Fibroblasts naturally play an important role in wound healing and reproduction

Figure 1 shows that the ethanol extract of kiriyuh leaf ointment can increase the number of fibroblast cells, which means it can accelerate the wound healing process. This phenomenon can be justified because several chemical compounds in the *Chromolaena odorata (L)* extract act as antiplatelet, anti-inflammatory, and antioxidants.[10] Sanchez (2018) reported that the Squalene compound is an antioxidant that can help increase the cell's defense response against infectious agents. While increasing the intensity of fibroblast cell production. In addition, an increase in fibroblast cells is also possible due to a decrease in infection or inflammation due to the influence of these chemical compounds.[12] Phenol compounds, (3S)-7-O-Methoxymethylvestitol and 9,12,15-Octadecatrienoic acid, methyl esters contained in *Chromolaena odorata (L)* can work as an anti-inflammatory. Decreased inflammation can help increase the migration of fibroblast cells to the wound area.

The wound-healing phase consists of hemostasis, inflammation, proliferation, and remodeling. Several factors can influence this physiological process, and abnormalities in these phases can lead to impaired wound healing.[14] The results of this study have illustrated the physiological role of *Chromolaena odorata (L)* in accelerating the formation of tissue or matrix synthesized by fibroblast cells. In addition, *Chromolaena odorata (L)* can act as an antithrombotic (antiplatelet), which works to prevent the accumulation of metabolic waste cells (coagulation) in the blood vessels.[15] so that the blood can remain thin. In general, based on the results of this study, *Chromolaena odorata (L)* can be involved in the process of wound healing, starting from stopping bleeding (hemostasis) and inflammation (inflammation), forming new tissue, and strengthening the tissue.

Figure 2 shows the wound area where angiogenesis is formed, indicating that Chromolaena odorata (L) has a good effect in increasing Nitrix Oxide production, increasing VEGF release, and activating the actin signaling pathway. The research findings from Figure 2 also show that *Chromolaena odorata (L)* can increase the mobilization of endothelial progenitor cells (fibroblast cells) and accelerate reendothelialization after vascular injury (Figure.3). In addition, *Chromolaena odorata* (L) may be angiogenic by initiating the release of protease enzymes from activated endothelial cells.[16] The formation of vascular blood vessels is characterized by degradation of the extracellular matrix, migration, and proliferation of endothelial cells, and the production of new extracellular matrix, followed by controlled and modulated blood vessel maturation/stabilization to meet tissue needs.[17] This

phenomenon could be possible that *Chromolaena odorata (L)* can reduce wound healing time while simultaneously increasing collagen deposition, fibroblast number, and blood vessel density (Figure. 3). In addition, it has been reported that chemical substances/compounds from *Chromolaena odorata (L)* function as pro-angiogenic agents during wound healing and wound repair by regulating TGF-β.[18]

Theoretically, excessive angiogenesis occurs when abnormal cells produce high amounts of angiogenic factors that inhibit the effect of angiogenesis inhibitors. Conversely, if the production of angiogenic factors is low, it can inhibit the regeneration of blood vessel cells, resulting in atherosclerosis, stroke, infertility, ulcers, and delayed wound healing. Abnormalities can result in tissue death.

Angiogenesis is also reported as forming new blood vessels from pre-existing blood vessels through developing or remodeling existing blood vessels.[20] The mechanism of blood vessel formation by angiogenesis begins with cell division facilitated by angiogenic substances and then travels to nearby blood vessels and activates its endothelial cell receptors

Figure 3 shows a graph of the relationship between fibroblast cell variables and angiogenesis. Based on the chart, it is explained that fibroblast cells can play a role in increasing angiogenesis in the healing of cuts in the nasal mucosa of rabbits. These results show fibroblasts secrete factors other than VEGF that induce an angiogenic phenotype in endothelial cells. Fibroblasts and fibroblasts-CM support endothelial cell growth and lumen formation.[21] Meanwhile, VEGF (Vascular Endothelial Growth Factor) promotes angiogenesis through several mechanisms, including increased proliferation and survival of endothelial cells; increased migration and invasion of endothelial cells; increased permeability of existing blood vessels, forming a lattice network for endothelial cell migration; and increase chemotaxis and homing.

This study's findings also reported that fibroblast cells increased by 69% in general while angiogenesis reached 60%. It means that the activity of fibroblast cells determines the development of angiogenesis. Tracy (2016) reported that fibroblasts' specific contribution to angiogenesis is still largely unknown. Still, derivatives of fibroblasts may act as angiogenic in endothelial cells to increase matrix rigidity.[23]

The role of fibroblasts in wound healing was reported by Tonnesen (2000), where angiogenic capillary buds invaded the wound clot, which is rich in fibrin/fibronectin and, within a few days, formed a microvascular network throughout the granulation tissue. When collagen accumulates in the granulation tissue of a scar, it reduces the density of blood vessels.[24] Angiogenesis plays a significant role in wound healing. The angiogenic response is required to deliver immune cells, remove debris, and provide nutrients for tissue regeneration. Defects in the regulation of vascular growth can lead to dehiscence and ulceration.

Angiogenesis plays an essential role in the development of many diseases. Disruption of the balance between pro-angiogenic and anti-angiogenic factors causes excessive or inadequate levels of angiogenesis.[26] *Chromolaena odorata (L)* extract used in this study tends to act pro-angiogenic because it is required for wound healing. It is believed that several active compounds contained in it have worked based on pharmacodynamic and pharmacokinetic properties, where their role, apart from increasing bioavailability, also increases their solubility and rate of absorption without changing their biological effects while providing a healing response.[27] In general, several natural compounds in plant or herbal extracts have angiogenesis-modulating effects.[28] These plant-derived compounds are mostly phytochemicals that have significant physiological effects on the body. This molecule acts as an antioxidant, stimulates enzyme activity, mimics hormones, interferes with DNA replication, or binds to cell wall proteins.[29] Several research reports also describe the synergistic effect of plant-based compounds as anti-angiogenic or pro-angiogenic agents.[28]

The polyphenols in *Chromolaena odorata (L)* have a proliferative effect to enhance wound healing. In addition, flavonoids, including flavones, flavonols, flavanones, anthocyanins, and isoflavones, belonging to another class of polyphenols, also play a pro-angiogenic role as well as play a role in wound healing.[16] A number of compound groups in Chromolaena odorata (L) plants increase angiogenic capillary bud to invade wound clots rich in fibrin/fibronectin to form granulation tissue.[27] The Phyto-response properties of *Chromolaena odorata (L)* can increase collagen accumulation in granulation tissue in scars and reduce blood vessel density.[24]

Molecularly Tonnesen (2000) reported that this healing process occurs due to interactions between endothelial cells and angiogenic cytokines, such as FGF, VEGF, TGF-beta, angiopoietin, mast cell tryptase, and the extracellular matrix environment.[11,24] These references are strengthened by Feng (2013) explained that endothelial cell-extracellular matrix receptors are crucial for morphogenetic changes in blood vessels during

wound repair. In particular, alpha(v)beta3, the integrin receptor for fibrin and fibronectin, is involved in wound angiogenesis. Alpha (v)beta3 is expressed at the bud tips of angiogenic capillaries that invade the wound clot and inhibit the formation of granulation tissue.[11,24,29] Recent findings have been reported that the extracellular matrix in the wound area can regulate angiogenesis by modulating the expression of integrin receptors.[30] Levels of mRNA alpha(v)beta3 in human skin microvascular endothelial cells either coated with fibronectin or covered with fibrin gel were higher than in cells coated with collagen or coated with collagen gel.[31] Wound angiogenesis is also regulated by the interaction of endothelial cells with the extracellular matrix environment in the wound area.[31]

CONCLUSION

Based on the research objectives, it can be concluded that the ethanol extract of kirinyuh leaves (*Chromolaena odorata (L)*) ointment applied topically improves the healing of cuts on the nasal mucosa of rabbits (*Lepus curpaneums*).

CONFLICT OF INTEREST

There is no conflict of interest in this research

ETHICAL STATEMENTS

Number of ethical contributions KEPPKN Registration Number: 1171012P Descrıptıon of ethıcal exempted "ethıcal exempted" Number: 130/EA/FK-RSUDZA/2020

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Original Research

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Root morphology analysis of posterior teeth using intraoral periapical radiograph

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ABSTRACT. Oral and dental health problems are still a part of ongoing national health issues in Indonesia. Through endodontic treatment, also called root canal treatment, a decayed tooth can still be preserved and restored to its original form. It is done if the dental infection has spread into the pulp or the tooth has become nonvital due to trauma or accident. Root morphology is one of the main concerns before performing endodontic treatment. A periapical radiograph taken with bisecting and paralleling techniques is the first method in intraoral radiographic examination to assist dental diagnosis and case management. This research aimed to analyze the mean length of posterior teeth using an intraoral periapical radiograph and the difference in posterior teeth length in different vertical angulations. A total of four extracted lower premolars and four extracted lower molars served as samples in this analysis. They were mounted in an occluder, and a periapical radiograph was obtained using paralleling and bisecting technique with vertical angulation of -20°, -15°, -10°, -5°, 0°, +5°, +10°, +15°, and +20°. The tooth length was measured from the crown's highest point to the tooth apex's lowest end. Data obtained was then calculated using SPSS. The result showed that the mean length of the lower premolar and molar was longer if the vertical angulation reached +20°. Analysis with one-way ANOVA for the difference in the length of premolars, mesial root, and distal root of lower molars between a direct measurement with digital caliper and measurement on periapical radiographs taken with paralleling technique and bisecting technique in all vertical angulations showed a p-value of > 0,05. There were no significant differences in the mean length of lower premolars and the mesial and distal root of lower molars between direct measurement using a digital caliper and measure on periapical radiographs taken with paralleling technique and bisecting technique in vertical angulations of -15°, -10°, -5°, 0°, +5°, +10°, +15°, and +20°.

KEYWORDS: Vertical angulation, posterior tooth root morphology, periapical radiograph

INTRODUCTION

Dental and oral health problems are still a part of ongoing national health issues in Indonesia. Based on the results of Basic Health Research (Riskedas) in 2018, the leading dental problems in Indonesia were tooth decay, cavities, and pain (45.3%), while 19% were the absence of a tooth because of extraction/falling out. The prevalence of people in Indonesia with edentulousness was 51.4%. It showed many cases of missing/extracted teeth, and one of the leading causes was dental caries.[1]

Dental and oral health can influence an individual's quality of life. Endodontic procedures can preserve a decayed tooth and restore its original form. According to Riskedas, in 2018, 57.6% of the Indonesian population experienced dental and oral problems, but only 10.2% sought treatment from dental professionals.[1]

Endodontic treatment is a treatment of root canal, performed if the infection has spread to the pulp or if the tooth has become nonvital due to trauma or accident. Root morphology is one of the main aspects that must be considered before initiating endodontic treatment. Dashrath et al. studied the root morphology of maxillary first premolars in the Nepal population, which consisted of tooth length, root length, and root number. The mean length of the maxillary first premolars was 21.0 mm, and the mean of its root length was 12.76 mm. They found a varied number of roots, with 58% having one root, 20% having two roots, 21% fused roots, and 1% having three sources.[2-5]

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Periapical radiograph taken with bisecting or paralleling technique is often viewed as the first choice of radiograph techniques in dental case management. Bisecting technique is more often used in dentistry because of more straightforward patient adaptation. However, the drawback of this technique is distortion due to errors in vertical and horizontal angles. Mistakes in vertical angulation in the bisecting process caused vertical distortion, which appeared as a shortening or lengthening of the tooth.[6,7]

Several kinds of literature have stated that vertical angulation for mandibular premolar was around -10° to -15°, and for mandibular molar was -5° or 0° to -5°. White and Pharoah studied vertical angulation in several populations, including North America, India, and Brazil, and found varied values of vertical angulation. For mandibular premolar, vertical angulation in North America was -10°, in India -15°, and in Brazil -10° to -5°. For mandibular molar, vertical angulation in North America was -5°, India -10° to 0°, and Brazil -5° to 0°. [6-8]

Based on that explanation, it was in our interest to analyze the root morphology of posterior teeth by digital periapical intraoral radiograph using paralleling and bisecting techniques with varied vertical angles.

MATERIALS AND METHODS

This research was an experimental, analytical study. It was conducted in the Installation of Dental Radiology in Dental and Oral Hospital of Universitas Sumatera Utara from February to December 2020. The sample population in this research consisted of all lower posterior teeth, excluding extracted third molar. According to inclusion and exclusion criteria, the selected samples in this research were four lower premolars and four lower molars. Inclusion criteria were teeth with intact crowns, caries that had not spread into the pulp, and teeth with good apical foramen. Exclusion criteria were teeth with fractured root.

Samples were mounted on an occluder, and then a periapical radiograph was taken with paralleling and bisecting techniques in vertical angulations of -20°, -15°, -10°, -5°, 0°, +5°, +10°, +15°, and +20° with target-object distance (TOD) of 3 – 8 cm. The resulting radiographs were measured from the highest point of the crown to the lowest point of the apex. Normality and homogeneity tests were performed on the resulting data, and one-way ANOVA was subsequently completed.

RESULTS

The result showed that the mean length of lower posterior teeth as measured by digital caliper was 21.65 ± 1.79 mm for premolars, 21.07 ± 1.05 mm for the mesial root of the molars, and 20.04 ± 1.13 mm for the distal root of the molars (Table 1). Measurement on periapical radiographs, which were taken by paralleling technique, showed that the mean length of the lower posterior teeth was 22.87 ± 1.68 mm for premolars, 21.27 ± 0.91 mm for the mesial root of the molars, and 20.55 ± 0.82 mm for the distal root of the molars (Table 2). The following tables showed the mean length of premolars (Table 3), the mesial root of molars (Table 4), and the distal root of molars (Table 5), measured on periapical radiographs taken by a bisecting technique using varied vertical angulations.

Table 1. The mean length of posterior teeth was measured using a digital caliper

Teeth	n	Min	Max	Mean ± SD (mm)
Premolars	4	19,63	24,00	21,65 ± 1,79
Mesial root of molars	4	19,75	22,17	21,07 ± 1,05
Distal root of molars	4	18,75	21,17	20,04 ± 1,13

Table 2. The mean length of posterior teeth as measured on periapical radiographs taken by paralleling technique

Teeth	n	Min	Max	Mean ± SD (mm)
Premolars	4	21,00	25,10	22,87 ± 1,68
Mesial root of molars	4	20,40	22,20	21,27 ± 0,91
Distal root of molars	4	19,50	21,50	20,55 ± 0,82

Table 3. The mean length of premolars as measured on periapical radiographs taken by a bisecting technique using varied vertical angulation in 3 – 8 cm distance.

Angle	Mean ± Standard Deviation (mm)					
	3 cm	4 cm	5 cm	6 cm	7 cm	8 cm
-20°	22,00 ± 1,57	22,00 ± 1,52	22,27 ± 1,39	22,12 ± 1,58	21,85 ± 1,45	22,20 ± 2,03
-15°	22,45 ± 2,11	22,22 ± 1,91	22,17 ± 1,83	22,30 ± 1,92	22,40 ± 2,07	22,37 ± 1,83
-10°	22,62 ± 1,87	22,57 ± 1,75	22,35 ± 1,87	22,72 ± 2,15	22,50 ± 2,05	22,40 ± 2,03
-5°	22,57 ± 1,93	23,00 ± 1,68	23,17 ± 1,20	23,10 ± 1,24	22,85 ± 1,43	23,35 ± 2,46
0°	22,87 ± 1,57	22,90 ± 1,53	22,85 ± 1,33	22,67 ± 1,56	22,67 ± 1,43	22,80 ± 1,43
+5°	22,77 ± 1,75	22,60 ± 1,80	22,60 ± 1,80	22,60 ± 1,88	22,60 ± 1,81	22,35 ± 1,98
+10°	22,65 ± 1,86	22,97 ± 1,58	22,55 ± 1,76	22,77 ± 1,72	22,77 ± 1,80	22,30 ± 0,94
+15°	23,12 ± 1,62	22,92 ± 1,62	22,90 ± 1,51	22,82 ± 1,58	22,80 ± 1,80	22,72 ± 2,01
+20°	22,95 ± 1,86	22,75 ± 1,74	22,80 ± 1,85	23,22 ± 2,36	23,10 ± 2,41	23,40 ± 1,89

Table 4. The mean length of the mesial root of the molars as measured on periapical radiographs taken by a bisecting technique using varied vertical angulation in a 3 – 8 cm distance

Angle	Mean ± Standard Deviation (mm)					
	3 cm	4 cm	5 cm	6 cm	7 cm	8 cm
-20°	20,97 ± 1,48	20,67 ± 1,21	20,60 ± 1,14	20,77 ± 1,27	20,72 ± 1,26	20,65 ± 1,09
-15°	21,15 ± 1,22	21,15 ± 1,16	21,12 ± 1,20	21,02 ± 1,13	21,15 ± 1,27	20,97 ± 1,13
-10°	21,45 ± 1,10	21,40 ± 1,15	21,42 ± 1,18	21,45 ± 1,16	21,42 ± 1,07	21,40 ± 1,22
-5°	21,75 ± 1,22	21,70 ± 1,30	21,65 ± 1,24	21,62 ± 1,26	21,70 ± 1,32	21,77 ± 1,36
0°	22,35 ± 1,32	22,00 ± 1,21	21,97 ± 1,27	21,77 ± 1,28	22,02 ± 1,34	21,80 ± 1,31
+5°	21,92 ± 1,40	22,25 ± 1,30	22,07 ± 1,29	22,30 ± 1,51	22,20 ± 1,21	22,15 ± 1,38
+10°	22,52 ± 1,55	22,62 ± 1,46	22,30 ± 1,52	22,32 ± 1,33	22,40 ± 1,34	22,77 ± 1,90
+15°	23,02 ± 1,71	22,82 ± 1,79	22,80 ± 2,01	23,07 ± 1,64	22,80 ± 1,68	22,65 ± 1,84
+20°	23,35 ± 1,69	23,52 ± 2,05	23,50 ± 1,40	23,12 ± 1,73	22,95 ± 1,66	23,10 ± 1,74

Table 5. The mean length of the distal root of the molars as measured on periapical radiographs taken by a bisecting technique using varied vertical angulation in a 3 – 8 cm distance

Angle	Mean ± Standard Deviation (mm)					
	3 cm	4 cm	5 cm	6 cm	7 cm	8 cm
-20°	20,20 ± 1,16	19,95 ± 0,91	19,92 ± 1,14	20,02 ± 1,02	19,90 ± 1,03	19,92 ± 0,97
-15°	20,37 ± 1,00	20,32 ± 0,86	20,20 ± 0,93	20,17 ± 0,99	20,30 ± 0,96	20,17 ± 1,00
-10°	20,37 ± 1,00	20,32 ± 0,86	20,20 ± 0,93	20,17 ± 0,99	20,30 ± 0,96	20,17 ± 1,00
-5°	20,85 ± 1,04	20,77 ± 1,02	20,72 ± 0,89	20,77 ± 1,02	20,62 ± 0,98	20,95 ± 0,98
0°	21,40 ± 1,12	21,07 ± 1,16	20,97 ± 1,28	20,75 ± 1,10	21,07 ± 1,15	20,87 ± 1,13
+5°	21,12 ± 1,26	21,30 ± 1,15	21,10 ± 1,19	21,22 ± 1,44	21,30 ± 1,19	21,15 ± 1,35
+10°	22,52 ± 1,55	22,62 ± 1,46	22,30 ± 1,52	22,32 ± 1,33	22,40 ± 1,34	22,77 ± 1,90
+15°	21,85 ± 1,52	21,72 ± 1,61	21,80 ± 1,86	21,90 ± 1,46	21,55 ± 1,34	21,37 ± 1,52
+20°	22,20 ± 1,83	22,52 ± 2,17	22,45 ± 1,70	22,10 ± 1,83	21,92 ± 1,79	22,02 ± 1,85

The normality test by the Shapiro-Wilk test on all groups showed that the data were normally distributed (p>0.05). Lavene Test to analyze the homogeneity of more than two data groups resulted in a p-value>0,05, meaning the data groups came from a population with the same variants. One-way ANOVA was subsequently performed, and the result showed no significant differences in the mean teeth length between a direct measurement with a digital caliper, measurement on periapical radiographs taken by paralleling technique, and measurement on periapical radiographs taken by bisecting approach in vertical angulations of -20°, -15°, -10°, -5°, 0°, +5°, +10°, +15°, and +20°. However, the TOD distance was 3 to 8 cm (p>0,05).

Table 6. P-value of the teeth length measurements between digital caliper, paralleling technique, and bisecting technique with TOD 3-8 cm in vertical angulation of -20°, -15°, -10°, -5°, 0°, +5°, +10°, +15°, and +20°

Teeth	*p-value*					
	3 cm	**4 cm**	**5 cm**	**6 cm**	**7 cm**	**8 cm**
Premolar	0,991	0,983	0,985	0,988	0,99	0,981
Molar, mesial root	0,230	0,144	0,143	0,187	0,285	0,334
Molar, distal root	0,261	0,161	0,174	0,249	0,279	0,343

DISCUSSION

This research showed that the mean length of lower premolars and molars was longer if the vertical angulation reached +20°. Incorrect vertical angulation would result in distortion. The greater the vertical angulation, the more teeth will appear shorter, and vice versa. The smaller the vertical angulation, teeth will appear longer. Bisecting angle technique produced an inherent distortion of the resulting image. Hence there are vertical angulation values that are still tolerable and do not impact the vertical length measurement of the tooth. [9,10]

In this research, no significant differences were found in tooth length between a direct measurement with a digital caliper, a measure on a periapical radiograph taken with paralleling technique, and a measurement on a periapical radiograph taken with bisecting approach with a significance level above 0.5. In paralleling procedure, the film was positioned parallel to the tooth, and the beam traveled perpendicular through the tooth and film with the aid of a film holder. Alothmani et al. stated that there were fewer errors in work length determination by using paralleling technique than bisecting technique.[11] Proper film positioning in the bisecting method before exposure was probably one of the reasons this research found no significant differences in tooth length measurement compared with the direct size with a digital caliper. If vertical angulation was becoming more negative, the upper side of the film was positioned higher, up to 1 cm, from the surface of the tooth object.

Conversely, if vertical angulation was becoming more positive, the upper side of the film was positioned slightly lower than the tooth surface to avoid cone cutting. The difference in tooth length in bisecting technique with varied vertical angulation, from -20° to +20°, was less than 2 mm. If vertical angulation was becoming more positive, the differences in tooth length could exceed 1 mm.

This result followed the research of Heryanto et al., which was done on mandibular premolars. If the vertical angle was more positive, the height of the cups became lower. Hence the length of the tooth became shorter, and superimposition became bigger. Vertical angulation that was significantly acceptable was -20° to +15° with a p-value of >0.05, while vertical angulation +20° showed a p-value of <0.05, indicating a significant difference with the direct measurement.[12]

This research also measured the length of the mesial and distal roots of mandibular molars. OneWay ANOVA test showed no significant differences between the actual tooth length and the estimated tooth length on the periapical radiograph obtained with paralleling and bisecting techniques in vertical angulation of 20°, -15°, -10°, -5°, 0°, +5°, +10°, +15°, +20° and TOD 3-8 cm (p>0,05).

Alothmani et al. recommended the paralleling technique in determining the endodontic working length. Vertical angulation in bisecting approach should be customized according to the anatomy of the patient's jaw. Variations in anatomy such as proclined teeth should be noticed and the vertical angulation adapted by the radiographer before acquiring a radiograph with bisecting technique.[13]

In this research, the mean length of mesial and distal roots of mandibular molars on radiographs taken with bisecting technique in vertical angulations between -5° to -20° was less than 1 mm. Some literature stated that vertical angulation for mandibular molars was -15°, -10°, and -5°. Several researchers said that although there were differences in vertical angulation for mandibular molars in pieces of literature, they still presented tooth length with distortion of less than 1 mm. The mean tooth length for paralleling technique in this paper was distorted between 0.2-0.5 mm. Therefore, we also agreed that paralleling process was better for usage on mandibular molars than bisecting technique because the resulting distortion was more negligible.

Faraj, in his research, concluded that working length estimation could be made closest to its actual clinical canal length by using a digital periapical radiograph obtained in paralleling technique, in addition to Cone Beam Computed Tomography.[14] According to Frommer, paralleling gave more accurate images and protected the thyroid gland area and eye lens more than bisecting technique. The direction of the x-ray in bisecting approach with steep vertical angulation can pass through the thyroid gland and eye lens area.[15,16]

At present, the bisecting technique is still widely used. One reason is that the film holder used in the paralleling procedure is more rigid and firm. In contrast, in bisecting strategy, the film is held with the aid of the patient's finger, hence more comfortable for both the patient and operator. In this research, the variation in vertical angulations from -20° to +20° can still be applied according to the anatomical condition of the patient. If the floor of the patient's mouth is shallow and causing the film to be positioned higher than the surface of the tooth, then the vertical angulation can be positioned up to -20°. If the floor of the patient's mouth is deep, causing the patient to push the film at least to the same level or lower than the surface of the tooth, then the vertical angulation can be positioned up to +20°. Distortion in the form of shortening or lengthening of the tooth at a perpendicular angle of +20° can reach ± 2 mm.

CONCLUSION

The measurement of the length of mandibular premolars and mesial root and distal root of mandibular molars based on radiographs obtained with paralleling technique and bisecting technique in vertical angulation of -20° to +20° showed no significant differences. This research found that paralleling technique was better than bisecting technique because the resulting distortion was more negligible and more protective of the patient's thyroid gland and eye lens.

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Original Research

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The effect of ethanol extract of kirinyuh leaf (*Chromolaena odorata* L) on the allergy healing of rabbit (*lepus curpaneums*)

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ABSTRACT. Allergic rhinitis is one of the world's most common diseases and remains alive. The following diseases are often found in otorhinolaryngology and constitute a significant health problem worldwide. Symptoms of nasal hyperactivity and hyperresponsiveness are caused by the role of eosinophils, basophils, neutrophils, and other inflammatory mediators. The development of medicinal plants in other countries is overgrowing due to many adverse drug reactions. *Chromolaena odorata (L)* has been used as a traditional medicine because it has ethnopharmacology effects such as alkaloids, essential oils, phenolics, flavonoids, steroids, phenolic compounds, glycosides, phytates, saponins and tannins which are suppress allergenic. To determine the effect of ethanol extract ointment of kirinyuh leaves (*Chromolaena odorata (L)*) on the levels of eosinophils, basophils, and neutrophils of allergic that induced in a rabbit nasal mucosa. Experimental research with a pretest-posttest control group design uses rabbits as a research subject. The extract of ethanol ointment from *Chromolaena odorata (L)* leaf can reduce the nasal mucosa's allergen response. One-way ANOVA statistical analysis showed no significant difference in the number of inflammatory cells between basophils, eosinophils, and neutrophils ($p>0.05;1.00$). Furthermore, based on the analysis of the Kruskal Wallis Test, it showed that there were differences in the number of inflammatory cells based on the concentration of the test material, related to the allergen response ($p<0.05$; 0.024) with a strong relationship ($r=0.907$). Extracting ethanol ointment from Chromolaena odorata (L) leaf, which is applied topically, can reduce allergic reactions and inflammation.

KEYWORDS: allergic rhinitis, eosinophils, basophils, neutrophils, *Chromolaena odorata (L)*

INTRODUCTION

Allergic rhinitis is one of the most common diseases in the world and usually persists throughout life.[1,2] The self-reported prevalence of allergic rhinitis is estimated to be around 2% to 25% in children and 1% greater than 40% in adults.[1] According to the World Allergy Report 2008, the prevalence of allergic rhinitis in low- and middle-income countries in the Asia Pacific region is estimated at around 5–45%.[2] Unfortunately, the prevalence rate in Indonesia is still unknown. Although rarely life-threatening, allergic rhinitis causes poor sleep and a lack of productivity in industry and education

Allergic rhinitis is an inflammatory disease with symptomatic abnormalities in the nose induced by inflammation caused by immunoglobulin E (IgE) due to exposure to foreign substances called allergens. It is characterized by one or more symptoms of nasal pruritus, sneezing, discharge, and congestion. In addition, the sense of smell and taste is also impaired.[1,4] Most allergens are between 5 and 20µm in diameter, allowing inhalation by the nose.[4] Allergens that cause hypersensitivity responses in individuals with atopy are proteins or protein-bound chemical substances. Typical allergens include protein in pollen, house dust

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mites, animal dander, food, mold, and drugs such as the antibiotic penicillin. Inhalant allergens play a significant role in the occurrence of allergic rhinitis.[4]

There have been many studies that have studied to treat allergic rhinitis. There are three options for the management of allergic rhinitis: (1) avoidance and environmental control, (2) pharmacotherapy, and (3) immunotherapy.[4] One alternative treatment that can be used is herbal medicine.[6,7] The development of medicinal plants at home and abroad is growing due to many adverse drug reactions.[8] The World Health Organization (WHO) recognizes traditional medicine as one of the essential elements of primary health care: *Saving plants saves lives*.[9,10]

Chromolaena odorata (L), commonly known as the Siamese weed, belongs to the sunflower family Asteraceae. *Chromolaena odorata (L)* is an important medicinal plant that can easily be found in the tropics of Asia, West Africa, and parts of Australia.

Several literature studies reveal several important pharmacological activities such as antiallergic, anticancer, antidiabetic, antidiarrheal, anticonvulsant, anti-inflammatory hepatoprotective activity, antihelmintic, antimalarial, analgesic, anti-inflammatory, antipyretic, antispasmodic, antioxidant, antigonorrhea, antimycobacterial, insecticide, fungicide, wound healing and hemostatic, diuretic, blood clotting, antibacterial[11] and antitrypanosomal, as well as antihypertensive.[12] The value of medicinal plants lies in bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds, which produce specific physiological actions in the human body.[13]

Quercetin is an active bioflavonoid found in many plants and is very safe for treating allergic rhinitis. Flavonoids inhibit enzymes that increase histamine release from mast cells and basophils: cAMP phosphodiesterase and calcium-dependent ATPase. Large amounts of the cAMP act by blocking the intracellular histamine reservoir. Calcium-dependent ATPases also degrade ATP to release energy to facilitate the Ca^{2+} gate across cell membranes: high intracellular Ca^{2+} levels also cause histamine release from cellular storage granules. Quercetin is a flavonoid with a strong affinity for mast cells and basophils. Quercitin stabilizes their cell membranes, thereby preventing them from shedding histamine. By inhibiting the release of histamine and leukotrienes into the bloodstream, quercetin prevents allergy symptoms such as swollen nasal passages, stuffy nose, sneezing, watery eyes, and itchy eyes and nose.

According to the results of research conducted by Oluwaseun Ruth Alara in 2019, significant variables include microwave power (400-800 W), irradiation time (1-5 minutes), and ethanol concentration (20-60%) at a constant temperature (70°C) and solvent ratio (10:1) mL/g) can be further optimized to achieve maximum yield of total phenolic and flavonoid content (TFC) from *Chromolaena odorata (L)* leaves.[15] Animal allergy experiments usually use ovalbumin (OVA). One of the uses of ovalbumin is stimulating allergic reactions in various experimental animals. Allergy induction with ovalbumin is given gradually, starting with sensitization and continuing with allergen provocation (allergen challenge).[16,17] Murat Kar et al., 2019 stated 0.4% OVA solution is prepared with OVA emulsified in Tween 80 with $Al(OH)_3$ in 100mL saline. Experimental animals were sensitized by intraperitoneal administration of 5mL OVA on days 0, 2, 4, and 6. Furthermore, 10µL of a similar solution was administered intranasally once a day for seven days.[18] From the description above, the researcher wanted to know the effect of giving kirinyuh leaf (*Chromolaena odorata (L)*) ethanol extract ointment on the levels of eosinophils, basophils, and neutrophils in the nasal mucosa of rabbits.

MATERIALS AND METHODS

This research is an experimental laboratory study with a pretest-posttest control group design using rabbits as the research subject. The time plan for this research is from October 2020 to March 2021.

This research was conducted at the Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, to maintain and treat experimental models. Preparation of ointments from the ethanol extract of kirinyuh leaves (*Chromolaena odorata (L)*) at the Faculty of Pharmacy, Syiah University Kuala Banda Aceh.

The subjects of this study were male rabbits with the inclusion criteria for male rabbits, body weight between 1000-1500 grams, and rabbits in good health (ears erect and clean, eyes round and clear, dry nasal surface, strong teeth).

While the exclusion criteria were that the experimental animals looked sick during the adaptation, decreased body weight during adaptation >10%, experimental animals died during the experiment. This study had five groups: three treatment groups, one positive control group, and one negative control group. The sample of this

research is five samples for each treatment, so the required selection is 25 rabbits.

The research procedure includes: 1.) Sterilization of tools done by steam sterilization. All tools to be used are washed and cleaned. 2.) The preparation of the ethanol extract of kirinyuh leaves is air-dried for 72 hours. After drying, it is ground using a vortex to become powder. Dry kirinyuh leaf powder was extracted by maceration employing fine kirinyuh leaf powder soaked in 7 liters of 70% ethanol for 72 hours to obtain the first filtrate. Furthermore, the fine powder of kirinyuh leaves was rewashed with 7 liters of 70% ethanol for 72 hours, so the second filtrate was obtained. Then the first and second filtrates were combined, and the solvent was evaporated with a rotary evaporator to obtain a thick extract of 20 grams. 3.) Making a variable concentration of kirinyuh leaves, the extract that has been obtained is then diluted with distilled water to obtain concentrations of 5%, 10%, and 15%. 4.) Preparation of ointment Evaluation of the ethanol extract of kirinyuh leaf ointment in this experiment was carried out to find out the results of the tests parallel to the theoretical results which included organoleptic and pH tests, homogeneity, viscosity, and spreadability tests of the ointment. 5.) Preparation of Experimental Animals The research samples, before treatment, were weighed and adapted for two weeks, homogenized in the cage, and the temperature in the cage was set at room temperature. Every day the rabbits were fed in the form of standard feed/standard food (carrots, kale, pellets), and drinking water was provided ad libitum. 6.) Assessment of eosinophils, basophils, and neutrophils before and after treatment. Assess the number of eosinophils, basophils, and neutrophils of the nasal mucosa by scraping the nasal mucosa of rabbits and staining with Giemsa and viewing using an electron microscope with 400x magnification as much as 100x field of view at histology laboratory, Faculty of Veterinary Medicine Unsyiah Banda Aceh. 7.) Sensitization of Experimental Animals with Allergens In experimental animals, rabbits were sensitized with ovalbumin. Experimental animals were given 0.4% ovalbumin solution emulsified in Tween 80 with $Al(OH)_3$ in 100 mL saline. Experimental animals were sensitized by intraperitoneal administration of 5mL ovalbumin on days "14, 16, 18, and 20". Then 10µL of a similar solution was administered intranasally once a day for seven days, until day 27.[18] 8). Animal Treatment Experiments Rabbits were divided into five groups, each group consisting of 5 rabbits, with three groups being treated with 5% kirinyuh leaf ethanol extract ointment, 10% and 15%, and 1 group as a positive control and 1 group as a negative control. The experimental animals were sensitized with ovalbumin, then given an ethanol extract of kirinyuh leaves (*Chromolaena odorata (L)*) ointment with three preparations. Group 1 was given an ethanol extract of kirinyuh leaves 5 %, group 2 was given 10%, group 3 was assigned 15%, 1 group was a positive control, and 1 group was a negative control. The ointment was applied to the nasal mucosa of the experimental animals as much as 0.1 g each p the next day, carried out for seven days. All data is processed using one-way ANOVA.

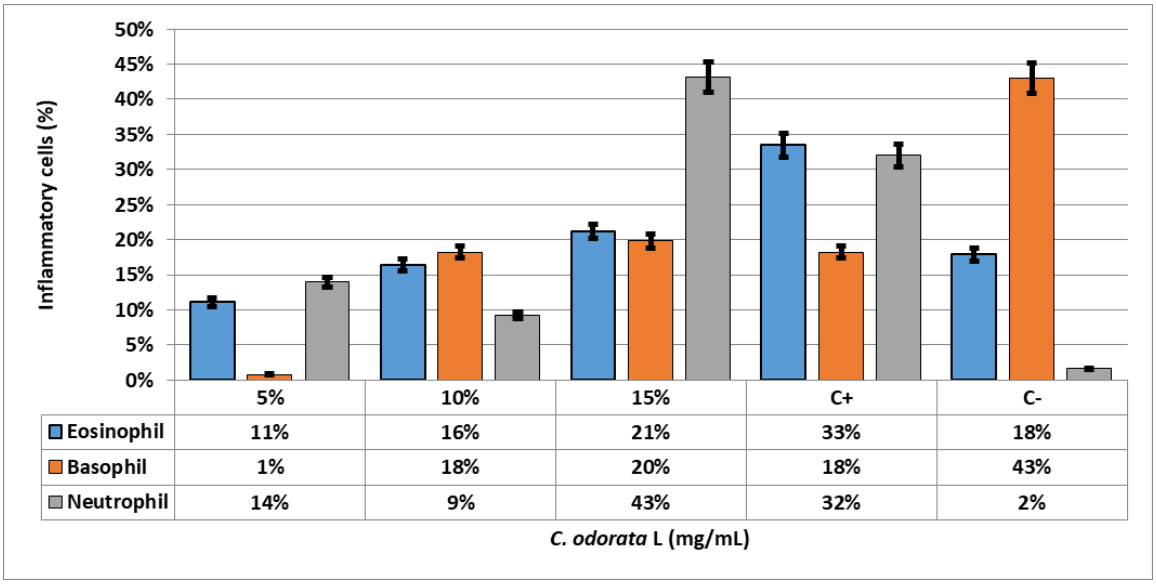
RESULTS

The study's results reported that the ethanol extract of kirinyuh leaves (*Chromolaena odorata (L)*) ointment with concentrations of 5%, 10%, and 15% could reduce inflammatory cells for seven days of treatment. The decrease in inflammatory cells was positively correlated with the allergic response elicited during the administration of kirinyuh leaf ethanol extract ointment. A reduction in basophil cell response marks a decrease in allergies. Basophil cells have been reported as precursors of the IgE response in the pathogenesis of the allergic reaction.

The study's results described the phenomenon that kirinyuh leaf ethanol extract ointment can be involved in reducing the response of immune cells involved in allergies, especially in the mucosal area. Thus preventing the interaction of allergen responses with the immune system as well as functioning as immunoprotection.

The 5% concentration has a better ability to reduce inflammatory cells. A decrease in inflammatory cells correlates with a reduction in allergies, characterized by a decline in basophil cells (**Figure 1**).

Figure 1 shows that all concentrations of the ethanol extract of kirinyuh leaf ointment used in this study had a response to reduce allergic infections in the nasal mucosa of rabbits as animal models in this study. The concentration of 5% has a better ability than the concentration of 10% and 15%. Basophil cells and neutrophil cells experienced a dominant decrease compared to eosinophil cells. This indicates that allergic and inflammatory responses have decreased because these two cells predominantly work on allergic and inflammatory reactions by the host immune system.



	5%	10%	15%	C+	C-
Eosinophil	11%	16%	21%	33%	18%
Basophil	1%	18%	20%	18%	43%
Neutrophil	14%	9%	43%	32%	2%

Figure 1. Inflammatory cell response in rabbits induced by allergens after being given kirinyuh leaf ethanol extract ointment for seven days.

Kirinyuh leaf ethanol extract ointment gave a maximum response to basophil cells and neutrophil cells by 300%. It decreased to 400% response to respond to allergens that trigger allergies in the nasal mucosa. Meanwhile, neutrophil cells are still increasing (**Figure 2**). Figure 2 shows A scatter diagram showing the role of the ethanol extract of kirinyuh leaf ointment with various concentrations during the immune system's response for protection against allergens. Basophils and neutrophils have an operational phase (spread) reaching 300% peak phase and decreasing again at an operational level of 400%. The seven-day treatment time is considered the maximum response time of inflammatory cells to decrease allergen response in modeling nasal mucosal allergy. The morphology of inflammatory cells can be seen in **Figure 3**.

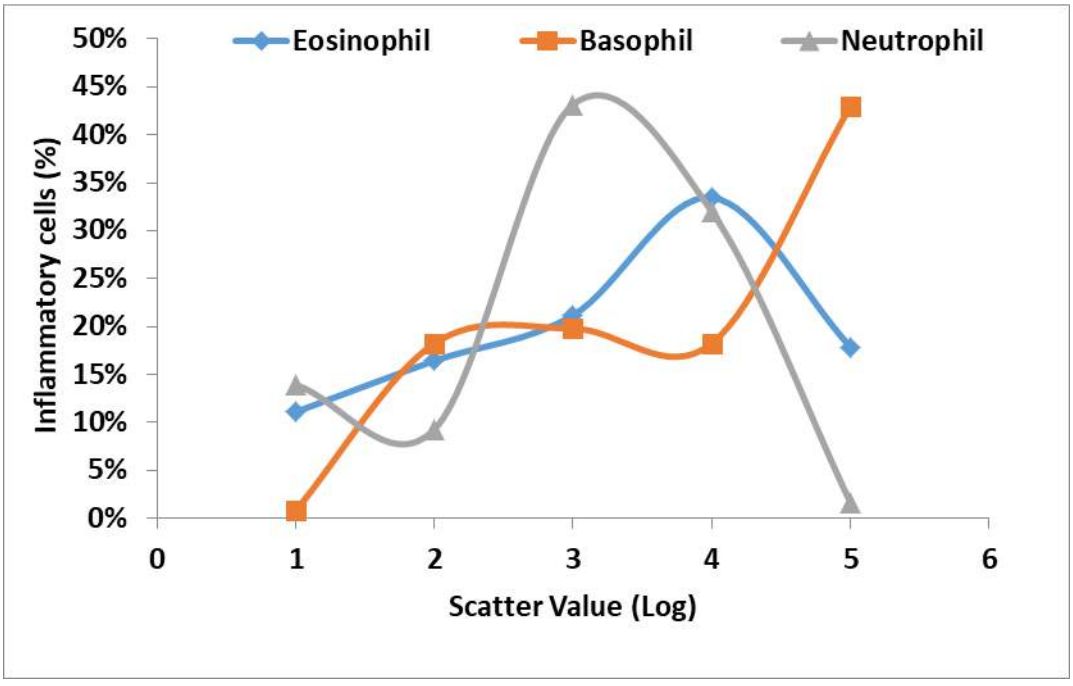


Figure 5.2. Scatter diagram of the relationship between inflammatory cells during the inflammatory cell response in the host's body for 7 days.

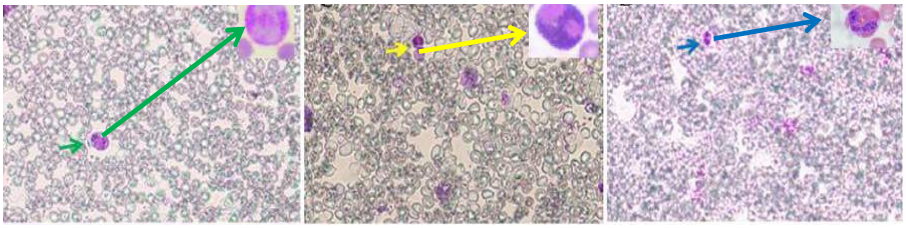


Figure 3. Inflammatory cell histopathology. Green arrows (basophils), yellow arrows (neutrophils), and blue arrows (eosinophils). Images were taken at 400x magnification using a light microscope

Table 1 shows the total distribution and frequency of inflammatory cells affected by the concentration of the ethanol extract of kirinyuh leaf (*Chromolaena odorata (L)*) ointment.

Table 1. Frequency and distribution of inflammatory cells from mucosal scraping preparations

Cons	N	Eosinophil				Basophil				Neutrophil			
		Cell	SDV	Freq	Scale	Cell	SDV	Freq	Scale	Cell	SDV	Freq	Scale
5%	5	132	37.26	20%	Moderate	0.2	0.45	2%	Low	3.4	5.94	28%	Low
10%	5	132	30.30	20%	Moderate	0.4	0.55	3%	Low	1	1.73	8%	Low
15%	5	124	15.17	19%	Low	0.8	0.45	6%	High	3.8	1.30	31%	Moderate
C+	5	55.8	21.00	8%	Low	0.4	0.55	3%	Low	3.6	1.52	30%	Moderate
C-	5	224.6	237.26	34%	High	11	14.70	86%	High	0.4	0.89	3%	Very Low

Table 2 describes the presence of inflammatory cells after being induced with various concentrations of ethanol extract of kirinyuh leaf ointment. This value will be used as a reference for the response of inflammatory cells to allergens. Value 40% (high), 30-39% (moderate), and value <30% (low). This value was used to measure the distribution of inflammatory cell activity affected by the ethanol extract of kirinyuh leaf ointment from each concentration for seven days of animal model treatment. Values on a low scale indicate a better role for the test material in reducing the frequency of inflammation and allergies. While the large scale shows the response to reduce inflammation and allergies is not maximal.

Table 2. Total distribution and frequency influenced by the concentration of (*Chromolaena odorata (L)*)

Cons	N	Eosinophil	Basophil	Neutrophil	r	p
5%	5	11%	1%	14%		
10%	5	16%	18%	9%		
15%	5	21%	20%	43%	0.907	0.024
C+	5	33%	18%	32%		
C-	5	18%	43%	2%		

Oneway ANOVA statistical analysis showed no significant difference in the number of inflammatory cells between basophils, eosinophils, and neutrophils p>0.05;1.00). Furthermore, the Kruskal Wallis Test analysis showed differences in the number of inflammatory cells based on the concentration of the

test material, related to allergen response (p <0.05; 0.024). with a strong relationship (r=0.907). Based on the analysis of the Independent Samples T Test, it showed that there was no significant difference between the activity of eosinophil cells and neutrophils (p> 0.05; 1.00), as well as the activity of basophil cells with neutrophils and basophil cells with eosinophils (p> 0.05; 1.00)

DISCUSSION

This study assessed the effect of giving kirinyuh leaf ethanol extract (*Chromolaena odorata (L)*) ointment on eosinophil, basophil, and neutrophil levels of rabbits induced by allergens in the nasal mucosa. The results of this study reported that the ethanol extract of kirinyuh leaves provided benefits for reducing inflammatory cells, especially eosinophil cells and basophil cells, as indicators of decreased allergies, while neutrophils as indicators of reduced inflammation during seven days of treatment.

Basophils play an essential role in allergic inflammation related to IgE activity. This process occurs because basophils migrate to sites of inflammation and induce the secretion of various mediators, including cytokines, chemokines, and proteases. Upon encountering an antigen, IL-3-stimulated basophils release several effector molecules that may contribute to allergic inflammation.[19] Eosinophils and basophils are reported to be involved in allergic inflammation and circulate at relatively low levels in the blood, forming 0.1-1 % and 1-5% of white blood cells.[20] Studies in humans using allergens in vivo have shown the ability of basophils to induce increased expression of CD63 associated with allergic responses. However, there are no clinical symptoms of an allergic reaction.

An increase in the number of eosinophils is associated with histamine release. In contrast, neutrophils have been reported as essential histamine producers in allergic responses. Neutrophils are estimated to store 0.29 pg/cell and release 50% of the histamine content. The release of these allergic response substances highly depends on the allergen response to stimulation with other neutrophil agonists.

Like mast cells, basophils activated due to cross-linking between IgE and FceRI receptors will rapidly degranulate and release their cellular contents. In addition, inflammatory mediators such as complement factors C5a and C3a, MBP, PAF, and chemokines can start basophils without cross-linking IgE.[23] Also, basophils contain the anticoagulant heparin, which prevents blood from clotting too quickly, and the vasodilator histamine, which increases blood flow to the network. Neutrophils are reported to be important effector cells of the immune system. These cells work to prevent the development of pathogens in the body through phagocytosis to trap and kill pathogens that attack the host.

Results of the study reported that, in general, the ethanol extract of kirinyuh leaf ointment with various concentrations reduced inflammatory cells for seven days of treatment. This decrease in inflammatory cells is associated with reduced allergic reactions in animal models. Pathophysiologically, an allergy reduction is characterized by a decline in the response of eosinophil and basophil cells, which these two cells are reported to trigger the IgE response to allergies. In comparison, the decrease in neutrophils is associated with the production of histamine as a protein in allergic reactions. Specifically, Figure 1 shows a concentration of 5% has a better ability than concentrations of 10% and 15%.

According to Warrington (2012), this ability is related to the drug absorption response during an immune response to allergens.[25] This means that this test material can reduce the immune response and increase the action of the active compounds contained in the ethanol extract ointment of kirinyuh leaves while working to inhibit allergen activity. Specifically, kirinyuh leaves contain flavonoid compounds that function as antioxidants

Flavonoids are reported to inhibit histamine release, IL-4, IL-13 synthesis, and CD40 ligand expression by basophils.[27] Flavonoids indirectly work to reduce basophil activity. This study is in line with the research findings reported by Kawai (2007). In addition, Park (2008) explained that flavonoids could suppress the release of inflammatory mediators such as histamine and proinflammatory cytokines through their function as antioxidants, cytoprotective and anti-inflammatory mechanisms. Pharmacologically, flavonoids show their potential activity for treating allergic inflammatory diseases through the down-regulation of mast cell activation.[28] So, it can be assumed that the flavonoids in the ethanol extract of kirinyuh leaves can interact with the immune response that binds to mast cells to reduce the release of inflammatory mediators. Such as histamine, thereby suppressing allergic activity.

In this study, the concentration of the ethanol extract of kirinyuh leaf ointment at a concentration of 5% could better suppress the development of allergies than other concentrations. As a comparison, Alexandrakis (2003) reported that Flavones and kaempferol at 100 mL could inhibit mast cell proliferation by more than 80% on days 3, 4, or 5 of treatment. These results indicate that flavonoids can inhibit the proliferation of Molecule-histo Compatibility (MHC)-1 in inducing the development of secretory granules and the accumulation of inflammatory mediators.[29] In this study, a decrease in basophil cell activity was found, possibly because the flavonoids from the ethanol extract of kirinyuh leaf ointment can inhibit the expression of IL-4 and CD40 ligands mediated through their inhibitory action on activated T cell nuclear factor activation.

The results of this study also describe neutrophils have increased because one of the functions of plant flavonoids is to modulate increased neutrophil work to prevent infection or inflammation. Busse (1984) has previously reported that flavonoids are natural plant compounds that have been shown to have various anti-inflammatory effects. The role of flavonoids in the inflammatory response are lysosomal enzyme release, chemiluminescence (CL) response, and superoxide anion production.[31]

In this study, the ethanol extract ointment of kirinyuh leaves succeeded in suppressing the development of eosinophil and basophil cells and activating the intensity of neutrophil work. The ability to stop the growth of these three cells is possible because the flavonoid compounds in the ethanol extract ointment from kirinyuh leaves can inhibit factor mediators from activating IgE and mast cell responses by increasing the role of neutrophil cells.

This study has reported the role of ethanol extract ointment from kirinyuh leaves (*Chromolaena odorata (L)*) as an anti-inflammatory agent in the allergic response of the nasal mucosa. In general, it can accelerate inflammatory healing and reduce allergic reactions based on eosinophil, basophil, and neutrophil cell profiles. This potential can be made possible by using this plant as a therapeutic ingredient in healing allergies and inflammatory reactions.

CONCLUSION

Based on the research objectives, this study can be concluded that the ethanol extract ointment of kirinyuh leaves (*Chromolaena odorata (L)*) applied topically can reduce allergic reactions and reduce inflammation. Kirinyuh leaf ethanol extract ointment ((*Chromolaena odorata (L)*) can reduce eosinophil, basophil, and neutrophil inflammatory cells in the nasal mucosa in rabbit allergy modeling for seven days. A 5% concentration of kirinyuh leaf ethanol extract ointment (*Chromolaena odorata (L)*) has an effect that is better in responding to the activity of eosinophil, basophil, and neutrophil inflammatory cells in the nasal mucosa in rabbit allergy modeling for 7 days.

ETHICAL STATEMENTS

Several ethical contributions KEPPKN Registration Number: 1171012P. Descrıptıon Of Ethıcal Exempted "Ethical Exempted" Number: 004/EA/FK-RSUDZA

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Potential of chitosan oligosaccharide gel as a cavity cleanser against adhesive restoration adhesive on the cavity wall

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ABSTRACT. Cavity cleanser is a cavity cleaner with ideal properties such as being biocompatible, antibacterial, removing smear layers, and not interfering with the adhesive bond. This study aims to examine the potential of gel chitosan oligosaccharide (COS) as a cavity cleanser on the adhesion of the restoration to the cavity wall. Thirty-two mandibular premolars with class V restorations are divided into two groups with 2% COS gel and 2% chlorhexidine digluconate (CHX). Samples were immersed in 2% methylene blue, cut longitudinally, observed under a stereomicroscope with 1x magnification, and scored 0-3. The Mann-Whitney test was used to see the potential of gel COS as a cavity cleanser. The results showed a significant difference between the two groups at the edge of the enamel junction restoration (p=0.038) and the dentin junction restoration (p=0.027). The mean microleakage score in group 1 showed better results at the enamel junction (1.19± 1.328) and the dentin junction (2.00±1.211). It shows that 2% COS gel has the potential as a cavity cleanser. There was a significant difference between the two groups as a cavity cleanser on the adhesion of adhesive restorations to the cavity wall.

KEYWORDS: cavity cleanser; chitosan oligosaccharide; chlorhexidine digluconate; microleakage

INTRODUCTION

Caries is a multifactorial disease mainly caused by an imbalance of oral flora (biofilm).[1] The development of caries can be prevented by restorative treatment. Restoration of class V cavities is a challenge in dentistry because frequent failures caused microleakage in the restoration, especially at the gingival margin close to the cementoenamel junction (CEJ). Factors that can influence the occurrence of this microleakage are that at the cervical margin, there is little or no enamel which makes adhesive more difficult because dentin contains more organic components and water content than enamel. It prevents restorative materials and adhesives from adequately penetrating the dentin.[2,3]

Before filling the dental restorative material in the cavity, preparation was carried out.[4] During the preparation process, the dentin was covered by bacteria, debris, and a smear layer which later became a significant problem in restoration. The smear layer comprises organic and non-organic components with a thickness of 5-10 m. The smear layer may prevent the adhesion of cavity wall adhesive restorations. [5,6]

Cavity cleanser is a cavity cleanser before dental restorative procedures that can clean, moisten, and disinfect the smear layer remaining on the dentin-enamel junction after cavity preparation. The ideal cavity cleanser should be of low or no toxicity to pulp cells while not interfering with the adhesive bond of the restorative material.[6,7]

Chlorhexidine digluconate (CHX) 2% is the most widely used cavity cleanser in dentistry because it is considered the "gold standard" and is the most commonly used antimicrobial agent. CHX 2% has high antibacterial effectiveness against gram-positive and negative bacteria. CHX 2% has drawbacks, namely a lower value because it cannot

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dissolve organic tissue in the smear layer, has disadvantages in long-term use, which causes discoloration of teeth and tongue, has an unpleasant odor, and can be allergenic in continuous contact.[8] [9,10,11]

Previous research used chitosan as an irrigant to remove the smear layer on the dentin of the root canals. Chitosan is a natural material resulting from the deacetylation of chitin through chemical and biochemical reactions in the shells of shrimp, crabs, and crabs. Chitosan has biodegradable, biocompatible, non-toxic properties, broad antimicrobial effect, and chelation ability which can remove smear layers with inorganic components. In the study of Ayu and Trimurni et al. (2013) regarding the ultrastructural description of the root canal wall using SEM, it was found that 0.2% chitosan solution was able to lift the smear layer, while the combination of 2.5% NaOCl plus 0.2% chitosan, there was more smear layer In the dentinal tubules. [9,12]

Research on chitosan has been modified with the development of chitosan oligosaccharides**.** Chitosan oligosaccharide (COS) is a hydrolysis of chitosan consisting of 2-10 D-glucosamine, which can be made by chemical and enzymatic hydrolysis. However, enzymatic hydrolysis of chitosan is considered more effective and easier to control. In the study of Ernani et al. (2015), COS has a higher solubility and is easier to apply.[13] Several studies have reported that COS is also proven to be a promising antimicrobial, immune stimulant, and antitumor. In the study of Suzuki et al. (2014), it was confirmed that COS dissolved in citric acid was able to remove the smear layer significantly with minimal erosion after soaking for five minutes. [14–16]

Currently, research on chitosan has been widely used in root canal treatment to remove the smear layer. However, the effect of COS in the form of a gel as a cavity cleanser is not yet known, especially its impact on restoration adhesion. Therefore, researchers are interested in seeing the potential of COS as a gel as a cavity cleanser on the bonding of adhesive restorations to the cavity wall by looking at the microleakage.

MATERIALS AND METHODS

This experimental study was carried out using a posttest-only control group design. Ethical clearance was approved by the Ethical Research Committee Faculty of Medicine Universitas Sumatera Utara (USU). It was conducted in the Laboratory of Analytical Chemistry FMIPA, USU, Department of Dental Conservation FKG USU, Department of Prosthodontics FKG USU, Basic Biology Laboratory UPT PP LIDA USU

A sample of 32 premolars that had been extracted was cleaned and immersed in saline solution. Then, they were randomly grouped into two groups, each with 16 illustrations, and planted on plaster beams.

COS powder was obtained from the Center for Innovative Excellence, University of North Sumatra. The making of 2% COS gel was done by dissolving 1 gram of chitosan oligosaccharide powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic stirrer for 10 minutes. Next, 2% hyaluronic acid was made by dissolving 1 gram of hyaluronic acid powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic bar for 30 minutes to form a hydrogel preparation. Then, 30 ml of 2% hyaluronic acid was mixed with 2.6298 g of NaCl while dropping 14 ml of COS 2% solution with a dropper gradually and stirred until homogeneous for 24 hours, then refrigerated for 24 hours.

Sample preparation was carried out by making an outline form for a Class V cavity design of 3 mm x 2 mm x 2 mm with the cervical edge 1 mm above the CEJ using a diamond bur (round bur and tapered fissure bur). Wash and dry the prepared cavity surface. Restoration of the sample was carried out in 2 groups. In group 1, a cavity cleanser (2% COS gel) was applied for 20 seconds. In group 2, a cavity cleanser (CHX 2% (Bisco, USA) was used for 20 seconds with a micro brush, then washed and dried. Next, both groups were applied with an adhesive system (total-etch two steps) by applying etching material with a micro brush for 15 seconds, then rinsed. Then use bonding (Esbond, Korea) for 15 seconds, lightly blow for 10 seconds, and cure for 20 seconds. Apply a flowable composite resin (Esflow, Korea) with a bulk-filled insertion technique in the cavity, then cure for 20 seconds. The restored teeth were contoured and finished using a finishing bur and polished using rubber silicone on the surface of the restoration.

The sample was soaked in a saline water container for 24 hours. Then a thermocycling process was carried out using a water bath (Memmert, Japan) by inserting the sample into a glass beaker containing ice at a temperature of 5°C, left for 30 seconds, and then transferring within a transfer time of 10 seconds into a water bath at 55°C, went for 30 seconds and repeated for 250 seconds. Round times. Next, the apex of the sample was

covered with wax and the tooth surface was coated with 2 coats of nail polish (acetone) except the restoration surface and 1 mm around the edge of the restoration and then allowed to dry in the open air.

RESULTS

The mean microleakage score at the edges of enamel junction restorations showed that the mean microleakage score in group 1 (1.19±1.328) was better than in group 2 (2.13±1.258). The same thing was also seen at the edge of the dentin junction restoration, which showed the microleakage's mean score.

Table 1. Microleakage score mean

Edge	Groups	N	Microleakge score (Mean)
Enamel Junction	1	16	1,19± 1,328
	2	16	2,13± 1.258
	Total	32	
Dentin Junction	1	16	2,00±2,00
	2	16	2,75±0,775
	Total	32	

Information: Group 1: COS gel 2%; Group 2: CHX 2%

Then, it was soaked in a 2% methylene blue solution for 24 hours at room temperature. Next, all teeth were cleaned until dye are removed in running water and dried. The sample was split longitudinally using a disc bur. Microleakage observations were carried out by observing the penetration of 2% methylene blue dye through a stereomicroscope (Olympus, Japan) with 1x magnification.

The sample was then assessed using a standard scoring system with a score of 0-3 as in the study conducted by Moosavi et al. (2013) as follows: Score 0: No dye penetration Score 1: dyes penetration <1/2 cavity wall depth Score 2: Penetration of dye >1/2 the depth of cavity wall Score 3: Penetration of dye has reached the axial cavity wall

The data obtained will be processed and analyzed using the Statistical Package for the Social Sciences (SPSS). The Shapiro-Wilk test was conducted to determine whether the data were normally distributed. The data obtained were tested for normality with the Shapiro-Wilk test p-value <0.05. Because the data were not normally distributed, the analysis was carried out using a non-parametric statistical test, namely the Mann-Whitney test.

Table 2. Statistic test result with *Mann Whitney*

Microleakage score				
Edge	Groups	N	Microleakage score (Mean)	P
Enamel Junction	1	16	1,19± 1,328	0,038*
	2	16	2,13± 1.258	
	Total	32		
Dentin *Junction*	1	16	2,00±2,00	0,027*
	2	16	2,75±0,775	
	Total 32	32		

Table 1 shows that group 1 (2,00±1.211) was better than group 2 (2.75±0.775) and Table 2 shows that there were significant differences between the COS 2% gel and CHX 2% groups at the edges of the enamel junction restoration (p=0.038) and dentin junction restoration (p=0.027).

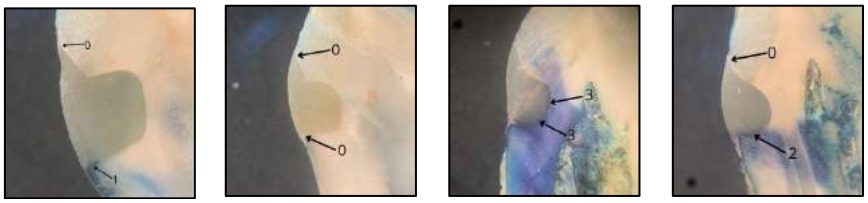

Figure 1. Stereomicroscope photos in group 1

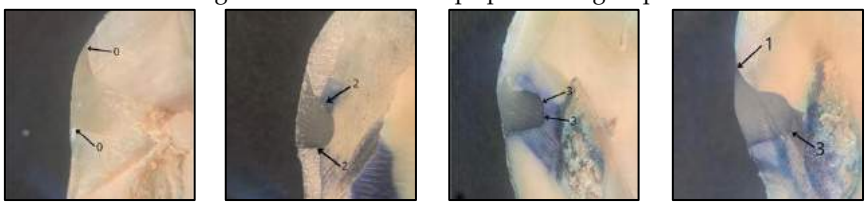

Figure 2. Stereomicroscope photos in group 2

DISCUSSION

This study used 2% COS gel as an alternative to cavity cleansers derived from natural ingredients, which are expected to have the potential as cavity cleansers and affect the adhesion of adhesive restorations to cavity walls that were applied before adhesives were used. Table 2, obtained $p < 0.05$, which proves that 2% COS gel has the potential as a cavity cleanser that can affect the adhesion of adhesive restorations to cavity walls.

COS is a hydrolysis of chitosan that has a shorter polymer structure, so it has a lower molecular weight and higher solubility than high molecular chitosan.[17,18] COS above, it can be seen that chitosan oligosaccharide has the potential as a cavity cleanser because it has the ideal properties that a cavity cleanser should possess. Research done by Varshneya et al. (2017) shows that chitosan has the potential as an effective disinfectant before restoration because it can prevent microleakage and does not interfere with existing adhesive bonds.[19] The same result was also found by Paschoini et al. (2021), showing that chitosan can increase the adhesive strength of the material better than the untreated control.[20]

This study used a chemical modification of COS in the gel preparation. The gel is a polymeric bond that is physically/chemically bonded to each other, which traps the liquid phase so that it is a viscoelastic semisolid. The water contained in the hydrogel is a type of imbibition water that can enter a material and will increase the volume of the material. Guibal et al. (1997) research show that the chemical modification of chitosan into chitosan gel can increase the absorption power because the gel form has a larger pore volume than the flake form.[21] In additionPaschoini et al. (2021) research shows that chitosan in the form of a gel can also increase and maintain adhesion bonds.[20]

The results of statistical tests with Mann Whitney in Table 5 show that there is a significant difference ($p<0.05$) between the COS 2% gel and CHX 2% groups at the edges of the enamel junction and dentin junction restorations ($p = 0.027$) as a cavity cleanser against adhesion of adhesive restorations to cavity walls so that the hybridization layer formation on composite resin restorations is well established. Silva et al. (2017) show that the chelating properties of chitosan solution removed the inorganic smear layer in root canal treatment. Although the exact mechanism is unknown, it is believed that this is due to the adsorption properties, ion exchange effects, and chelating properties that form complexes of chitosan substances with metal ions. The same results were also found by.[22] Besides being able to remove the smear layer, as a cavity cleanser, it must also have ideal antibacterial properties. Kaur et al. (2020) also proved that the combination group of COS with COS solution was the highest in removing the smear layer from other groups and had high antibacterial effectiveness.[17]

The microleakage scores in Table 1 show that the restoration at the dentinal margin was higher than that of the enamel in all treatment groups, with an average value of groups 1 and 2. It was because, at the cervical margin, there was little or no enamel in the Class V cavity, so bonding was achieved. Dentin also has a smaller hydroxyapatite structure and is arranged in a criss-cross pattern making it more challenging to form micromechanical bonds with dentin.[2] In addition, it can be caused by heat caused by friction of the bur with the teeth during cavity preparation which causes the interprismatic enamel to break and the

collagen in the dentin to collapse, polishing after filling the cavity. [23] The thermocycling process can also cause a high microleakage score. The extreme temperature changes in the oral cavity will affect the difference in expansion and contraction between the restorative material and the tooth structure, which causes the restoration surface to become weak.[24,25]The occurrence of microleakage is also related to shrinkage during polymerization caused by the C-factor. Class V cavities have a C-factor with a value of 5. The higher the C-factor, the higher the potential for polymerization shrinkage.[26]

CONCLUSION

Chitosan oligosaccharide 2% has the potential as a cavity cleanser material when developed as a gel and affects the adhesion of adhesive restorations to cavity walls. The 2% chitosan oligosaccharide could be a cavity cleanser material resulting in the lowest mean microleakage score both at the edge of the enamel junction restoration and at the edge of the dentin junction restoration.

The results showed a significant difference between 2% oligosaccharide chitosan gel and 2% chlorhexidine digluconate as a cavity cleanser material on the adhesion of adhesive restorations to cavity walls so that the formation of hybridization layers on composite resin restorations was well formed.

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Level of Knowledge on Radiation Protection in Roentgen Photo-taking among Clinical Dental Students

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ABSTRACT. Radiography in dentistry is a device that is often used. The images produced from radiographs are significant for dentists, especially to see any abnormalities that are not visible or unclear on clinical examination so that they can assist in making a diagnosis, determining treatment plans, and assessing treatment success. Besides having benefits, radiography can also cause damage to normal human cells or tissues. The danger caused by this radiation can be overcome by radiation protection. This study aimed to determine the knowledge of clinical clerkship students regarding radiation protection when taking dental x-rays at the Dental and Oral Hospital of Universitas Syiah Kuala (RSGM). This type of research is descriptive, with a total of 305 subjects. The data was Collected through the distribution of questionnaires. The results showed that there were 44 (14.4%) students who had a high level of knowledge, 159 (52.1%) moderate, and 102 (33.4%) low. It was concluded that most young dentists at RSGM had an intermediate level of knowledge about radiation protection when taking x-rays.

KEYWORDS: Radiography, radiation protection, level of knowledge, Clinical Clerkship Students

INTRODUCTION

Utilization of X-rays (roentgen) in the field of medicine is one way to improve health. This application has various uses, from diagnostic tools to therapy (radiotherapy).[1] Radiotherapy is an example of treatment using X-rays widely used to treat cancer. X-ray radiotherapy is used to treat oral cavity cancer and surrounding areas such as the tongue, the soft palate, and the lips.[2]

Diagnostic radiology (radiography) applications widely used include X-ray photos that function for imaging body organs. Radiography in dentistry is a device that is often used. According to (cit. Goaz et al., 1992), the picture produced by radiography is significant for dentists, especially to see any abnormalities that are not visible or less obvious on clinical examination so that they can assist in making a diagnosis, determining a planned treatment and assess the success of the treatment that has been carried out on patients.[3]

Besides having benefits that can be used in diagnosis and therapy, X-ray radiation can also cause damage to normal human cells or tissues due to atomic interactions at the cellular level. X-ray radiation can cause erythema, atrophy, ulceration, sterility, cancer, and genetic disorders.[4] The danger from this radiation can be overcome by doing radiation protection.[5] Radiation protection is an effort to protect someone from receiving or being exposed to radiation in the smallest possible dose.

All individuals who interact in an X-ray exposure environment need proper and continuous protection, such as the use of film badges, Thermo luminescence dosimeters, taking radiographs as far as possible from the X-ray source, using radiation protection devices such as lead (Pb) aprons, Pb gloves, Pb goggles, Pb thyroid protectors, radiation measuring instruments and shorten radiation time. Operators must have a thorough knowledge of radiation hazards and protection protocols.[7,8]

Mojiri and Moghimbeigi (2011) stated that although most operators know that it is mandatory to use a film badge and routine inspections every six months, there are still operators who do not use radiation protection when taking x-rays and do not carry out routine check-ups every six months.[8] Research by Yucel et al. (2009) in Turkey stated that

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knowledge about X-ray radiation among medical students and doctors who requested radiological procedure treatment was very lacking.[6] Shahab et al. (2010) also published that learning and behavior related to the application of oral radiology safety standards in most students and dentists did not meet expectations. Most dentists do not understand technological advances and still perform procedures that cause radiation overexposure to themselves and their patients.[9] Scientific publication by JKM Apps (2010) at the Ghent University School of Dentistry explained that students' knowledge of dento-maxillofacial radiology was very lacking. Both in terms of radiation use and protection.[10] Suwargiani (2007) demonstrated that in Indonesia, the knowledge and awareness of clinical clerkship students regarding the principles and techniques of radiation protection when taking pictures and the dangers that may arise from taking dental radiographs, in general, can be categorized as good.

Based on these conditions, the authors feel the need. They are interested in researching the level of knowledge of clinical clerkship students regarding radiation protection when taking X-rays at the RSGM, where later, those who act as operators must understand the importance of radiation protection very well so that operators and the patient can avoid the dangers of excessive radiation.

MATERIALS AND METHODS

Types of research

This type of research is descriptive research with a cross-sectional approach. Descriptive research is a method carried out with the main objective of making an objective picture or description of a situation. This study will describe the knowledge of clinical clerkship students about radiation protection measures when taking x-rays at RSGM Unsyiah Banda Aceh.

Inclusion Criteria

1. Clinical clerkship student at RSGM Universitas Syiah Kuala Banda Aceh, Indonesia
2. Willing to be a respondent.
3. Cooperative

Population and Research Subjects

The population in this study were all clinical clerkship students at RSGM RSGM Universitas Syiah Kuala Banda Aceh, totaling 332 students. Subjects are part of the population taken from the whole being studied and are considered to represent the 332 population. The subjects in this study were all clinical clerkship students at RSGM. The subject-taking method is done in total sampling with a non-probability approach. Taking subjects with a non-probability sampling technique is taking subjects based on calculated possibilities.

Data Collection Procedures

The researcher visited the research subjects according to the inclusion criteria, then asked about the subject's willingness to fill out an informed consent form. Researchers will distribute questionnaires after the subject agrees. Subjects were given approximately 10 minutes to fill out a questionnaire that had been designed. After completion, the researcher collected questionnaires for data analysis.

Trials of Research Instruments

The validity test aims to determine the extent to which a measure or value indicates the level of validity of a measuring instrument by measuring the correlation between variables and the total score of variables which can be seen according to the corrected item-total correlation (r), provided that if the value of r count > value of r table, then declared valid and vice versa.

The reliability test aimed to determine the extent of the reliability of a measuring instrument. The calculation of the reliability test for the agency for each variable can be seen from Cronbach's alpha coefficient.

Research Data Analysis

The data analysis in this study was descriptive and univariate to describe the knowledge of clinical clerkship students at the Faculty of Dentistry Universitas Syiah Kuala Banda Aceh about radiation protection when taking x-rays using SPSS (Statistic Package for Social Science (SPSS) 15.0). The data will be displayed in a frequency distribution table with a percentage.

Univariate analysis, namely analysis to see an overview of the frequency distribution of each variable, and all data were analyzed with the help of a computer and concluded descriptively.

Furthermore, the variables are categorized in the following criteria: 12

a. Good, if $x > 75\%$ is obtained
b. Enough, if $75\% \leq x \geq 56\%$ is obtained
c. Less, if $x \leq 55\%$ is obtained

Furthermore, the data that has been entered into the frequency distribution table is determined by the percentage of gain for each category using the formula, namely:

Information: p = percentage, fi = observed frequency, n = the number of respondents who are subjects.

RESULTS

This research was conducted at RSGM Universitas Syiah Kuala Banda Aceh on 22-24 September 2014. The study subjects were all young dentists who attended the hospital, as many as 305 people out of 332 total young dentists. Twenty-seven young dentists did not become research respondents because they were currently in the public health stage clerkship at the public health center. The research was conducted on the knowledge of young dentists about radiation protection in taking x-rays at RSGM. The data collection technique was carried out by distributing questionnaires. Young dentists were asked to agree to participate in this study by filling out the distributed questionnaires. Univariate analysis was used to see the frequency distribution of knowledge of young dentists about radiation protection when taking x-rays at RSGM.

Table 1 shows that most of the research subjects were female, with a total of 231 people (37.4%), and subjects male sex, as many as 74 people (24.7%).

Table 1. Demographic data of research subjects based on gender

Sex	Amount (%)
Male	74 (24,7%)
Female	231 (75,3%)
Total	305 (100%)

Table 2 shows that most young dentists at RSGM know about radiation protection in taking x-rays in the moderate category, namely 159 (52.1%). A total of 44 (14.4%) young dentists knew the high category, and 102 (33.4%) young dentists knew the low category.

Table 2. Level of knowledge of young dentists about radiation protection when taking x-rays at RSGM Unsyiah

Level of knowledge	Amount (%)
Hight	44 (14,4%)
Medium	159 (52,1%)
Low	102 (33,4%)
Total	305 (100%)

Table 3 shows that young male dentists at RSGM are more likely to have a percentage of knowledge about radiation protection when taking x-rays in the high category, namely 41.9%. Young female dentists at RSGM have a portion of knowledge in the high category, namely 30.7%.

Table 3. Level of knowledge of young dentists about radiation protection in taking x-rays at RSGM Unsyiah by gender

Knowledge	Female	Male	Amount (%)
Tinggi	71 (30,7%)	31 (41,9%)	102 (33,4%)
Sedang	121 (52,4%)	38 (51,4%)	159 (52,1%)
Rendah	39 (16,9%)	5 (6,80%)	44 (14,4%)
Total	231 (100%)	74 (100%)	332 (100%)

DISCUSSION

Using ionizing radiation such as x-rays is one of the most important diagnostic aids in the medical world. The benefits obtained from ionization energy are enormous. However, there are still potential risks that cannot be ignored because the radiation harms body tissues. It makes radiation protection measures necessary for all individuals involved, especially health workers who work with radiation [11].

Every worker must monitor how much radiation dose he has received and protect himself from radiation adequately. They must also receive education and training on good self-protection measures. The level of knowledge and awareness about radiation greatly influences the behavior of operators in protecting themselves, so an overview of radiation protection knowledge for operators needs to be known[12].

Table 2 shows that most young dentists know radiation protection in the moderate category, 159 (52.1%). These results follow previous research by Shahab et al. (2012), which states that the knowledge of most doctors about radiation protection is not very good [9]. Research Yucel et al. (2009) also explained that most respondents in their study did not have good knowledge about radiation protection[6]. Yurk et al. (2014) added in their research that most of the research subjects had poor knowledge about radiation protection[13]. Sadigh et al. (2014) also presented the same results where most residents who studied had limited knowledge about radiation protection[14].

This result is inversely proportional to the research of Mojiri and Moghimbeigi (2011), which stated that the majority of research respondents had good knowledge about radiation protection8. It is probably related to the experience of the radiographers, most of whom have worked long enough and have experience in the field of radiology.

The results of this study indicate that most respondents know radiation protection in the moderate category related to the information received by the operator. Mubeen et al. (2008) stated that inadequate information about something would lead to destructive behavior. Good education and education must be given to operators to protect themselves adequately. The addition of materials on radiation into the education curriculum is needed so that the operator's understanding of the dangers of radiation becomes better[15].

In addition to information, knowledge about radiation protection by operators is also related to the length of education undertaken. Research by Rabhat et al. (2011) stated that the longer the education period, the better the radiation protection knowledge and behavior1. The level of knowledge of clerkship students about radiation protection is best obtained from final-year students, followed by students at the lower level. It is related to work experience. Shah et al. (2007) stated that work experience is related to knowledge and adherence to taking radiation protection measures when taking x-rays[16].

Table 3 shows that the percentage level of knowledge of young male dentists in the category is 41.9% compared to young female dentists, namely 30.7%. This result follows the study of Hagi and Khafaji (2011), which stated that 57% of male respondents had a good level of radiation protection knowledge compared to 42% of female respondents. Research conducted before and after that also showed the same results where the comparison was 51% versus 43%[17]. Research Sadigh et al. (2014) also showed the same results, where the knowledge of male respondents was higher than that of female respondents[14]. It is thought to be related to psychology. Female respondents often do not concentrate on their ability to take good photos and protect against radiation, causing them to often not take action properly due to fear and nervousness.

Green in Notoatmodjo (2003) states that there is a link between knowledge and behavior. The knowledge that is not good will make someone tend to do an action that is not good too. In this study, the understanding of female respondents was lower than that of male respondents, so women tended not to take radiation protection measures properly[18].

The data collection process in this study was carried out by distributing self-administered questionnaires, thereby increasing bias. Filling out the questionnaires carried out by the respondents without being interviewed by the researchers made the respondents tend to fill out the questionnaires unobjectively.

CONCLUSION

Based on the research that has been done, it can be concluded that the majority of young dentists at RSGM Universitas Syiah Kuala Banda Aceh know radiation protection in taking x-rays in the medium category, namely 159 people (52.1%). Young dentists at RSGM who knew about radiation protection in the high class were 44 people (14.4%), young dentists at RSGM who knew about radiation protection in the low category were 102 people (33.4%)

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Association between the number of bilateral free-end posterior tooth loss and mastication performance in RSGM USU patients

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ABSTRACT. Mastication is the process of mechanically crushing food to form soft particles, facilitating the ingestion process in providing nutrients to the body. One of the objectively assessed mastication functions is mastication performance. The number of tooth losses is one factor that affects mastication performance; the more tooth loss, the lower the mastication performance. Posterior teeth play an important role in mastication function, and loss of posterior teeth decreases mastication performance. The research aims to determine the association between the number of bilateral free-end posterior tooth loss and mastication performance. This type of research is descriptive-analytic with a cross-sectional design. The subjects in this research were 17 people. Data were collected by examining the oral cavity and color-changeable chewing gum to assess mastication performance using a colorimeter. Data were analyzed using the Mann-Whitney test. The results showed that all subjects with bilateral free-end posterior teeth loss in one and both arches had poor mastication performance. The statistical test results showed no association between the number of bilateral free-end posterior teeth loss and mastication performance in one and both arches with p = 1,000 (p> 0.05). It can occur because this research does not pay attention to the number of occlusion pairs of posterior teeth and the results of the assessment of mastication performance are not quantitative. The number of bilateral free-end posterior tooth loss can be considered in assessing mastication performance as an educational basis for denture care.

KEYWORDS: Posterior Bilateral Free End, Mastication Performance

INTRODUCTION

Tooth loss is the loss of one or more teeth from their socket or place due to various causes, including periodontal disease, dental caries, and external injury. Its prevalence increases with age. Based on the Basic Health Research (Riskesdas) in 2018, the prevalence of tooth loss in Indonesia was 19%, with the highest percentage at the age of 65 years and over, which was 30.6%, followed by the age of 55-64 years, which was 29%. Tooth loss can cause disturbances in aesthetics, phonetics, and mastication. Therefore, denture treatment is needed to rehabilitate and maintain masticatory, aesthetic, and phonetic functions.[1,2,3]

Mastication is the process of mechanically crushing food to form a small bolus to facilitate the swallowing process in providing nutrients for the body. The components of mastication consist of the dentition, temporomandibular joint, nervous system, and masticatory muscles. The functions of mastication include cutting, crushing, mixing food, and stimulating salivary secretion.[4,5] Mastication is influenced by several factors, including saliva, gender, age, supporting tissue, and the amount of tooth loss. One assessment of masticatory function that can be objectively assessed is masticatory performance.[6]

Mastication performance is a measure of the distribution of food that can be achieved under standardized testing conditions and indicates the comprehensive ability required for mastication. Mastication performance consists of cutting, crushing, and mixing food, but none of the three functions can be evaluated at once. Nasseri's (2017) study found a relationship between crushing and food mixing ability, so both can be used to evaluate mastication performance.[6,7,8,9] There are several

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methods available to objectively evaluate mastication performance, one of which is using color-changeable chewing gum. This method is more focused on food mixing ability and has the advantages of being simple and fast, and has proven reliability and validity to evaluate masticatory performance by measuring gum color changes.[10,11,12,13]

One factor that affects mastication is tooth loss. Permanent teeth in adults consist of 32 teeth, 16 in the upper jaw and 16 in the lower jaw. The total number of anterior teeth is 12, and the posterior teeth are 20 if the entire M3 erupts. Tooth loss can be classified based on the number of tooth loss quadrants, Kennedy's classification, and the amount of posterior tooth loss. Dental quadrant is a term used in the division of the jaw into four equal parts, starting from the center line of the dental arch or the contact point of the central incisor and extending towards the last tooth at the back of the mouth, consisting of four quadrants, namely, the upper right, upper left, lower left and lower right quadrants. Kennedy's classification is based on the relationship of the edentulous space to the supporting teeth. This classification divides all toothless conditions into class I (bilateral free end), class II (unilateral free end), class III, and class IV. Posterior tooth loss is prone to occur in a person due to difficulties in performing efficient dental hygiene and generating considerable occlusal forces when used, so the loss of posterior teeth has a major impact on masticatory function.[14,15,10,16] If in a bilateral free end state, it will cause difficulty and limitations in mastication so that a person tends to advance the chin and result in occlusion instability which has an impact on masticatory function and jaw movement processes.[17,18,19,20]

Good occlusion should allow the mandible to translate without occlusal resistance during functional movements, especially the posterior part, so that the load distribution is more even.[21] Occlusion and mandibular position will be expected and stable if each masticatory component can carry out its activities, usually with harmonious and balanced interactions. It is because a disturbance in one component of the masticatory system will impact other components.[22] In Krista's research (2016) showed that loss of posterior teeth that are not replaced will cause impaired function of the masticatory muscles, impacting the mastication process.[23] Another thing that can occur is a decrease in the thickness of the masseter muscle in someone who loses a tooth, causing a decrease in bite force.[24]

In the study of Hayato et al. (2014), there were differences in mastication performance between the bilateral posterior tooth loss group and the group that did not lose posterior teeth, which showed a decrease in mastication performance in the bilateral posterior tooth loss group. Still, in this study, there was no division of bilateral posterior tooth loss by number.[15] Muslita's research (2018) shows that there is an effect of the number of teeth that still have occlusal contact in the oral cavity with masticatory performance, while Ikebe et al.'s research (2011) shows that the more the number of missing teeth and the less the number of remaining teeth in the oral cavity, the lower a person's masticatory performance, but in these two studies there was no division of missing teeth based on location.[4,7] Wardhana et al.'s research (2015) divided the number of posterior tooth losses into <3 and ≥3 teeth, showing the results that individuals who lost more than three posterior teeth experienced interference, limitation, or pain in the function of the oral cavity, but there was no division of tooth loss based on location.[15,21] Based on the description of these studies, there is a relationship between the number and location of tooth loss with masticatory performance.

MATERIALS AND METHODS

This type of research is descriptive-analytic using a cross-sectional research design. This research was conducted at the Department of Prosthodontics in February-March 2020. The population of this study was 2019-2020 USU RSGM patients. Sampling using purposive sampling technique, namely setting inclusion criteria and exclusion criteria. The inclusion criteria in the study were patients with bilateral free end posterior tooth loss ≥2 - 4 and >4 teeth in one jaw arch, bilateral free end posterior tooth loss ≥4 - 10 and >10 teeth in both jaw arches, willing to be respondents in the study, not paying attention to Kennedy's classification modification. Exclusion criteria are patients using maxillary and mandibular GTP (full tooth loss) and patients who are not willing to be respondents. The subjects in this study totaled 17 people. The tools and materials used in the study were standard intraoral examination tools (mouth glass, tweezers, sonde), preparation glass, colorimeter, color scale, hand tally counter, and color-changeable chewing gum.

Examination of the Number of Bilateral Free-end Posterior Tooth Losses

Conduct an oral examination to see the number of bilateral free-end posterior tooth losses and record and classify the examination results based on the tooth loss group.

Mastication Performance Check

Subjects were instructed to rinse their mouth, then chew color-changeable chewing gum 120 times. The chewed gum was taken and flattened on polyethylene film and then compressed between two glass preparations to a thickness of approximately 1.5 mm to see the even distribution of color change. The color change was measured using a colorimeter. Chewing gum was measured immediately after leaving the patient's mouth. The measurement results were converted into the formula:

$$\Delta E = \sqrt{(L \times -72{,}3)^2 + (a \times +14{,}9)^2 + (b \times -33{,}0)^2}$$

The ΔE value indicates the color change to see the mastication performance. The ΔE value is divided into 0 to 70 with an interval of 7 and a color scale ranging from 1-11 shades. Color shades 1-3 are score 1 (green), color shades 4-5 are score 2 (yellow), color shades 6-7 are score 3 (pink), color shades 8-9 are score 4 (red), color shades 10-11 are score 5 (dark red). Then the subjects were categorized into mastication performance scores, namely good (score 4-5) and bad (score1-3).

***Figure 1*. Color-changeable chewing gum[25]**

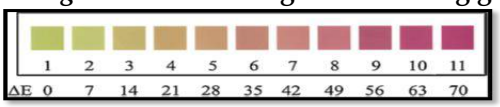

Figure 2. Color scale based on ΔE value[25]

Data Analysis

Descriptive data, namely the percentage of bilateral free-end posterior tooth loss in one jaw arch and both jaw arches and mastication performance values, were presented in tabular form. Analytical data, namely the relationship between the amount of bilateral free-end posterior tooth loss and mastication performance, were analyzed using the Mann-Whitney test to see the relationship between variables ($p < 0.05$).

RESULTS

The results of data collection on the prevalence of bilateral free-end posterior tooth loss in **Table 1** show that in one jaw arch from a total of 9 patients, four people (44.4%) had a loss of ≥2-4 bilateral free-end posterior teeth. Five people (55.6%) had a loss of >4 bilateral free end posterior teeth. **Table 2** data in both jaw arches showed that out of a total of 8 patients, one person (12.5%) had a loss of ≥4-10 posterior teeth bilateral free end, and seven people (87.5%) had a loss of >10 posterior teeth bilateral free end

Table 1. Prevalence of Bilateral Free-end Posterior Tooth Loss in One Jaw Arch

Jaw Arch	Number of Bilateral Free-end Posterior Tooth Losses				Total
	≥2-4 Tooth		> 4 Tooth		
	n	%	n	%	
One jaw arch (maxilla/ mandible)	4	44,4	5	55,6	9 (100%)
Total	**4**	**44,4**	**5**	**55,6**	**9 (100%)**

Table 2. Prevalence of Bilateral Free-end Posterior Tooth Loss in Both Jaw Arches

Jaw Arch	Number of Bilateral Free-end Posterior Tooth Losses				Total
	≥4-10 Tooth		> 10 Tooth		
	n	%	n	%	
Both jaw arches (maxilla and mandible)	1	12,5	7	87,5	8 (100%)
Total	**1**	**12,5**	**7**	**87,5**	**8 (100%)**

The results of data collection on the prevalence of mastication performance in **Table 3** patients who lost bilateral free end posterior teeth in one jaw arch showed a total of 9 people all had poor mastication performance (100%) and in both jaws showed a total of 8 people all had poor mastication performance (100%).

Table 3. Prevalence of Mastication Performance in Patients with Bilateral Free-end Posterior Tooth Loss

Jaw Arch	Mastication Performance				Total
	Good		Bad		
	n	%	n	%	
One jaw arch (maxilla/ mandible)	0	0	9	100	9 (100%)
Both jaw arches (maxilla and mandible)	0	0	8	100	8 (100%)

Based on **Table 4** of the statistical analysis results below, one jaw arch shows no relationship between the amount of bilateral free-end posterior tooth loss and mastication performance, with a p = 1.000 (p>0.05). Both jaw arches also showed no relationship between the amount of bilateral free-end posterior tooth loss and mastication performance, with a value of p = 1.000 (p>0.05)

Table 4. Association Between the Number of Bilateral Free-end Posterior Tooth Loss and Mastication Performance

Number of Bilateral Free-end Posterior Tooth Losses	Mastication Performance				Total		p
	Good		Bad				
	n	**%**	**n**	**%**	**n**	**%**	
One jaw arch (maxilla/ mandible)							
≥2-4 Gigi	0	0	4	44,4	4	44,4	
>4 Gigi	0	0	5	55,6	5	55,6	1,000
Total	**0**	**0**	**9**	**100**	**9**	**100**	
Both jaw arches (maxilla and mandible)							
≥4-10 Gigi	0	0	1	12,5	1	12,5	
>10 Gigi	0	0	7	87,5	7	87,5	1,000
Total	**0**	**0**	**8**	**100**	**8**	**100**	

DISCUSSION

This study examined the number of bilateral free-end posterior tooth losses. The examination was carried out in patients with bilateral free-end posterior tooth loss in one jaw arch and two jaw arches because at least 20 teeth are the antagonistic pair needed to ensure good mastication. The occlusal contact of the remaining posterior teeth is key in predicting the decline in mastication ability.[12,26] Mastication performance is a measure of the distribution of food that can be achieved under standardized testing conditions and indicates the comprehensive ability required for mastication, consisting of cutting, crushing, and mixing food.[6] In this study, the assessment of mastication performance was divided into two, namely, good and bad. The amount of tooth loss is one factor that affects masticatory performance, which means that the greater the amount of tooth loss, the lower the masticatory performance.[6,7] Posterior teeth, namely molars and premolars, play an essential role in masticatory function, so the loss of posterior teeth will have an impact on reducing masticatory performance. If in a bilateral free end state, a person tends to use anterior teeth to replace their function so that they tend to advance the chin and cause instability.[18,19,20] The number of remaining teeth in occlusion also affects masticatory function because the smaller the number of remaining teeth in occlusion, the lower the masticatory performance.[26]

The results of the study in tables 1 and 2 show that the prevalence of bilateral free end posterior tooth loss in one jaw arch is more dominant in losing >4 teeth (55.6%) and in both jaw arches is more prevalent in losing >10 teeth (87.5%). The results of this study are in line with the research of Peres et al. (2014), which shows the prevalence of tooth loss is more dominant in losing >4 teeth or the number of remaining teeth <10 teeth in one jaw arch, while in both jaw arches the prevalence of tooth loss is more dominant in losing >10.[27]

The results of the study in table 3 show that the prevalence of mastication performance experiencing bilateral free end posterior tooth loss ≥2 teeth in one jaw arch and ≥4 in both jaw arches has poor mastication performance (100%). The

results of the statistical analysis in table 4 show that there is no relationship between the number of bilateral free-end posterior tooth loss and masticatory performance in one jaw arch with p = 1.000 (p>0.05) and no relationship between the number of bilateral free end posterior tooth loss and masticatory performance in both jaw arches with p = 1.000 (p>0.05). These results indicate that a small or large amount of bilateral free-end posterior tooth loss has poor masticatory performance. This may occur because this study did not pay attention to the number of remaining occluded posterior teeth. After all, in addition to the number and location of teeth that affect masticatory performance, the number of occlusion pairs of posterior teeth also affects masticatory performance.

The results of the mastication performance assessment were also categorized in qualitative form, namely good or bad, not in quantitative form (numbers), so it did not show that the greater the amount of tooth loss, the lower the mastication performance score. The results of this study are in line with research by Wardhana et al. (2015), showing that a person who loses more than three posterior teeth in one jaw arch experiences interference, limitation, or pain in the function of the oral cavity, and this study the function of the oral cavity is included in the function of mastication.[21] This shows that mastication performance in bilateral free-end posterior tooth loss in one jaw arch also has the same effect on both jaw arches. This occurs because the mastication process requires occlusion contact in both jaw arches, so the loss of occlusion contact from the antagonist's teeth causes a decrease in mastication performance.

The results of this study are not in line with the research of Ikebe et al. (2011), which shows that there is a relationship between the number of remaining teeth in the oral cavity and masticatory performance, but in that study, there was no division of the number of remaining teeth in the oral cavity based on the location of the teeth and had a large sample size, while in this study dividing the amount of tooth loss based on location, namely posterior bilateral free end by not paying attention to the amount of tooth loss in other locations and having a minimal sample size so that it might affect the accuracy of the results of this study. In Muslita's research (2018) there was an influence on the number of occlusion pairs of posterior teeth with masticatory performance in that study subjects with the criteria had no loose teeth, no tooth loss, or only lost one tooth and had good occlusion, while in this study it did not pay attention to the number of occlusion pairs of posterior teeth with subject criteria, namely no loose teeth, good occlusion, and more than one tooth loss, so it might affect the accuracy of the results of this study.[4,7]

The weaknesses in this study are the minimum sample size due to the limited number of patients who fit the inclusion criteria, the number of free-end bilateral posterior tooth loss does not take into account the number of occlusion pairs of posterior teeth, the results of mastication performance assessment are not in quantitative form (numbers), uncontrolled variables such as saliva, systemic diseases, psychological effects and modification of Kennedy's classification. It may affect the accuracy of the results. Therefore, if this study is continued, it is necessary to control these variables with a larger sample size and pay attention to the number of occlusion pairs of the posterior teeth and the results of the mastication performance assessment in quantitative form (numbers) to show lower or higher mastication performance.

CONCLUSION

Based on the results of this study, in addition to the number and location of teeth affecting masticatory performance, the number of occlusion pairs of posterior teeth also affects masticatory performance. The amount of bilateral free-end posterior tooth loss can be taken into consideration in assessing masticatory performance as a basis for education to perform denture treatment

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The relationship between static and dynamic occlusion based on the relationship between anterior and posterior teeth and the occlusion scheme in dentistry students

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ABSTRACT. Occlusion is important in the mastication process. Tooth occlusion is generally classified into static occlusion and dynamic occlusion. Static occlusion is visible through the relation of both anterior and posterior teeth. Incisor relationships were classified according to the British Standard Institution (BSI), while Angle was classified based on the relationship of the first molars in permanent teeth. The classification of occlusion according to Angle and BSI is based on the description of the shape of the arch, tooth position, and tooth contact in the intercuspal position. This study aims to determine the distribution of static occlusion based on the relationship between anterior and posterior teeth as well as dynamic occlusion distribution based on the occlusion scheme and the relationship between static and dynamic occlusion in students of the Faculty of Dentistry, University of North Sumatra. It is a descriptive-analytic with a cross-sectional study design. The sample consisted of 100 students with a complete set of teeth. Each sample was examined using shim stock and articulation paper. The results of statistical using the chi-square test showed a significant relationship between static and dynamic occlusion based on the relationship between anterior teeth ($p < 0.05$) and the relationship between posterior teeth ($p < 0.05$).

KEYWORDS: occlusion, static occlusion, dynamic occlusion

INTRODUCTION

Occlusion is the intercuspal contact between the maxillary and mandibular dentition in all positions and movements of the mandible. Occlusion is controlled by components of the neuromuscular and masticatory systems viz: teeth, periodontal structures, maxilla and mandible, temporomandibular joint, muscles, and ligaments. Normal occlusion of the dentition can be categorized into two aspects: static occlusion and dynamic occlusion.[1]

A static occlusion is a contract between the maxillary and mandibular teeth that occurs when the jaw is stationary. Static occlusion can be seen through the anterior and posterior teeth relationship.[2] The most common occlusal relationship can be seen through the relationship of the posterior teeth. Angle characterizes static occlusion based on the relationship of the first molar in the permanent dentition into three, namely: class I, class II and class III.[2,3] The relationship of the incisors is classified based on the British Standard Institution (BSI), namely: class 1, class II Division 1, Class II Division 2, and class III. The classification of occlusion according to Angle and BSI is based on the description of the arch's shape, the teeth' position, and tooth contact in the intercuspal position.[2,4] In the study of Al-Hiyasat et al. (2004), stated that the prevalence of static occlusion with class II jaw relations, namely: 29% in class II molars and 37% in class II incisors (20% Division 1 and 17% Division 2).[4]

Dynamic occlusion is the occlusal contact that results when the mandible moves relative to the maxilla, either anteriorly, laterally, or posteriorly. There are two functional occlusions of the posterior teeth during lateral movement of the mandible: canine protection and group function.[3,5] According to the Glossary of Prosthodontics Terms, canine protection is a mutually protective and beneficial form of articulation in which the vertical and horizontal overlap of the canines prevents the posterior teeth from contacting during excursive mandibular movement. The group function is the amount of contact between the mandibular and

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maxillary teeth on the working side during lateral movement, where the simultaneous connection of some teeth acts as a group to distribute occlusal forces.[5]

Occlusion is significant in the mastication process. Occlusion abnormalities can interfere with efficiency in masticatory function and if in a more severe condition, can disrupt the temporomandibular joint. Research by Al-Hiyasat et al. (2004) states that the relationship between static occlusion and dynamic occlusion shows that canine protection is most prevalent in class II (for molar and incisor relationships) while the least associated with type I and class III.[4] In research by Al-Nimri et al. (2010) on the relationship between static and dynamic occlusion in dental students with an age range of 21-30 years stated that in the 0.5 mm position, the dynamic occlusion pattern is different from static occlusion, but not significant. At the 3 mm position, the active occlusion pattern was significantly related to the incisor teeth. According to the study of Tipton and Rinchuse (1991), cited from Al-Nimri et al. (2010), there is no relationship between static occlusion and dynamic occlusion.[6] This study confirms that changes in the occlusion scheme of canine protection and group function are found in the transition stage from adolescence to adulthood during the post-graduate period, which can be related to food habits, psychological factors, and stress.[7]

Some literature still shows differences in the relationship between static occlusion and dynamic occlusion. So the author is interested in researching the connection between static occlusion and dynamic occlusion in the natural teeth of students of the Faculty of Dentistry, University of North Sumatra.

MATERIALS AND METHODS

The type of research conducted in this study was descriptive-analytic using a cross-sectional research design, where the case sample was only observed once, and the variables were measured according to the state or status at the time of observation. This research was conducted through direct interviews and clinical examinations. The population in this study is a large number of subjects. It has specific criteria, such as students still actively studying in 2013-2018 at the Faculty of Dentistry, University of North Sumatra.

Sampling was carried out using a purposive sampling technique with a simple random sample by following the inclusion criteria; all students who are actively studying at FKG USU aged over 17-22 years with complete dental conditions and not in orthodontic treatment, patients who are willing to participate in research activities and as exclusion criteria; patients with caries or fillings and the presence of deciduous teeth. The number of samples was added by ± 10% of the specified sample. Therefore the number of samples used in this study was 100 people.

The researcher examined the patient's oral cavity by instructing the patient to wear a cheek retractor. The researcher made visual observations when the subject occluded the anterior teeth by measuring the overbite and overjet on the incisors. Then examined the molar teeth using a mouth glass. The researcher made a straight line on the labial surface of the maxillary and mandibular anterior teeth. The subject was instructed to move the mandible to the labial about 3 mm. The movement was seen from the straight line that had been made on the surface of the anterior teeth. The researcher positioned the articulating paper between the occlusal plateau of the canine and posterior teeth. Then the subject was instructed to perform the maximum bite. The operator held the articulating form while the subject performed the bite. The tooth surface area with a mark on the occlusal surface is tried again with a shim stock to see the contact. The method is the same as the articulating paper, but only the area with a mark. When the shim stock is pulled, there is resistance or tearing, which indicates that contact has occurred.

Patient data were obtained from the questionnaire and presented by calculating the percentage distribution. Then a significant test was carried out with chi-square to test the relationship between two categorical variables and measure the strength of the relationship between one variable and another categorical variable. Based on the chi-square test results, it can be determined which variables show a significant relationship ($p<0.05$).

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RESULTS

Based on this study obtained data, in static occlusion with anterior tooth relationships, 45 people (45%) class I, 21 people (21%) class II Division 1, 21 people (21%) class II Division 2, and 13 people (13%) class III. While in the static occlusion of posterior tooth relationships, there were 45 people (45%) in class I, 42 people (42%) in class II, and 13 people (13%) in class III. (**Table 1**)

Table 1. Distribution of Static Occlusion Based on the Relationship of Anterior and Posterior Teeth in Students of the Faculty of Dentistry, University of North Sumatra

Static Occlusion		Amount	
		N	%
Relation of Anterior	Class I	45	45
	Class II (Division 1)	21	21
	Class II (Division 2)	21	21
	Class III	13	13
Relation of Posterior	Class I	45	45
	Class II	42	42
	Class III	13	13

Based on the results of the dynamic occlusion examination conducted on 100 people, it was found that 59 people (59%) had a canine protection occlusion scheme, 35 people (35%) had a group function occlusion scheme, and 6 people (6%) had a mixed occlusion scheme between canine protection and group function. (**Table 2**).

Table 2. Distribution of Dynamic Occlusion Based on Occlusion Schemes in Students of the Faculty of Dentistry, University of North Sumatra

Dynamic Occlusion	Amount	
	N	%
Canine protection	59	59
Group function	35	35
Mixed Canine protection dan group function	6	6
Total	**100**	**100**

In **Table 3**, the results of the study of occlusion schemes based on anterior tooth relationships are from 59 people (59%) who have a canine protection occlusion scheme found 24 people (24%) class I, 15 people (15%) class II Division 1 and 17 people (17%) Division 2, 3 people (3%) class III.

Table 3. Distribution of Dynamic Occlusion Based on Occlusion Schemes in Students of the Faculty of Dentistry, University of North Sumatra

Static Occlusion		Dinamic Occlusion								p
		Canine protection		*Group function*		*Mixed canine protection* dan *group function*		Amount		
		n	%	n	%	n	%	n	%	
Relation of Anterior	Class I	24	24	20	20	1	1	45	45	0,011*
	Class II Division 1	15	15	4	4	2	2	21	21	
	Class II Division 2	17	17	3	3	1	1	21	21	
	Class III	3	3	8	8	2	2	13	13	
	Amount	**59**	**59**	**35**	**35**	**6**	**6**	**100**	**100**	
Relation of Posterior	Class I	24	24	20	20	1	1	45	45	0,003*
	Class II	32	32	7	7	3	3	42	42	
	Class III	3	3	8	8	2	2	13	13	
	Amount	**59**	**59**	**35**	**35**	**6**	**6**	**100**	**100**	

A total of 35 people group have a function occlusion scheme, 20 people (20%) were found in class I, 4 people (4%) in class II Division 1 and 3 people (3%) in class II Division 2, and 8 people (8%) in class III. Total 6 people (6%) who had a mixed occlusion scheme between canine protection and group function, 1 person (1%) was found in class I, 2 people (2%) in class II Division

1, 1 person (1%) in class II Division 2 and 2 people (2%) in class III. Based on statistical tests using the chi-square test, it was found that there was a significant relationship between static and dynamic occlusion based on anterior tooth relationships with a value of $p=0.011$ ($p<0.05$). In Table 3, the results of the study of occlusion schemes based on posterior tooth relationships are

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out of 59 people (59%) who have a canine protection occlusion scheme found 24 people (24%) class I, 32 people (32%) class II and 3 people (3%) class III. Total 35 people who had a group function occlusion scheme, 20 people (20%) were class I, seven people (7%) were class II, and 8 people (8%) were class III. In the mix between canine protection and group function from 6 people (6%), there is 1 person (1%) in class I, 3 (3%) people in class II, and 2 (2%) people in class III. Based on statistical tests using the chi-square test, it was found that there was a significant relationship between static and dynamic occlusion based on posterior tooth relationships with a value of $p=0.003$ ($p<0.05$).

DISCUSSION

Static occlusion can be identified based on Angle's classification in 1907 and subsequently by Andrews in 1972. This classification is based on the description of arch shape, tooth position, and tooth contact in the intercuspal position. The examination of static occlusion in this study was carried out visually to see the connection between the maxillary and mandibular teeth that occurs when the jaw is not moving.[4] In this study, static occlusion obtained the highest number of subjects was Class I relationships, as many as 45 people (45%) both anterior and posterior, and the least number in class III, namely 13 people (13%). The results of this study are the same as research conducted by Touzi et al. (2015), where the number of subjects obtained was more in the class I relationship at 72.86% and the least class III at 3.02%.[3] In Class II Division 1 and Division 2, the same results were obtained, namely 21 people (21%), and 42 people (42%) posterior class II while in the research of Al-Hiyasat et al. (2004), the prevalence of class II was 37% based on anterior tooth relationships (20% Division 1 and 17% Division 2), 29% based on class II posterior tooth relationships. In the study by Touzi et al. (2015), there were 24.12% of class II.[4] (Table 1)

Table 2 shows that most research subjects have a canine protection occlusion scheme of 59 people (59%). The results of this study are the same as research conducted by Al-Hiyasat et al. (2004) on 447 subjects showing a 57% canine protection percentage. Still, there are differences in results for group function with a percentage of 13%, while 17% have mixed canine protection and group function occlusion schemes. The difference in the results of this study is due to the much younger age group of the research subjects. The younger age in this study is the reason for the difference in research. It is to previous research, which states that occlusion schemes correlate with age.[4] In this study, the samples examined were 17-22 years. Therefore, the results correlate directly with existing research where canine protection occlusion schemes are more commonly found at a young age.

The theory of the canine protection occlusion scheme is based on the concept that the canines are the most suitable teeth to guide the movement of lateral excursion. Therefore, all teeth do not contact except the maxillary canines with the mandible on the working side during the lateral excursion. Some reasons why canine tooth contact is ideal for guiding mandibular movement are because the canine teeth have an excellent root-crown ratio to absorb occlusal pressure, and the roots of the canine teeth are longer. They have a wider root surface area than the other teeth, so the periodontal ligaments are more numerous and mechanoreceptors, which receive stimuli in the form of mechanical tension and pressure on the teeth. The palatal surface of the maxillary canine teeth is concave or concave, very suitable for guiding lateral movement.[8]

The study of Touzi et al. (2015) showed different results, and it was seen that the most occlusion scheme was 45.9% in a group function, while canine protection was 24.09%.[3] In the study of Aswaworit et al. (2011), where the majority of the population in their study had a group function occlusion scheme, this is the same as

epidemiological data from Byron's research (1964) (cited from Aswaworit et al. 2011), showing that adult Australian aborigines have a group function occlusion scheme. Weinberg (1964) (awarded from Aswaworit et al. 2011) found 81% of his study belonged to a group function occlusion scheme, of which only 5% were canine protection occlusion schemes.[9] The dissimilarity across studies is due to differences in population examination, culture and dietary intake received, and the influence of the materials used to evaluate contact.

The study conducted by Athiban (2014) on 239 subjects selected between the age group of 17-22 years showed that the majority of 92.3% and 88.37% had canine protection in the age group of 17 and 18 years while above the age of 19 years, there was an increase in the prevalence of group function occlusion, which was about 77.19%, 100%, 88.37% of individuals in the age group of 20, 21, 22 years. This study confirms that the change in occlusion scheme from canine protection to group function found in the transition stage from adolescence to adulthood can be related to dietary habits, psychological factors, and stress. This study found the group function occlusion scheme with less presentation. This result is in line with several existing studies which state that group function is found in various age groups but is most prevalent in older age groups. The group function is known to be more stimulating to the periodontium than other occlusion schemes because it balances the jaw arch as we age.[10]

This study's results were mixed between canine protection and group function of as many as six people (6%). It is the same as Al-Hiyasat's research which shows that subjects with mixed occlusion schemes between canine protection and group function are found less. In the results of statistical tests using the chi-square test, significant results were obtained between occlusion schemes based on anterior tooth relationships with $p = 0.011$ ($p < 0.05$). This study found 24 people (24%) in class I, who had the most canine protection occlusion scheme. From 35 people who had a group function occlusion scheme found, 20 people (20%) were class I, the results of this study were the same as those conducted by Al-Hiyasat et al. (2004) there was a canine protection occlusion scheme 114 (56%) class I, 60 (67%) class II Division 1, 62 (82%) class II Division 2, 17 (21%) class III. Group function occlusion scheme 25 (12%) class I, 10 (11%) class II Division 1, 6 (8%) class II Division 2, 16 (20%) class III while in mixed between canine protection and group function 45 (22%) class I, 7 (8) class II Division 1, 6 (8) class II Division 2, 18 (23%) class III.[4]

In table 3, the occlusion scheme based on the anterior tooth relationship is from 59 people (59%) who have a canine protection occlusion scheme found 24 people (24%) class I, 15 people (15%) class II Division 1, and 17 people (17%) Division 2, 3 people (3%) class III. Of the 35 people who had a group function occlusion scheme, 20 people (20%) were found in type I, four people (4%) in class II Division 1 and 3 people (3%) in class II Division 2, and 8 people (8%) in class III. Of the 6 people (6%) who had a mixed occlusion scheme between canine protection and group function, 1 person (1%) was found in class I, 2 people (2%) in class II Division 1, 1 person (1%) in class II Division 2 and 2 people (2%) in class III. In the results of statistical tests using the chi-square test, significant results were obtained between occlusion schemes based on anterior tooth relationships with $p = 0.011$ ($p < 0.05$). From the results of this study, who had the most canine protection occlusion scheme found 24 people (24%) class I, and from 35 people who had a group function occlusion scheme found, 20 people (20%) class I.

Several studies have shown that in healthy dentition, canine protection is an ideal relationship during extrinsic movement of the mandible for the dentition, muscles, and temporomandibular joint.[11] In adolescence, the shape of the canine teeth is generally still pointed and sharp, resulting in steepening and increasing the prevalence of canine protection occlusion schemes. In the canine protection occlusion scheme, the canine teeth guide the movement of the lower jaw.[12]

In Class II occlusion, the anterior teeth show a vertical overlap protrusion which causes the steepness of the anterior teeth to prevent the posterior teeth from making minimal lateral contact, so the most significant prevalence is canine protection. It can be seen in class II occlusion is most affected by partial excursion and shows a significant decrease in canine protection. Class II occlusion tends to be dominated by canine safety, while the prevalence of canine protection is low in Class III occlusion. In contrast, class III is less affected by the degree of excursion, as the posterior teeth play the most dominant role in controlling the occlusion during the partial expedition. The role of the posterior teeth will continue until the complete excursion. Class III occlusion tends to show minimal anterior tooth overlap, edge-to-edge relationship, or crossbite, reducing the influence of the anterior teeth on lateral occlusion. Therefore the greatest prevalence

of class III occlusion is group function and balanced occlusion.[12]

A good occlusion should allow the mandible to translate without occlusal resistance during functional movements, especially in the posterior segment, so that the efficiency of mastication on the working side is not lost, the axial distribution is more even and can avoid overloading the temporomandibular joint. In the field of prosthodontics, one of the goals of denture manufacturing is to restore function. Therefore, the prosthodontist requires a good understanding of occlusion to rehabilitate the occlusion and achieve dynamic function.[13]

Aswaworit et al. (2011) concluded that several factors influence the lateral occlusion scheme. The first factor is the lateral excursion distance; the occlusal morphology is complex, and the location and magnitude of tooth contacts can be influenced by the degree of excursion. There are two categories of occlusal contacts; partial (0.5-1.5mm) and full (2-3mm). The second factor is the individual's age; the functional teeth also increase as age increases, affecting tooth contact in static and dynamic positions. The third factor is the static occlusion relationship, and some studies mentioned that anterior teeth and arch size could affect the occlusion scheme relationship.[9,12]

The most fundamental difference between the two occlusion schemes is which teeth contact during lateral movement. Both occlusions have multiple posterior tooth contacts in the intercuspal position (centric occlusion), preventing posterior tooth contact on the non-working side during lateral movement and preventing posterior tooth contact when the anterior teeth come into contact with the protrusive movement. Several studies have examined the possible relationship between static occlusion and functional occlusion. Balance occlusion appears much more significant and appears to be more predominant in subjects with normal (ideal) static occlusion or Class I occlusion compared to Angle malocclusion. When jaw movements are examined from the frontal plane, subjects with normal occlusion tend to have more superficial and less crossed activities than subjects with malocclusion.[12,14]

The jaw relationship refers to the position of the mandible concerning the maxilla and can be said to be the relationship of the teeth between the maxilla and the mandible. The maximal intercuspal position is a tooth-to-tooth relationship that does not depend on how the jaw muscle or joint anatomy would position the mandible. The centric relation is the maxillary-mandibular relationship obtained when the individual cheats and swallows. The ideal occlusion relationship results in a harmonious condition between the jaw muscles. The position of the condyles concerning the disc and fossa and the maximum contact of the dentition. In normal occlusion, there is a reflex function of the neuromuscular system, which results in movement of the mandible, thus avoiding premature contact. It leads the mandible to the maximum intercuspation position with the condyles in the optimal position. It will result in hypertonic symptoms from the surrounding muscles or trauma to the TMJ. In pathological occlusion, there can be signs of trauma and damage. There is attrition on the occlusal surface due to overuse, bulge fracture, and tooth mobility. Therefore the dentist must know the occlusion and be able to analyze it.[14]

Obstacles in centric occlusion can sometimes be seen in cases with Class II Division 2 relationships and among patients with restorations on the anterior teeth, leading to limited or no movement when the posterior teeth come into contact, thus having a locking effect on the patient's jaw. It is not well tolerated and can manifest as pain and discomfort, restoration failure, and adverse effects on supporting structures, and can result in unwanted tooth movement.[15]

In normal dentition, mutually protected or canine-protected occlusion is the ideal relationship in the extrinsic movement of the mandible on the teeth, muscles, and temporomandibular joint. The canine can promote optimal stress distribution between the anterior and posterior teeth. Several studies with the same methodology as this study also showed signs of TMJ dysfunction conditions that are more commonly found in group function occlusion schemes that offer the incidence of various types of centric occlusion on the relationship of healthy teeth and with abnormalities of the odontoid-stomatognathic system. Proper selection and arrangement of teeth should also follow the ideal occlusion pattern according to the existing teeth' static occlusion and dynamic occlusion conditions.

CONCLUSION

Based on the results of this study, there is a significant relationship between static and dynamic occlusion based on anterior tooth relationships with a value of p = 0.011 (p < 0.05), and there is a substantial relationship between static and dynamic occlusion based on posterior tooth relationships with a value of p = 0.003 (p < 0.05). In this study, there are clinical implications. Namely: the results of this study can be used to help diagnose the patient's occlusion scheme before treatment

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Original Research

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Comparative Study of Micro-Leakage Between Glass Ionomer Cement Restoration Materials and Alkasites on Cavitas Class I (GV. Black)

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ABSTRACT. Microleakage is a gap that allow clinically undetectable passage of bacteria, fluids, chemical substances between the tooth and its restoration. Microleakage can occurs whether in a cavity restored with Glass Ionomer Cement or Alkasite. The objective of this study is to compare the microleakage between GIC and Alkasite after 1 day, 7 days and 30 days polymerization. This study used 30 specimens divided into six groups. Group A is restored with GIC and Group B restored with Alkasite. Group A1 and B1 restored and conditioned in incubator for 1 day, group A2 and B2 for 7 days, group A3 and B3 for 30 days. The specimens isolated using varnish nail except the restored area and immersed in methylene blue 1% for 1 day, then all specimens were washed and cut longitudinally. The results were observed using a stereomicroscope. The observation results analyzed using nonparametric test Kruskal-Walis test, showed there's no significant difference in each material with different conditioning times ($p > 0.05$). Post hoc test using Mann-Whitney likewise, showed no significant difference between GIC and Alkasite with similar conditioning time. Descriptive statistics showed that all the mean of microleakage in GIC is bigger than Alkasite and microleakage mean score in both materials conditioned for 30 days in incubator is smaller than conditioned for 1 day and 7 days. This study concluded that microleakage in Alkasite is smaller than Glass Ionomer Cement

KEYWORDS: Microleakage, Glass Ionomer Cement, Alkasite, Class I

INTRODUCTION

Glass Ionomer Cement (GIC) is a restoration material that has often been used since its introduction by Kent and Wilson in 1972.[1] Glass Ionomer Cement is a water-based and self-adhesive material where contains fluoroaluminosilicate glass and a matrix in the form of polymers or copolymers of carboxylic acids.[2] Glass Ionomer Cement has several such advantages are relatively easy to manipulate and most importantly its nature that is able to release fluorine ions.[1] GIC material obtaining its attachment to the tooth structure is by forming a chemical bond between carboxylic acid in the polymer and calcium in the tooth structure. [3]

Glass Ionomer Cement has disadvantages, one of which is that it has a high viscosity level so that it can cause micro-leakage, George's research (2018) shows the average value of GIC micro-leaks after restoration for 1 day is 3.00. [1] In addition to micro-leakage GIC also has disadvantages, namely its opaque appearance is considered less aesthetic so that Alkasite is introduced, this material has the ability to release fluorine and has a more aesthetic clinical appearance. [4,5]

Alkasite is a dental material consisting of a liquid containing dimetharcrylate and an initiator and powder containing glass filler, initiator and pigment. Alkasite is a self-curing material and light curing as another option. [6]Alkasite has the ability to release fluorine and calcium hydroxide ions which function as anti-cariogenics as well as prevent demineralization of tooth structures. [7] This material also contains filler which has the ability to minimize the occurrence of shrinkage during polymerization so as to reduce micro-leaks that occur, but research by Burgess (2015) shows that micro-leaks can also occur in alkasite with an

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average value of micro leakage in Alkasite after restoration for 1 day, which is 0.5. [4] Micro-leakage will be the entry point of oral fluids, ions and bacteria that cause post-restoration sensitivity, pulp inflammation and secondary caries. [8]

Several studies have been conducted to see the average comparison of micro-leakage values between GIC and alkasite after soaking for 1 day. Research by Samanta et al (2017) states that the average micro-leakage value in the V GIC class cavity is greater (1,280) than alkasite (0.283). [6] Research by George et al (2018) also states the same thing that the average micro-leakage value in class II GIC cavitas is greater (3.00) than Alkasite (0.10).

Based on the research above, it turns out that there is still a difference in the average micro-leakage value in the two restoration materials and no research has been found on the comparison of micro-leaks between GIC restoration and Alkasite restoration after 7 days and 30 days of polymerization. GIC bonding to enamel due to interaction with the mineral hydroxyapatite will increase within a few days and will continue for several months which issuspected to decrease the micro-leakage value. [9,10,11] Alkasite material contains PEG-400 DMA in its liquid which plays a role in increasing the binding strength of this material and research by Tran et al (2020) shows that alkasite can form hydroxyapatite and the percentage of *hydroxyapatite-forming* components increases over time, this is thought to reduce the value of micro-leakage. [4.12 13]

Therefore, researchers are interested in re-examining the comparison of micro-leakage of Glass Ionomer Cement and Alkasite restoration materials as restoration materials in class I cavitas of premolar teeth after 1 day, 7 days and 30 days of polymerization.

MATERIALS AND METHODS

This type of research is Experimental Laboratory with Post-Test Only Design method, to see a comparison of micro leakage images in Alkasite restoration and *Glass Ionomer Cement* on cavitas class I premolars using a stereomicroscope.

The specimens in this study are premolars of the upper jaw which have no caries, no restoration, the crown is intact and there is no fracture and has been cleaned with alcohol and then stored in *saline* solution. The cleaned teeth will then be prepared in the form of class I cavitation with details of a mesiodistal length of 4 mm, a buccal-palatal width of 2 mm and a depth of 4 mm.

The number of specimens used in this study amounted to 30 specimens which were divided into six groups, namely Group A1 (restoration with GIC which was carried out storage of specimens in humid conditions in the incubator for 1 day), Group B1 (*Alkasite* restoration carried out which was carried out storage of specimens in humid conditions in the incubator for 1 day), Group A2 (restoration with GIC which is carried out storage of specimens in humid conditions in the incubator for 7 days), Group B2 (*Alkasite* restoration which is carried out storage of specimens in humid conditions in the incubator for 7 days), Group A3 (restoration with GIC which is carried out storage of specimens in humid conditions in the incubator for 30 days), Group B3 (restoration *Alkasite* storage is carried out in a damp in the incubator for 30 days).

Teeth that do not have caries, fractures or restorations will be prepared with a mesiodistal width of 4 mm, a buccal-palatal width of 2 mm and a depth of 4 mm. Specimens that will be restored with GIC will be prepared which begins with using spherical bur to obtain the depth of cavitation then widened cavities with using cylindrical bur. Specimens that will be restored with *Alkasite* will be prepared which begins with using spherical bur to obtain the depth of the cavity then the cavitation is widened using cylindrical bur and makes a bevel of the edge of the restoration with bur *fissure*.

Cavity depth was measured using periodontal probs and ensured the smoothness of the prepared walls with sonde *half moon*. The teeth are then divided into 6 groups with 5 teeth in each group.

Prepared teeth that are included in group A1, group A2 and group A3 will be prepared which begins with applying *dentin conditioner,* with a *microapplicator* for 20 seconds. After that, it is rinsed using water sprayed with a *syringe* and then dried using *a cotton moplet* until the cavity is moist.

After that, prepare GIC with a ratio of powder and liquid is 1: 1, namely one measuring spoonful for powder and one drop for the liquid on the *paper pad* above the *mixing slab.* Then the powder is divided into two equal parts with a plastic spatula. Then the powder is mixed and stirred with the liquid for 10 seconds with a pressing and folding motion then put the remaining powder into the mortar and mix the whole material and collected within 20 seconds, so that the total stirring is 30 seconds until the material is homogeneous, then collect it at the end of the spatula and take the

material with a *plastic filling instrument* then put into the cavity. GIC hardening will occur for 7 minutes from the start of stirring the material and then applied with *cocoa butter* to isolate the material.

Prepared teeth that are included in group B1, group B2 and group B3 will be restored with *Alkasite* which begins with applying adhesive materials by rubbing using a *microapplicator* then thinning with a *chip blower* and a *light curing* procedure for 20 seconds.

After the *light curing* procedure is complete, proceed with preparing the powder and liquid in a ratio of 1: 1, namely one measuring spoonful for the powder and one drop for the liquid on the *paper pad* above the *mixing slab*. The powder and liquid are mixed with a small portion of the powder to the liquid and mixed and stirred the whole powder slowly within 45 to 60 seconds and then put into the cavity with a *plastic filling instrument* and will be hardened in 4 minutes.

After each specimen group was restored with GIC and *Alkasite*, the six specimen groups were placed in plastic vials on cotton wool that had been moistened with aquabides solution and stored in an incubator for 1 day, 7 days and 30 days at 37° C. Moisture can be maintained by maintaining the state of cotton wool to keep it wet. [14] After the storage of the specimen in the incubator is completed, the specimen is coated with two layers of *varnish nail* on the tooth surface except on the 2 mm tooth surface from the restoration edge. After that, the six groups of specimens will be put into a plastic vial containing 1% *methylene blue* for 1 day.

After all six groups soaked for 1 day the teeth were rinsed with aquabides and dried with *a blower chip*. Then, the tooth is split into two parts longitudinally from the buccal - palatal direction using a micromotor and *carborandum disc*. After that, each specimen was observed for micro-leaks that occurred in class I cavitation using a stereomicroscope.

After the specimens were immersed in a plastic vial containing *methylene blue* solution for 1 day, the teeth in these six groups were washed thoroughly under running water and then cut in half, then observed using a stereomicroscope with a magnification of 20x.

Micro-leakage measurements can be performed by providing the following critiqued score:[15]

1. Score 0 : No dye penetration
2. Score 1 : Penetration up to half of cavity
3. Score 2 :Penetration exceeds. Half the depth of cavity
4. Score 3 : Penetration reach walls of the pulp

The results of micro-leakage observations between *Glass Ionomer Cement* and *Alkasite* in class I cavitation viewed with stereomicroscopes were analyzed withthe Kruskal-Wallis non-parametric test followed by *post hoc* tests with Mann-Whitney.

RESULTS

The results showed that the specimen with the highest micro-leakage score occurred in group A1 with a score of 3. Group A2 had a score of 1 on five specimens. In group A3 there was only one specimen that did not have a micro-leakage and the other four specimens had a microleakage with a score of 1. In group B1 had a score of 1 on five specimens. In group B2 there was only one specimen that did not have a micro-leak and the other four specimens had a micro-leak with a score of 1. Group B3 had 2 specimens that did not have micro-leaks and only had micro-leaks with a score of 1 in the other three specimens.

The results of statistical tests with the Kruskal-Wallis test conducted to determine the comparison of micro-leaks between treatments with durations of 1 day, 7 days and 30 days on each material that the *p* value obtained is greater than 0.05 can be concluded that there is no significant difference.

The average micro-leak score in GIC and *Alkasite* that was stored in the incubator for 30 days was smaller than the average value of the micro-leakage score that occurred in each of the restored materials for 7 days and 1 day.

The results of the *post hoc* test with the Mann-Whitney test conducted to determine the comparison of micro-leaks between each group also showed that the entire *p* value > 0.05. It can be concluded that there is no significant difference in the micro-leakage that occurs between *Glass Ionomer Cement* and *Alkasite* with storage time in the same incubator

DISCUSSION

Micro-leakage is a gap that allows the entry of bacteria, liquids, molecules and ions between the walls of the cavity and clinically invisible restoration material which is considered to be the main factor

affecting the length the age of a restoration. [16] The results of micro-leak observations using a stereomicroscope show that micro-leaks can still occur in *Glass Ionomer Cement* and *Alkasite*. Based on the results of statistical analysis, it was found that the average value of micro-leakage in each specimen treated with storage in the incubator for 30 days was smaller than specimens with storage in the incubator for 1 day and 7 days, but the results of the Kruskal-Wallis nonparametric test showed that the difference that occurred was not significant.

The average value of micro-leakage in GIC specimens with storage in the incubator for 1 day is most likely due to the maturation process that is still ongoing even though the material has been *clinically set up*. Nicholson (2018) said that the state of GIC after maturation for 1 day is still the same as it was 1 hour after restoration, so this is suspected to be the cause of micro-leaks that occur in GIC with the highest storage duration of 1 day. In the maturation process, there is an ion exchange between GIC and the tooth structure which will continue to increase so that over time it produces a bond between GIC and a better tooth structure so that the average value of micro-leakage in specimens with storage for 7 days and 30 days is smaller. However, the GIC maturation process is a slow-running process so it is suspected to be the thing that causes the difference in micro-leakage in GIC material between storage durations of 1 day, 7 days and 30 days is insignificant. [10]

Alkasites also undergo ion exchange with tooth structures due to contains calcium *barium fluorosilicate* and *calcium fluoro silicate glass*. Todd (2016) said that the ion exchange that occurs produces a layer of minerals calcium *fluoride* and *calcium phosphate*, this mineral layer will continue to increase over time so that *alkasite* bonds with tooth structure will also become better. This is suspected to be the cause of the average value of micro-leakage in *Alkasite* specimens with a storage duration in the incubator for 30 days smaller than specimens with a storage duration in the incubator for 7 days and 1 day. However, the difference in micro-leakage that occurs is not significant, this is suspected to be because the alkasite ion exchange process and tooth structure run slowly. [4]

The entire average value of micro-leakage in *Glass Ionomer Cement* with a treatment duration of 1 day, 7 days and 30 days is greater than *alkasite* with a treatment duration of 1 day, 7 days and 30 days according to previous research conducted by George et al (2018), this is suspected to be due to the difference in viscosity between the two materials. In this study, Fuji IX was used, which is a GIC material that has adequate physical properties due to the influence of the high molecular weight of the polymer, but this high molecular weight produces a high viscosity, while *Alkasite* has a lower viscosity because it contains monomers with low viscosity, namely *Urethane dimethacrylate* (UDMA), DCP and *Aromatic aliphatic* -UDMA. Materials with high viscosity will be difficult to wet the entire surface of the teeth dengan baik sehingga can result in micro-leakage, on the contrary, materials with lower viscosity will be easier to wet the tooth surface so as to reduce the formation of micro-leakage between the restoration material and the tooth structure. [4.17]

In this study, specimens that were restored with GIC were given *dentin conditioner* first in the form of 10% polyacrylic acid for 20 seconds. The provision of dentin conditioner aims to lift the *smear layer* in the form of debris produced from enamel instrumentation, dentin and cementum or is dirt that can block the interaction of the material with the tooth structure, besides that *dentin conditioner* also plays a role in demineralizing part of the tooth structure and forming microporosity to obtain bonds through *mechanical mechanisms interlocking* and improve the chemical interaction of *polyalcenoic acid* with hydroxyapatite. *Dentin* conditioner can increase GIC bonding to tooth structure for the better, this is as mentioned by Wardani et al (2018) that the strength of GIC bonds given *dentin conditioner* is 7.7 MPa and in GIC materials that are not given *dentin conditioner* is 3.3 MPa. [2,18,19]

In specimens that were restored with *Alkasite*, adhesive materials were given first with a *one-step self-etch* technique and the adhesive material used in this study wasTetric N Bond Universal. Adhesive materials this contain. *methylaryloyxydyl dihydrogen phosphate* (MDP) so that it has the ability to perform mild demineralization of enamel and allows the formation of *a hybrid layer* on dentin then the monomer will enter into the microporosity formed and after polymerization a good bond will be formed between enamel and dentin with adhesive materials and *alkasites*, in addition MDP also has the ability to chemically trigger chemical bonding of restoration materials with calcium in enamel and dentin, as well as the ability to prevent degradation between the surfaces of adhesive materials. The bond strength produced after the administration of this adhesive material is 17 MPa so it is greater than the material given *dentin conditioner* this causes the average value of micro leakage that occurs in GIC to be greater than that of *Alkasite*. [8.21]

Although the entire average value of micro-leakage in GIC is greater than that of *Alkasite* in both

storage with a duration of 1 day 7 days and 30 days, the difference that occurs is not significant, this is thought to be due to the almost the same properties of these two materials in terms of continuously undergoing ion exchange with the tooth structure so that the bond strength produced by both continues to increase. In addition, this is suspected to be due to the small shrinkage that occurs in both materials, the acid-base reaction in the GIC setting process produces a small shrinkage. The setting reaction that occurs in *Alkasite* occurs with a reaction between *free radicals* that react with monomers so as to form many polymer bonds. Polymerization of *Alkasite* can cause shrinkage, but shrinkage due to this reaction is prevented by *isofillers* that act like springs that resist shrinkage during polymerization. These two materials both have a small shrinkage so it is suspected to be the thing that causes the difference in micro-leaks that occur is insignificant. [4,10,22,23]

CONCLUSION

Based on the results of research on class I cavitation visually using a stereomicroscope, *Alkasite* material experienced a smaller micro-leakage than *Glass Ionomer Cement* but statistical analysis showed no significant differences and micro-leakage in each material with a treatment duration of 30 days smaller than with a treatment duration of 1 day and 7 days.

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Literature Review

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Bacterial profile in children with early childhood caries

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ABSTRACT. Early Childhood Caries (ECC) is the most common chronic infectious disease of childhood in the world, caused by the interaction of the host (oral cavity), substrate, microbes, and time. Bacteria as microbes that play a role in the formation of ECC have been widely studied. This study aims to determine the bacterial profile in children with ECC and to compare the oral microbial profiles in children with ECC and caries-free children (CF). **Methods:** This study uses a scoping review method. Article searches were performed on the PubMed, Science Direct, Cochrane, and Google Scholar databases, using the keyword and Boolean operator "(early childhood caries) AND (profile bacterial OR bacterial) AND (children)." The initial search obtained 1356 articles. All articles were then selected according to the inclusion and exclusion criteria using the PRISMA procedure to receive 14 articles. **Results:** Analysis of 14 articles showed that 11 articles reported that *Streptococcus mutans* had high levels of ECC in children. Other bacteria with lesser numbers were *Provetella*, *Veillonella*, *S. wiggsae*, *S. sobrinus*, *Lactobacillus*, *Leptrotrichia shahii*, and *Leptrotrichia IK04*. Neisseria, Streptococcus mitis, Streptococcus salivarius, and Leptotrichia buccalis were found in CF children. Other bacteria such as *Hemophilus paraphrohaemolyticus* HK411, *Neisseria* sicca 4320, *Neisseria sp. oral* clones AP132, Actinobacillus pleuro-pneumoniae MCCM 00189, and *Streptococcus sp.* ASCE06 oral clone was found in CF children but not in ECC children, while *Lactobacillus sp* C56 was found in half of ECC children and not in CF children. **Conclusion**: *Streptococcus mutans* levels in children with ECC are high, so they are assumed to be the main bacteria causing ECC. The bacterial profiles of ECC and CF children are different in species diversity.

KEYWORDS: Early Childhood Caries, Bacterial, Children

INTRODUCTION

Caries is a common disease for adults and children.[1] According to the American Academy of Pediatric Dentistry, children under six years of age with one or more carious lesions are categorized as individuals with Early Childhood Caries (ECC).[2] ECC affects 23 % of preschool children (<6 years) in the United States and can be observed in children under 12 months.[3] The prevalence of ECC in some countries in the Middle East is 12%-27% in children aged 2-3 years, and 27%-48% in children aged 4-6 years.[4] In Indonesia, the 2018 Riskesdas show the prevalence of ECC in children aged 3-4 years old is 81.1% and 92.6% in the age of 5-9 years.[5] These percentages show that ECC is an aggressive form of common caries in children.

In general, ECC is caused by four main factors: the host (oral cavity), substrate, microbes, and time. Bacteria that are part of microbes are one of the infectious factors in caries. *Streptococcus mutans* and *Lactobacillus spp*. have a major role in caries formation. These bacteria and Saliva will form a biofilm layer and get nutrients through a substrate in the form of carbohydrates which will be fermented into acid. The acid can cause demineralization of the teeth.[3,6,7]

Streptococcus mutans is considered to be the main cariogenic bacteria that is strongly associated with ECC. These bacteria are associated with a decrease in the oral cavity's pH under high sucrose concentrations.[8] Other microbes besides *Streptococcus mutans* were also found to be associated with ECC, such as *Lactobacillus spp., Candida ssp., Bifidobacteria spp., Actinomyces ssp.,* and *Veillonella ssp*.[2] Interestingly, several studies reported low levels of Streptococcus mutans in children with ECC. It suggests that other species may be responsible for the development of caries.[8–14]

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ECC spreads faster as a result of microbial development in children's teeth.[2] Children with ECC must get treatment immediately to prevent the expansion of the cavity, which requires expensive treatment costs.[3] In-depth knowledge of the bacteria that cause ECC is needed to avoid and treat ECC. This study examines the bacterial profile in children with ECC and the differences in the bacterial shape between ECC and CF (Caries Free).

RESULTS

A literature search using keywords in the four databases obtained 1,356 articles. Based on multiple data, the first screening received 1328 articles, and 28 articles were excluded. The second screening based on the topic title and abstract obtained 38 articles, and 1290 were excluded. The third screening or examination of article eligibility was reading the suitability of the full-text content of the article with the inclusion criteria, obtained 14 articles. The results of the analysis of the fourteen articles are shown in Table 1. One article is a Randon Controlled Trial (RCT) study, and 13 are Cohort Studies.

Table 1. Inhibitory Zone Diameter Category[8]

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	ECC
1	India	Kalpana *et al.* (2020)[15]	RCT	Total sample: 30 Children, Ages: 3-6 years Caries: n= 10 CF: n =10 RC: n=10	**According to Phylum**: 1. *Firmicutes* (46.56%) 2. *Proteobacteria* (CF-36.99%) 3. *Actinobacteria* (pada CF<(2.6%) SC) 4. *Fusobacteria* CF < ECC 5. *Bacteroidetes* **Two-way ANOVA**: CF Samples: ***Over-represented***: *Gemellaceae, Flavobacteriaceae* ***Under-represented***: *Gracilibacteria*_(GN02), *Absconditabacteria*_(SR1), *Campylobacteraceae, Prevotellaceae* ***Potential Biomarkers (LeFse analysis)***: CF: Dominated by *Negativicutes* followed by 4 different *Gracilibacteria*_GN02, 3 different *Actinomyces* sp., *Veillonella* sp._HMT_780, *Rothia mucilaginosa* and *Haemophilus parainfuenzae*	**According to Phylum**: 1. *Firmicutes* (3.25%) 2. *Proteobacteria* (32.98%) 3. *Actinobacteria* (pada CF< (2.6%) SC) 4. *Fusobacteria* ECC>CF 5. *Bacteroidetes* **Two-way ANOVA**: SC samples: ***Over***: *Micrococcaceae, Selenomonadaceae, Bifidobacteriaceae, Lactobacillales* ***Under***:*Abscondita bacteria*_(SR1)_[F1], *Fusobacteriaceae, Porphyromonadaceae, Campylobacteraceae* ***Potential Biomarkers (LeFse Analysis):*** ECC: 17 differentially abundant taxa that include *Bifidobacteriales, Pseudomonadales, Fusobacterium nucleatum subsp. vincentii, Lactobacillus salivarius, Enterococcaceae, Micrococcaceae, Carnobacteriaceae, Dialister invisus, Atopobium, Prevotella sp_HMT_313, Granulicatella adiacens, Granulicatella elegans, Megasphaera micronuciformis, Veillonellaceae, Prevotella* and *Neisseria subfava*

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	ECC
2	Ireland	Hurley *et al.* (2019)[16]	Cohort Study	Total Sample:138 Age <6 years; ECC: 68 Children CF: 70 Children	Saliva Sample (CF>CAS): 1. *Leptotrichia buccalis* 2. *Capnocytphaga gingivalis* 3. *Tannerella forsytia*	Dominant Phylum in: Caries Active Dentinal Microbiota 1. *Firmicutes* (33.5%) 2. *Bacteroidetes* (23.2%) 3. *Neisseria* (10.3%) *Prevotella* (10%) Caries Active Saliva Dominated by: 1. *Proteobacteria* (38.2%) 2. *Bacteroidetes* (27.8%) 3. *Neisseria* (16.3%) 4. *Porphyromonas* (9.5%). On Caries Lesions: High: *Prevotellaceae, Veillonellaceae, Bifidobacteriacae,* and *Streptococcaceae* Low: *Corynebacteriaceae, Carnobacteriaceae, Aerococcaceae,* and *Micrococcaceae* It is characterized by a high relative abundance of *Streptococcus mutans*, *Prevotella* spp., *Bifidobacterium,* and *Scardovia* spp.
3	Canada	de Jesus *et al.* (2020)[19]	Cohort Study	Sample: ECC: 40 Children CF: 40 Children Age: <72 Years	**LefSe Supragingival Plaque Bacterial Community Analyses*:*** **CF:** *Actinomyces, Neisseria, Corynebacterium* **Higher relative abundant of** *Actinomyces, Leptotrichia, Corynebacterium durum* and *Lautropia mirabilis*	**LEfSe Supragingival Plaque Bacterial Community Analyses:** **ECC:** *Veillonella, Neisseria,* and *Streptococcus* **Higher relative abundance of *Veillonella sp. oral* taxon 780*, V. dispar,* and *Streptococcus mutans*.** ***Neisseria*: male>female**
					16s rRNA: *Proteobacteria* CF>ECC **High abundance**: *Rothia, Actinobacillus,* and *Defluviitaleaceae* **Relatively increased abundant of 21 species:**	**16s rRNA**: *Firmicutes* ECC>CF (17) ***High abundance***: *Lactobacillus, Mogibacterium, Dialister, Veillonellaceae uncultured, Centipeda, Filifactor,* and *Anaeroglobus* **Relatively increased**

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	ECC
4	UK (United Kingdom)	Wang *et al.* (2017)[17]	Cohort Study	Sample: 21 CF and 20 Caries Children Age: 3-5 Years	*Neisseria* spp. *(Neisseria cinerea* ATCC 14685 and *Neisseria polysaccharea,* etc*,), Haemophilus* spp. *(Haemophilus parahaemolyticus* and *Haemophilus paraphrohaemolyticus* , etc*,), Streptococcus* spp.*, Rothia spp.,* and *Aggregatibacter aphrophilus,*	**abundant of 38 species:** *Veillonella* spp. (*Veillonella atypical* and *Veillonella denticariosi*, etc.), *Streptococcus* spp. (*Streptococcus mutans* and *Streptococcus sobrinus*, etc.), P*revotella* spp. (*Prevotella histicola, Prevotella multisaccharivorax*, and *Prevotella nigrescens*, etc.), *Lactobacillus* spp. (*Lactobacillus fermentum, Lactobacillus gasseri*, and *Lactobacillus mucosae*, etc.), *Selenomonas* spp., *Mogibacterium* spp., *Actinomyces viscosus*, and *Dialister pneumosintes*, etc. **Taxa related to ECC**: *Lactobacillus* spp., *Prevotella* spp., *Streptococcus* spp., and *Veillonella* spp., etc.,
					Found: *H. paraphrohaemolyticus* HK411, *Neisseria sicca* 4320, *Neisseria sp. oral* clone AP132, *Actinobacillus pleuropneumoniae* MCCM 00189, and *Streptococcus sp. oral clone* ASCE06	**Not Found**
					Lactobacillus sp. C56: **-**	*Lactobacillus* sp. C56: 10 Children
					Streptococcus mutans was found in 14.3% of CF children	*Streptococcus mutans* was found in 80% of ECC Children
5	United States	Han *et al.* (2021)[18]	Cohort Study	Sample: 136 Children Age: 3-6 Years	**16s rRNA**: Five dominant Phylum: 1. *Proteobacteria* (39.2%) 2. *Firmicutes* (24.7%) 3. *Bacteroidetes* (22.1%) 4. *Fusobacteria* (8.03%) 5. *Actinobacteria* (4.83%), There were 33 different Genus between the two groups	**16s rRNA**: Five dominant Phylum: 1. *Proteobacteria* (36.9%) 2. *Firmicutes* (25.3%) 3. *Bacteroidetes* (21.6%) 4. *Fusobacteria* (9.85%) 5. *Actinobacteria* (4.42%). There were 15 dominant genera and three Genera with the highest abundances: *Neisseria*, *Streptococcus,* and *Prevotella*

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	**ECC**
6	Melbourne (Australia)	Dashper *et al.* (2019)[20]	Cohort Study (VicGen) 16s rRNA	Sample: 134 Children Age: 1.9±0.8, 7.7±1.3, 13.2±1.2, 19.7±2.0, 39.0±3.2, 48.6±1.6 and 60±1.8 months	**Decrease as caries progression:** *Leptotricia* and *Actinobaculum* 12B759	**Increase as caries progress:** *S. mutans, S. sobrinus* and *V. parvula*
					Child's Saliva (1.9 month): 1. *Streptococcus mitis* group (100%) 2. *Gamella haemolysans* (100%) 3. *Streptococcus salivarius* group (92%) 4. *Campylobacter consisus* (92%) 5. *Rothia mucilaginosa* (93%) 6. *Staphylococcus caprae* (93%) 7. *Haemophilus parainfuenzae* (92%)	At 48 months-of-age *Leptotrichia shahii, Scardovia wiggsiae,* and *Leptotrichia* IK040 were also associated with the disease.
					7.7 months of age (14 species) Six of seven species are the same, but only *Staphylococcus caprae* decreasing below 90% prevalence.	Twenty-four children were found with *S. mutans* 1. **1,9 months:** *S. mutans* detected 2. **7.7 months:** decreased to 17% 3. **13.2 months:** increase to 25% 4. **19,7 months: i**ncrease to 42% 5. **39 months:** increase to 58%. *6.* **48.6 months:** decreased to 51%
					13.2 months of age increased to 28 species **19.7 months-of-age** 32 species were found and stable up to **48.6 months**	
					4 years-of-age 70% lead to 2 OTUs, namely *Streptococcus mitis* group and *S. salivarius group. S. mitis* is the group with the most OTUs in Saliva.	
					39 months-of-age *Fusobacterium periodonticum, Stomatobaculum longum* and *Bergeyella* 602D02 associated with health.	
					48.6 months-of-age *Prevotella shahii, Prevotella pallens, Stomatobaculum longum, Porphyromonas*	

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	**ECC**
					CW034 and *Capnocytophaga* AM20030 associated with health.	
					In nine CF children, *S. mutans* was not found and remained healthy	
7	Australia	Gussy *et al.* (2020)[21]	VicGen Prospective Cohort Study	Sample: 467 Children Age: 2-4 weeks of age and follow up until the age of 5 years		**Children with a bottle at bedtime have an increase in** *Streptococcus mutans.* **ECC children increased in** *S. mutans, S. sobrinus, Veillonella parvula, Leptotrichia shahi, Scardovia wiggsiae,* and *Leptotrichia* IK040.
8	USA	H. Colombo *et al* (2017)[22]	Cohort Study	Sample: 136 Children Age: 36-60 months CF: 47 Children ECC: 40 Children S-ECC: 49 Children	There is an increase of *S. salivarius* in CF children. There was no significant difference between *S. mitis, S. oralis, A. naeslundii*, and *Lactobacillus spp.* in both groups.	There was an increase of *S. mutans, S. sobrinus, Bifidobacterium spp.,* and *S. wiggsiae* with caries severity. (ECC>CF)
9	Canada	Lei Xu *et al* (2018)[23]	Cohort Study	Samples: Of the 60 children, 23 survived and were followed up one year later. H-H: 11 children H-C: 12 children Age: average 47.5 months	**Both are dominated by:**	
					Dominated by 6 Phylum: 1. *Firmicutes* (29,7%) 2. *Bacteroidetes* (27,3%) 3. *Proteobacteria* (27,1%) 4. *Actinobacteria* (9.4%) 5. *Fusobacteria* (3.6%) 6. *Candidate* divisi TM7 (2.5%)	**Dominated by 11 Genus:** 1. *Prevotella* (21.9%) 2. *Neisseria* (20.0%) 3. *Streptococcus* (10.5%) 4. *Rothia* (7.5%) 5. *Haemophilus* (5.2%) 6. *Porphyromonas* (3.2%) 7. *Leptotrichia* (2.0%) 8. *Actinomyces* (1.7%) 9. F*usobacterium* (1.5%) 10. *Alloprevotella* (1.4%)
					T0: *Rothia* and Shuttleworthia H-H > H-C	
					T1 (6 months later): H-H-T1: *Bergeyella, Capnocytophaga, Haemophilus, Neisseria, Porphyromonas,* and *Streptococcus*	**T1 (6 months later):** H-C-T1: *Atopobium, Megasphaera, Prevotella, Selenomonas,* and *Veillonella*
					T2 (12 months later): H-H: *Actinobacillus, Filifactor,* and *Peptococcus*	**T2 (12 months later):** H-C: *Dolosigranulum*

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	**ECC**
					T3 (18 months later): H-H: *Eikenella*	**T3 (18 months later):** H-C: *Atopobium, Megasphaera,* and *Veillonella*
					T4 (24 months later), there is a difference in the abundance of H-H: *Actinobacillus, Bergeyella, Fretibacterium, Haemophilus, Mycoplasma,* and *Propionibacterium*	**T4 (24 months later), there is a difference in the abundance of** H-C: *Alloprevotella, Atopobium, Lautropia, Megasphaera, Selenomonas,* and *Veillonella*
10	China	Wang *et al* (2019)[24]	Cohort Study	Sample: 19 children divided into two groups (i) "stay healthy" children (ii) "caries-onset" children Age: 3-5 years old	**Both are dominated by:**	
					At the phylum level: *Proteobacteria, Firmicutes, Bacteroidetes,* and *Actinobacteria* were the most common taxa in both groups.	**At the genus level**: *Neisseria, Prevotella, Rothia, Streptococcus, Veillonel*la, and *Haemopholis* were the most common genus in both groups
					At the species level: *Nesseria mucosa, Rothia mucilaginos*a, and *Prevotella melaninogenica* were the most common in both.	
					Nitrospirae and *Erysipelotrichaceae bacterium 5_2_54* FAA: CF> ECC CF: an increase of *Neisseria lactamica* or *Streptococcus australis*	Prevotella: ECC > CF ECC: 20 increased bacterial counts, including *Streptococcus mutans* and *Prevotella spp.*
11	Australia	Kennedy *et al.* (2019)[25]	Cohort Study	Sample: 93 children followed up at the age of 6,12,24 months. You are seeing normal bacteria.	Multiple Phylum (6 months - 24 months) *Firmicutes, Proteobacteria, Epsilonbacteraoeta,* and *Fusobacteria* At six months, phylum Firmicutes dominate and decrease at the ages of 12 and 24.	
					With age, *Bacteroidetes* increase, *Prophyromona*s also increase, and *Neisseriaceae.*	
12	Thailand	Ledder *et al* (2018)[26]	Cohort Study	Sample: Children aged 31-85 months		ECC >CF: Gram-negative anaerobes and *streptococcus, Lactobacilli*
13	Colombia	Gamboa *et al*	Cohort Study	Sample: 23 Children		**ICDAS score 3 (baseline;3 months;6**

No	Region	Authors (Year)	Study Design	Sample Characteristics	Result	
					Caries Free (CF)	ECC
		(2018)[27]		Age: 3- 7 years seen at baseline age, 3 months, and 6 months after		**months)**: *S. mutans* 91% (10/11), 27% (3/11) and 9% (1/11) **ICDAS score 6 (baseline;3 months; 6 months)**: *S. mutans* 100% (12/12), 100% (12/12) and 75% (9/12) Overall *S. mutans* 95.7% (22/23), 65.2% (15/23) and 43.5% (10/23)
14	China	Zhang *et al* (2020)[28]	Cohort Study	Sample: 354 Children (3-5 years) followed up in the next three months		*P. denticola* and *S. mutan*: ECC>CF The rest have no significant difference (Saliva)

Based on the above table of 14 articles discussing the bacterial profile in children with ECC, 11 articles (78.6%) reported high levels of *Streptococcus mutans*, followed by *Prevotella spp*. in 7 articles (50%) *Veillonella spp*., in 7 articles (50%) *Streptococcus sobrinus* and *Scardovia wiggsae* in 4 articles (28.57%), *Lactobacillus spp.* in 3 articles (25%), and *Leptrotrichia shahii* and *Leptrotrichia* IK040 in 2 articles (14.3%), while high levels of bacteria in CF children such as *Neisseria* were found in 5 articles (35.7%).

Streptococcus mitis in 5 articles (35.7%), *Streptococcus salivarius* in 2 pieces (7.14%), and *Leptotrichia buccalis* in 1 article (7.14%). Other bacteria such as *Hemophilus paraphrohaemolyticus* HK411, *Neisseria sicca* 4320, *Neisseria sp. oral clone* AP132, *Actinobacillus pleuropneumoniae* MCCM 00189, and *Streptococcus sp.* ASCE06 *oral clone* was found in CF children but not in ECC children, while *Lactobacillus sp* C56 was found in half of ECC children and not in CF children.

DISCUSSION

Based on 14 studies published as articles, it was found that the bacteria in children with ECC and CF is very diverse. Phylum *Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria,* and *Actinobacteria* appeared to dominate in the two groups of children.[15–18,23,24] Phylum *Firmicutes* had a higher percentage of children with ECC but had less diversity than CF children.[15–18]

Phylum *Firmicutes* is one of the most powerful taxonomies because it includes many bacteria with diverse characteristics (phylogenetic, phenotypic, chemotaxonomic, and pathogenic).[29] Phylum *Firmicutes* was dominated first by the Order *Lactobacillales* of the genus *Streptococcus (Streptococcus mutans* and *Streptococcus sobrinus)* and *Lactobacillus salivarius (Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus gasseri,* and *Lactobacillus mucosae)*.[15,17,19–22,24,26] Both are dominated by the Order *Veillonellales* consisting of the family *Veillonellaceae* with the genus *Anaeroglobus, Dialister (Dialister incisivus* and *Dialister pneumosintes), Megasphaera (Megasphaera microuciformis)* , and *Veillonella (Veillonella atypical, Veillonella denticariosi, Veillonella dispar, Veillonella sp. oral taxon 780,* and *Veillonella parvula)*.[15–17,19,21,23]

Phylum *Bacteriodetes* is a phylum with high diversity at each level, so it is called the "genomes of strains". Members of the *Bacteriodetes* are also very adaptable to rapidly changing living environments.[30] Observations of ECC children evidence this for 24 months that found high levels of the genera *Alloprevotella* and *Prevotella (Prevotella sp_HMT_313, Prevotella histicola, Prevotella multisaccharivorax,* and *Prevotella nigrescens)* both of which belong to the family *Prevotellaceae* and Phylum *Bacteriodetes*.[15–17,23,24]

Phylum *Fusobacteria* is a gram-negative anaerobic bacteria that generally inhabit the oral cavity.[31] This Phylum was found to have higher levels in children with ECC than in children with CF.[15,18] The species of *Fusobacteria* found was *Fusobacterium nucleatum subsp. Vincentii,* and there was an increase in *Leptotrichia shahii* and *Leptotrichia IK040.* This species is said to be associated with caries formation.[15,20,21] In children with CF, there is an increase of *Leptotrichia buccalis* species.[16] Phylum *Actinobacteria* is one of the oldest bacterial phyla. Many genera in this Phylum

cause various infections in humans.[32] In the phylum *Actinobacteria,* there is an increase in the Order *Bifidobacteriales* of the genus *Bifidobacterium, Scardovia wiggsiae,* and *Acrinomyces viscosus* in children with ECC.[15,16,17,22,20]

Phylum *Proteobacteria* is a gram-negative bacterial with lipopolysaccharide on its outer membrane. This Phylum is the largest in the bacterial domain and consists of 6 classes.[32] This Phylum was found to be high in CF children compared to ECC children.[17,18,25] The Phylum *Proteobacteria* found in high levels came from the genus *Neisseria (Neisseria cinerea ATCC 1468, Neisseria polysaccharea, Neisseria sicca 4320, Neisseria sp. oral clone AP132,* and *Neisseria lactamica).*[17,24] *Neisseria* has higher levels in boys than girls.[19]

Bacterial profiles in children with ECC and CF are different. The *Streptococcus* genus showed significant levels of differences between the two groups of children. Species of the genus *Streptoccus* such as *Streptococcus mutans* and *Streptococcus sobrinus* were increased in children with ECC compared to CF children.[17,20–22] Other species, such as *Streptococcus mitis* and *Streptococcus salivarius* were found in high numbers in children with CF.[20,22] In addition, *Haemophilus paraphrohaemolyticus* HK411, *Neisseria sicca* 4320, *Neisseria sp. oral clone* AP132, *Actinobacillus pleuropneumoniae* MCCM 00189, and *Streptococcus sp. oral clone* ASCE06 was found in CF children and not found in children with ECC. In contrast, *Lactobacillus sp* C56 was found in half of ECC children's total samples and not in CF children. The results of this study indicate that *Lactobacillus sp* C56 plays an essential role in the occurrence and development of caries.[17]

Eleven out of fourteen articles reported that *Streptococcus mutans* was the species found in high levels and was thought to be the leading cause of ECC. *Streptococcus mutans* can make biofilms and metabolize substrates into acids, decreasing pH in the oral cavity (acidic conditions). A low pH level will cause demineralization, so caries will form. The interaction between *Streptococcus mutans* and other microbes, namely *Candida albicans* can produce Gtfs and increase the virulence of the plaque biofilm.[34] Two articles reported differences with other investigators, that there were low levels of *S. mutans* in ECC children because *Streptococcus* consisted of many species, including *S. mutans, S. sanguinis, S. oralis, S. sobrinus, S. mitis*, and have different roles that show varying abundance during the development of dental caries.[15,23]

The results of different levels of bacteria are caused by various analytical methods that are being used. There are five ways: Two-way ANOVA and T-test, which is a statistical analysis method, and Lefse analysis, 16s rRNA/DNA, and qPCR, which is a bacterial analysis method. From the two bacterial analysis methods, 16s RNA/DNA and qPCR, it was found the similarity of *Streptococcus mutans* bacteria. The two statistical analysis methods did not find parallels in bacteria.

CONCLUSION

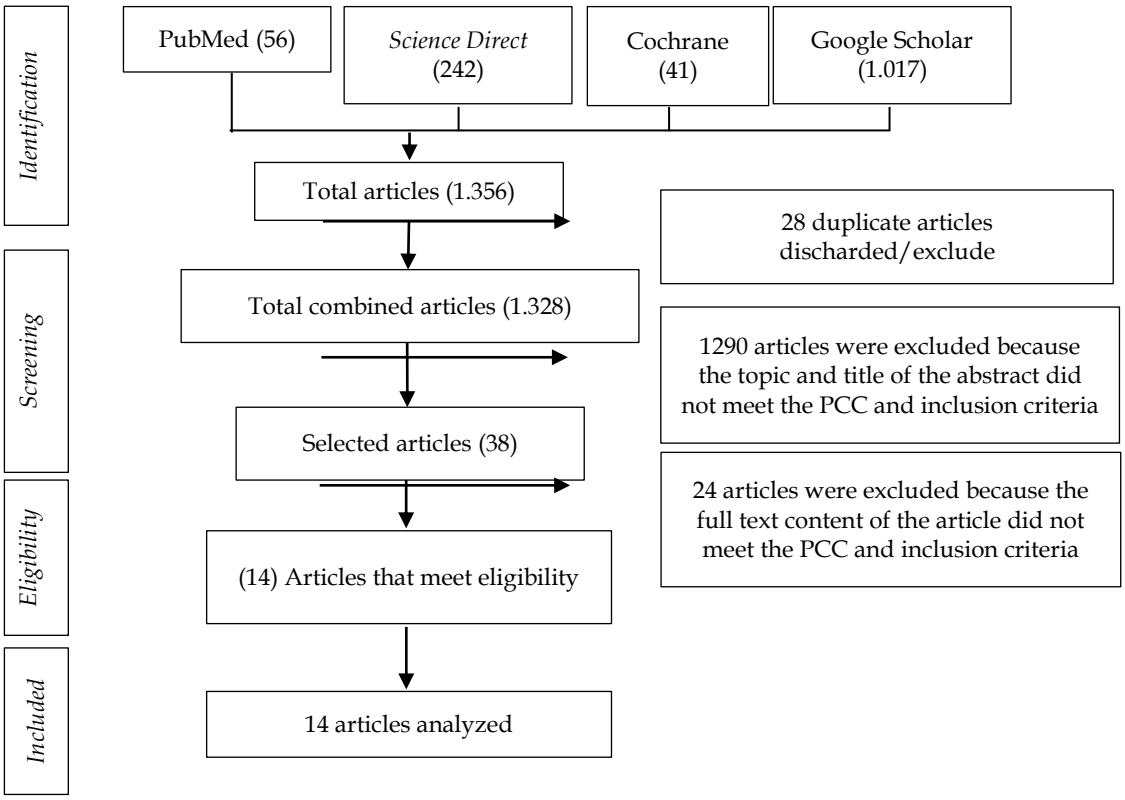
Based on the research, Streptococcus mutans have high levels in ECC children, so it is assumed to be the main bacteria causing ECC. In CF children, high levels of Neisseria, Streptococcus mitis are found, *Streptococcus salivarius,* and *Leptotrichia bucallis.*

RESEARCH METHODS

This scoping review uses the PRISMA-ScR (Preferred Reporting Items for Systematic reviews without Meta-Analyses extension for Scoping Reviews) tracing method. The research procedure focuses on the Population, Concept, and Context (PCC) analysis. The population used in this study were children with ECC and caries-free. Differences in bacterial profiles in children with ECC and caries-free as a concept and context in the form of cohort studies and randomized controlled trials. The databases used are PubMed, Science Direct, Cochrane, and Google Scholar. The search followed the steps of the PRISMA-Scr procedure using "Bloolean Operators" ('AND', 'OR', 'NOT'), and the keywords "(early childhood caries) AND (profile bacterial OR bacterial) AND (children)

Inclusion criteria used in this study are (i) Journals with a period of 5 years back, (ii) Children under 71 months (<6 years), (iii) Journals in English, (iv) Journals with types of research are Cohort Study and Random Controlled Trial. Exclusion criteria for research are (i) Journals with traditional literature review types of research (narrative review) (ii) Journals that cannot be accessed in full-text.

Chart 1 – PRISMA - ScR



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AUTHOR CONTRIBUTIONS

Concept – SDA., HTP., MG.,; Design – SDA., HTP., MG.,; Supervision – HTP., MG.; Resources – SDA., HTP., MG.; Materials – SDA., HTP., MG.; Data Collection and/or Processing – SDA.,; Analysis and/or Interpretation – SDA.,HTP., MG.; Literature Search – SDA.,; Writing – SDA.; Critical Reviews – SDA., HTP., MG.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest in the manuscript.

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Literature Review

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The *Streptococcus mutans* ability to survive in biofilms and during dental caries formation: scoping review

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ABSTRACT. Caries are the deterioration of dental hard tissue caused by acidic byproducts of bacterial carbohydrate fermentation. The formation begins within the bacterial biofilm that covers the tooth's surface. *Streptococcus mutans* is the dominant bacteria in the biofilm, forming a multispecies biofilm on the tooth surface, growing, and surviving within it. *S. mutans* colony formation and acid formation can lead to tooth demineralization. The purpose of this scoping review is to determine the ability of *S. mutans* to survive in biofilms and during the formation of dental caries using articles from the chosen database. Articles published from 2016 until 2021 were searched for using the keywords: "*Streptococcus mutans* and caries or dental caries and survival ability or survivability and survival factor" in the PubMed, ScienceDirect, Cochrane, and Google scholar databases. Using PRISMA-Scr, existing articles were chosen based on inclusion and exclusion criteria. There were ten articles found that were suitable for review. The data presented in the article vary according to the study's location, purpose, method, and samples. The finding revealed that *S. mutans* survive in biofilms and caries formation due to their ability to activate enzymes, virulence factors of *S. mutans*, and the environmental conditions of the oral cavity. Aciduric; acidogenic; quorum sensing; ability to form GTFs, GBPs, ATPase, CSP, eDNA; and the ability to produce bacteriocin and autolysins all contribute to *Streptococcus mutans*' ability to survive in biofilms and during the formation of dental caries

KEYWORDS: biofilm, caries, survival ability, survival factor, *Streptococcus mutans*.

INTRODUCTION

In their early stages, dental biofilms are non-cariogenic. *Streptococcus mitis*, *S. gordonii*, and *Actinomyces sp*. are the first colonizers of dental biofilm. They adhere to the tooth surface with the help of salivary proteins and form a non-cariogenic three-dimensional biofilm with neutral pH. Commensal bacteria will produce H_2O_2 and antimicrobial proteins to prevent pathogenic bacteria like *S. mutans* from overgrowing. *S. mutans* overgrowth in the biofilm causes an imbalance of microbial biofilms, and the biofilm becomes cariogenic, making *S. mutans* the primary etiologic agent in human dental caries. The biofilm becomes cariogenic, so *S. mutans* is the primary etiologic agent in human dental caries.[1] The main natural habitat of *S. mutans* is the tooth surface of the biofilm.[2] These bacteria multiply and survive in the natural ecosystem of the dental biofilm, being the dominant species in the multispecies biofilm along with other bacteria.

S. mutans dominance in dental biofilm development is characterized by the production of the enzyme fucosyltransferase (FTF), and three glucosyltransferases (GTFs); GTF-B, GTF-C, and GTF-D. *S. mutans* also encodes several cell surface-associated glucan binding proteins (GBPs) such as GBP-A, GBP-B, GBP-C, and GBP-D.[3] GTFs and GBPs activity contributes to the structure of the intracellular and extracellular matrix that underpins construction and biofilm development. *S. mutans* uses the GBPs enzyme to support sucrose-dependent tooth surface attachment as a biofilm foundation. Surface-adsorbed GTF-B and GTF-C use dietary sucrose to synthesize insoluble and soluble glucans and provide an insoluble matrix for biofilm formation. GTF-D forms a soluble polysaccharide. Then acts as a primer for GTF-B synthesis,

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increasing exopolysaccharide synthesis. GTF-produced glucan molecules serve as avid binding sites on surfaces for *S. mutans* (and other microorganisms), mediating tight bacterial clustering and adherence to tooth enamel.[4,5]

The presence of carbohydrates and sucrose in the oral cavity will cause *S. mutans* to ferment and produce lactic acid. An acidogenic factor is the ability to make this acid.[6] Lactic acid in large quantities in the oral cavity can cause a pH drop above the critical limit (4.5-5) and can demineralize teeth. *S. mutans* is also an aciduric bacteria, allowing it to live at this critical pH. And it evens developing existing biofilm structures and colonizing with more aciduric bacteria, increasing the acidity level of the oral cavity.[7] *S. mutans* ability to tolerate low pH or high acidity levels is a significant virulence factor in caries formation associated with dental biofilms.

The proton enzyme F-ATPase helps *S. mutans* survive in the acidic conditions of the dental biofilm. F-ATPase is a multimeric membrane-inserted enzyme that contains a functional subunit of the F0 protein and the F1 protein complex encoded by the *AtpF* and *AtpD* genes. The activity of F-ATPase keeps the biofilm's acidogenic and aciduric properties stable.[8] Bacteriocin (mutacin) and autolysins are two other factors that contribute to caries formation. Bacteriocin, a peptide with antimicrobial activity against other bacterial chains, is produced by the ***S. mutans*** mutant. Bacteriocin contributes to *S. mutans* competitiveness for survival in multispecies oral biofilms, so *S. mutans* is considered an important pathogen of dental caries.[9] Autolysis of *S. mutans* is required for biofilm maturation and biogenesis of a normal cell surface.[10]

The research findings show that *S. mutans* can survive in dental biofilms and is a dominant bacteria that cause dental caries. Valdez et al. found an increase in the prevalence of *S. mutans* with caries severity. *S. mutans* cell chains with a typical phenotype are found in higher concentrations in the biofilms of early caries children (ECC) and S-ECC (highly acid-tolerant) than in caries-free (CF) children. There was an association between the severity of children's caries and the number of ***S. mutans***.[11] According to Esberg *et al*., 48% of children infected with *S. mutans* at the onset of caries had a twofold increase in def-s scores at 12 and 17 years of age. As well as an increase in caries compared to uninfected children aged five years.[12] Ghazal, T. S. *et al*. conducted a study that compared DMF-T values per person in the presence of 94% *S. mutans*.[13]

According to Basic Health Research, tooth decay/cavities/pain due to caries account for 45.3% of dental health problems in Indonesia.[14] More than 90% of the world's population is affected by caries.[15] *S. mutans* is the dominant bacteria that cause caries, as previously stated. So, one way to address the problem of rising caries prevalence in Indonesia and around the world is to prevent *S. mutans* overgrowth in the oral cavity, particularly in the dental biofilm. Prevention of *S. mutans* growth in dental biofilm will be most effective if we understand how *S. mutans* survive in that environment. The ability of *S. mutans* to survive in the dental biofilm and during the formation of dental caries is described in this scoping review study.

MATERIALS AND METHODS

This study used scoping review method from January to May 2022. Figure 1 shows the research procedure was divided into three stages: preparation, implementation, and evaluation.[16] The preparation stage begins with research questions centered on the PCC criteria. The study population was *S. mutans* that survive in biofilm and during caries formation. The concept was to examine *S. mutans* ability to survive in biofilm and during caries formation. The research context was a cross-sectional study, prospective cohort study, and randomized controlled trials articles used for this study.

The implementation phase used a prism scoping review procedure that began with identification. Articles were searched using the keywords: "*Streptococcus mutans* and caries or dental caries and survival ability or survivability and survival factor" in the PubMed, ScienceDirect, Cochrane, and Google Scholar databases. The research inclusion criteria, which include: articles published between 2016 and 2021 in both Indonesian and English; study subjects aged 3-21 years in healthy teeth, never had caries, and had no restorations; investigate *S. mutans* survival ability during the early stages of caries formation; and articles in the form of observational and randomized controlled trials. Exclusion criteria included paid full-text articles, previously restored teeth, malocclusion, use of orthodontic appliances, and systemic disease. The article was screened through several stages, including screening for article duplication using the Mendeley application. Screening for article titles and abstracts using the Rayyan website (inclusion/exclusion criteria) and screening for article eligibility by reading the entire article.

The evaluation stage consists of extracting the final screening articles one at a time and summarizing the results in a table based on an analytical framework that concludes a summary of the research to answer research questions. The data extraction investigated is depicted in Table 1. Each chosen article was described separately based on predetermined criteria. The final findings included the existence of ***S. mutans*** during the caries formation process, from tooth attachment to the onset of caries formation. The effect of *S. mutans* and their virulence and what factors influence *S. mutans* ability to survive on biofilm formation and caries formation**.**

RESULTS

The results of the article identification stage yielded 1,379 articles with details in each database: 1,041 in PubMed, 40 in ScienceDirect, 134 in Cochrane, and 164 in Google Scholar. The database articles were then filtered for duplication, leaving 1,289 articles. The results of the duplication screening are then filtered based on the title and abstract's suitability with the previously determined inclusion and exclusion criteria. The second screening resulted in the removal of 19 articles. Ten articles were extracted to answer the research questions based on the eligibility criteria screening. Figure 2. depicts the article selection process using PRISMA's scoping review.

Table 1. contains the article extraction results, which include the title, author's name, year of publication, location, method, purpose, population or sample, and results of the selected research articles. Ten articles are obtained for extracting stage in this study. Five articles discussed the aciduric ability of *S. mutans*. Aciduric refers to *S. mutans*' ability to live in acidic or low-pH environments. It described that F-ATPase-mediated lipid membrane shift increases *S. mutans* virulence and viability in an acidic environment.[17–21] Five articles discuss ***S.*** *mutans*' acidogenic ability, or ability to produce acid. The formation of low pH-dependent GTFs aids acid formation.[19,22] The capability of *S. mutans* to produce acid from various fermentable sugars contributes to its virulence and tooth demineralization.[17,21,22]

Four articles discuss *S. mutans*' ability to form GTF enzymes. GTFs can produce lactic acid and provide tight binding sites for *S. mutans* and other microorganisms. GTFs catalyze the formation of water-soluble and water-insoluble glucans, which initiate biofilm attachment and colonization. Antibacterial compounds such as epigallocatechin-3-gallate (EGCG) and dextranase can be suppressed and increased in effective doses by GTFs protein.[17,22] GBPs are glucan-binding proteins associated with acidity by *S. mutans* discussed in three articles. GBPs are attachments to tooth surfaces, facilitating sucrose-dependent bacterial colonization and biofilm aggregation.[17,21,22] The formation of ATPase by *S. mutans* is discussed in five articles. The *atpF* gene encodes F-ATPase, which plays a vital role in acid tolerance in multispecies subunit complexes.[17–21] F-ATPase is an enzyme that degrades ATP molecules into ADP and Pi ions, releasing energy while moving cell metabolites and exporting toxins to inhibit normal cell functions.

Other bacteria added to the biofilm did not reduce the dominance of *S. mutans* and autolysin in the presence of low pH, which caused cells to die and increased biofilm formation. In response to adverse physiological changes such as antibiotic exposure, *S. mutans* produces an autolysin that degrades its cell walls.[17,22–25] ***S. mutans*** also produce bacteriocins, peptides with antimicrobial activity against other bacterial chains and help *S. mutans* survive in multispecies biofilms. Furthermore, *S. mutans* communicates with other bacteria via quorum sensing in the biofilm. The *S. mutans*' ability to communicate with other bacteria allows for the coordination of cell community in biofilm formation and expression of virulence gene.[17,23]

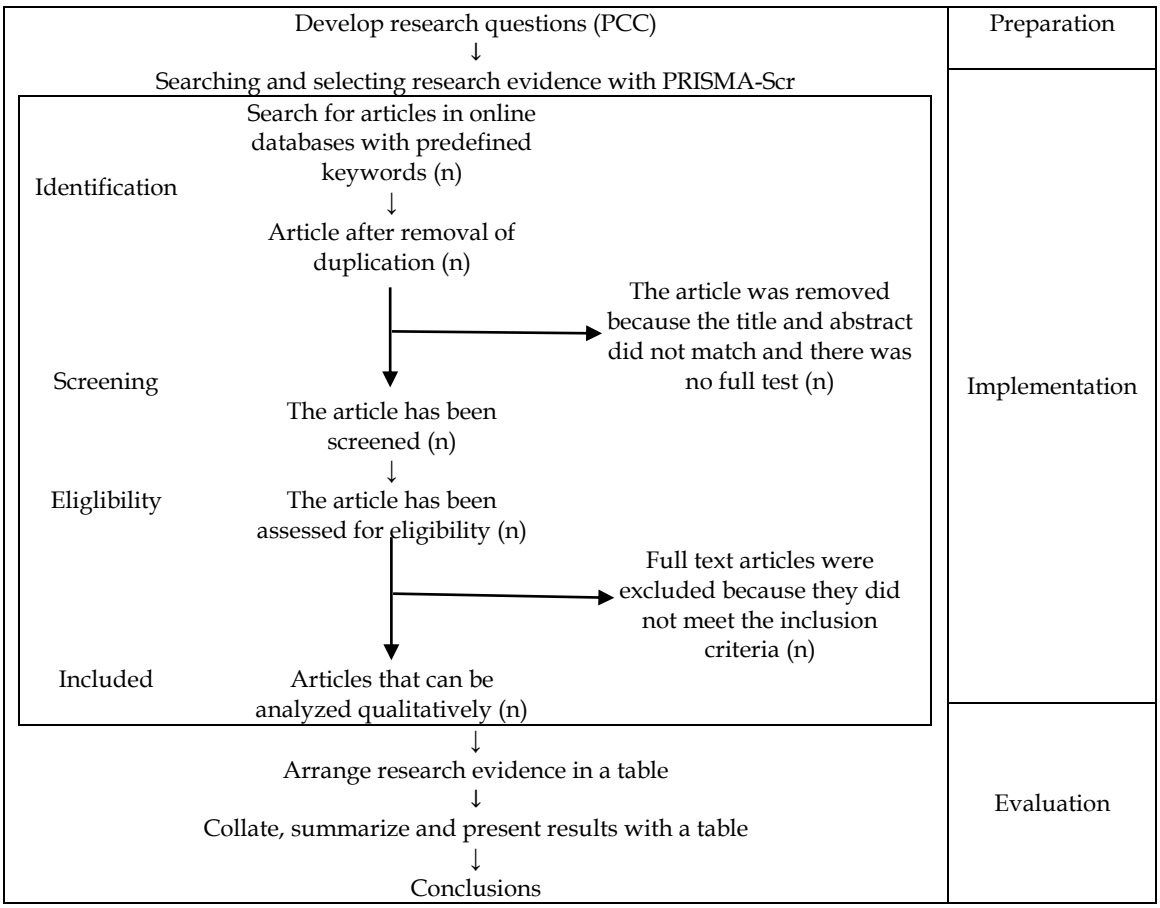

Figure 1. Stages of the research procedure

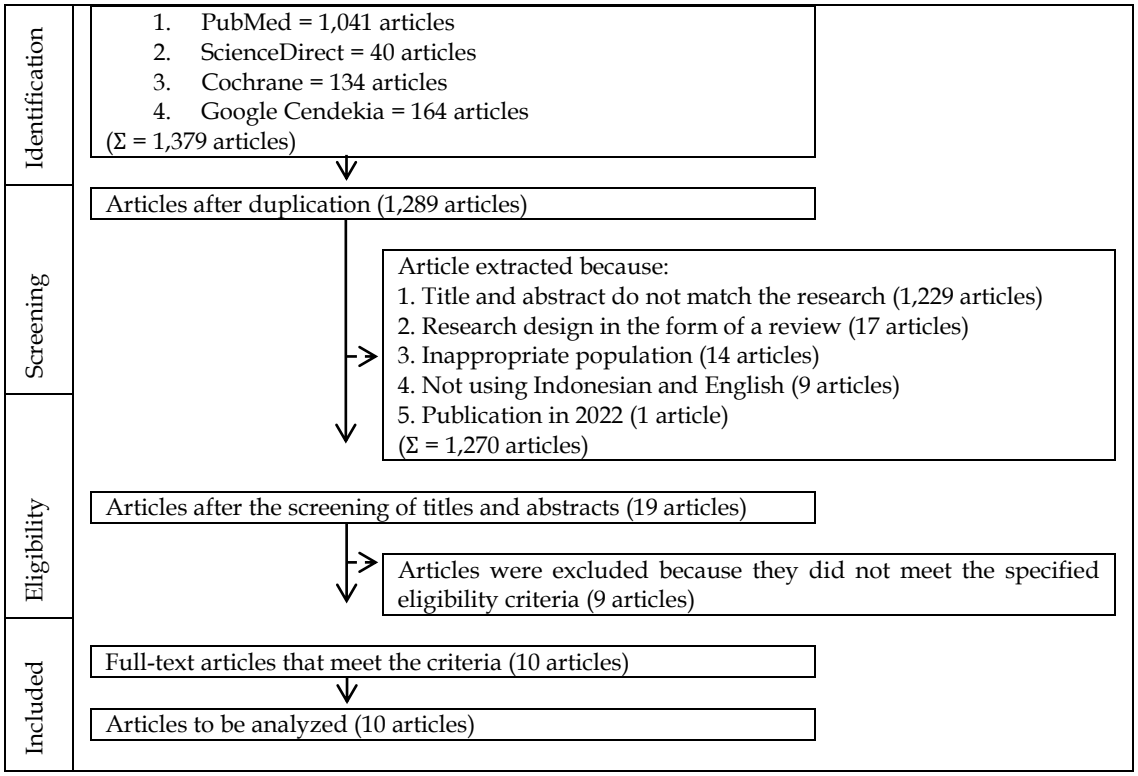

Figure 2. Flowchart of searching articles using PRISMA-Scr

Table 1. Extraction of data from 10 articles of final search

No	Author (Year)	Research Title	Research sites	Research methods	Research purposes	Population/Research Sample	Research result	
							Healthy teeth	Early dental caries
1	Rodrigu es et al. (2020)	Antimicrobial activity of *Lactobacillus fermentum* TcUESC01 against *Streptococcus mutans* UA159	Brazil	1. 16SrRNA gene sequencing 2. Statistical test: kruskal-wallist, anova, bonferoni	To evaluate the effect of the fermentation product of *L. fermentum* on the viability and virulence factors of *S. mutans* UA159 in planktonic and biofilm models.	1. *S. mutans* UA 159 2. *L. fermentum* TcUESC01	The fermentation product of *L. fermentum* did not reduce the cell, acid production, cell membrane permeability, acid tolerance, and polysaccharide production of *S. mutans*	The tested biofilms inhibited *S. mutans* virulence factors by biosurfactant production, showing impaired adhesion and formation of *S. mutans* biofilms.
2	Valdez et al. (2016)	Comparative in vitro investigation of the cariogenic potential of bifidobacteria	Brazil	Statistical test: anova and tukey	Assessed in vitro cariogenic potential of several species of bifidobacterium compared with caries-causing bacteria.	1. *Bifidobacterium species* 2. *Lactobacillus species* 3. *Streptococcus species* 4. *Actinomyces species*	The percentage of living cells decreased at pH 2.8, except for *B. animalis, B. dentium, L. acidophilus* and *L. casei*. The ability to form biofilms was lower as a single species compared to multiple and multispecies species.	Multiple species and multispecies biofilms cause loss of surface hardness. The aciduric nature of bifidobacteria allows them to live in acidic carious lesions.
3	Villegas, Arango, dan Arias (2018)	Effects of a food enriched with probiotics on *Streptococcus mutans* and *Lactobacillus spp.* salivary counts in preschool children: a cluster randomized trial	Colombia	Statistical test: mann whitney U dan X^2	Evaluated milk supplementation with probiotic bacteria and standard milk, measuring levels of *S. mutans* and Lactobacillus spp.	Children aged 3-4 years, without systemic disease, intolerance to milk and antibiotics, and brushing teeth with fluoride paste min. 1 time/day.	Differences in CFU/mL of *S. mutans,* dental plaque, and pH were insignificant, while salivary buffering capacity was significant in the probiotic and control groups after 9 months.	Consumption of probiotics did not show any significance. There was no relationship between consumption of probiotics and the appearance of early caries during the intervention.
4	Wu et al. (2018)	Inhibitory effects of tea catechin epigallocatechin-3-gallate against biofilms formed from *Streptococcus mutans* and a probiotic lactobacillus strain	Taiwan	1.RNA extraction and qPCR 2. Statistical test: ANOVA and Tukey test	To determine the effect of catechin epigallocatechin-3-gallate (EGCG) on biofilm formation by *S. mutans* and probiotic *Lactobacillus casei* (*LcY*) on yakult.	1. *S. mutans* 2. *LcY*	EGCG increased the pH of the *Sm+LcY* and *LcY* biofilm culture media but reduced the *Sm* and *Sm+LcY* biofilm biomass. The presence of *LcY* and Gtfb increased the effective concentration of EGCG in inhibiting biofilm formation.	-
5	Chi et al (2016)	Milk Sweetened with Xylitol: A Proof-of-Principle Caries Prevention Randomized Clinical Trial	Peru	Statistical test:: chi-square, anova, cochran-mantel-hazel	Evaluating the efficiency of xylitol-sweetened milk as a caries prevention strategy.	153 kindergarten to 4th grade elementary school children from low-income families.	Xylitol milk does not prevent tooth decay over a short period in children at high caries risk.	Constant sugar intake over time makes many carious lesions contain colonies of *S. mutans*
6	Sekiya et al. (2019)	Proton-pumping F-ATPase plays an important role in *Streptococcus mutans* under acidic conditions	Japan	Enzyme test and Bacterial survival ability test	Reported the inhibitory effect of E. coli proton pumping F-type ATPase on *S. mutans* enzyme activity, growth and survival of *S. mutans* under acidic conditions.	F-ATPase from *E. coli* and *S. mutans*.	Enzyme activity, growth, and survival under acidic conditions suggest that *S. mutans* F-ATPase plays an important role in acid tolerance. Piceatannol, curcumin, and DMC significantly inhibited *S. mutans* F-ATPase under acidic	-

No	Author (Year)	Research Title	Research sites	Research methods	Research purposes	Population/Research Sample	Research result	
							Healthy teeth	**Early dental caries**
							conditions.	
7	Miao, Wang, Zhang, Mode, dan Huangsh (2019)	Regulation of SmpB on acidogenic/aciduric ability and expression and activity of aciduric virulence factor in *Streptococcus mutans* from caries-sensitive children	China	Statistical test:: t test and anova	Exploring the role of SmpB on acidogenic and aciduric abilities and aciduric virulence factor expression in *S. mutans* from caries-sensitive children.	Saliva children aged 3-5 years.	Knockout SmpB inhibited the growth of *S. mutans*, SmpB deletion significantly reduced the acidogenic/aciduric ability of *S. mutans.*	SmpB regulates the acidogenic/ aciduric ability and aciduric virulence factor of *S. mutans* ECC, which is sensitive at low pH, causing tooth demineralization
8	Kawarai et al. (2016)	*Streptococcus mutans* biofilm formation is dependent on extracellular DNA in primary low pH conditions	Japan	Statistical test:: t test	Observed the effect of low pH on primary culture conditions comparing results at pH 6 and 7.	1. *S. mutans* MT8148 2. *S. mutans* MT8148 gtfb	Increased biofilm formation with stimulation of CSP, TSB with glucose; production of eDNA without insoluble glucans; and decreased insoluble glucans at pH 6.	-
9	Bojanich dan Calderon (2017)	*Streptococcus mutans* membrane lipid composition: Virulence factors and structural parameters	Argentina	Statistical test:: kruskal-wallis.	Analyzed the location of the dental biofilm associated with a shift in the fatty acid membrane profile, and whether the shift could affect certain virulence factors of the *S. mutans chain*.	*S. mutans* was isolated from dental biofilms of children with a mean age of 6.2 years.	The control chain showed cell growth at pH 5 with the percentage of unsaturated fatty acids>saturated fatty acids.	The acidic environment promotes a shift in the membrane lipid profile. It is associated with higher aciduric properties and more developed virulence of *S. mutans*.
10	Cai, Meng, Liu, Cao, dan Wang (2021)	Suppressive effects of gecko cathelicidin on biofilm formation and cariogenic virulence factors of *Streptococcus mutans*	China	1. *Real-time qPCR* 2. Statistical test: t test	To determine the effectiveness of the gecko cathelicidin Gj-CATH2 on biofilm formation and cariogenic virulence factors of *S. mutans*, and to examine the mechanism of its function.	Gj-CATH2 peptide	Gj-CATH2 prevents *S. mutans* UA159 biofilm formation by downregulating the expression of biofilm genes, acidogenicity-associated genes, and all QS system-associated genes.	The effect of Gj-CATH2 was insufficient to prevent tooth demineralization caused by bacterial acid because it controlled F-ATPase as the main determinant of acid tolerance in *S. mutans*.

DISCUSSION

The oral cavity is covered by saliva for masticatory activity and to keep it warm and moist at 35-36° C.[22,26] The oral cavity is at pH between 6.75 and 7.25, depending on the needs of microorganism growth. The salivary flow rate varies from person to person per day.[27] Physical and chemical changes in the composition of saliva, especially in buffering against dietary acids as well as metabolic acids and the ability to remineralize tooth enamel play a role in the development and progression of caries.[28] Consumption of a carbohydrate diet resulting in the acid of bacterial metabolism in the oral cavity causes a decrease in pH. The lower pH of the oral cavity causes the mineral crystals of the teeth to undergo local demineralization in the biofilm of the involved tooth surface.[29]

The oral cavity is also a habitat for various bacteria with different compositions and numbers, including *Streptococcus mutans*.[30] *S. mutans* exist as a biofilm complex with other species in response to changes in the oral environment. *S. mutans* is a normal opportunistic flora. The imbalance of normal flora and host immunity will support the rapid growth or multiplication of *S. mutans.* This situation initiates the formation of pathogenic or cariogenic biofilms that have the potential to damage teeth and cause caries. Caries is a chronic disease that develops over time, is initially reversible, and can be stopped even when the dentin or enamel is destroyed to form a cavity as long as the cause of caries is removed.[30] Caries cannot develop without a cariogenic (pathogenic) biofilm and frequent exposure to carbohydrates, particularly free sugar.[31] Caries in the oral cavity are frequently associated with bacterial metabolism in the biofilm, which causes the demineralization of teeth.[17]

The ability of ***S. mutans*** to produce GTFS enzymes is the first step in biofilm formation. GTFs secreted by ***S. mutans*** were incorporated into the pellicle and adsorbed on the bacterial surface, even without GTF production. GTF enzyme synthesis of both water-soluble and water-insoluble glucans of dietary sucrose. Changes in the amount of insoluble and soluble glucans between pH 6.0 and 7.0 are linked to a different reliance on biofilm formation and altered biofilm morphology.[23] Biofilm is attached to the tooth surface in two ways: dependent and independent of sucrose. The mechanism of GTFs in glucan synthesis initiates the sucrose-independent attachment to salivary components in the biofilm. While the sucrose-dependent extension is responsible for colonizing the tooth surface or bacterial immobilization on hard surfaces.[24,32] Increasing GTF and GBP expression, EPS production, and *S. mutans* adhesion sucrose-dependent, ultimately enhanced biofilm formation.[26] The maximum activity of GTF was seen between pH 5.5 and 6.5 and decreased at pH below 5.5.[23.] The expression levels of GTF-B and GTF-D biofilm *Sm+LcY* were higher than the Sm biofilm when grown with 250 g/ml EGCG. This expression is an interaction between *S. mutans* and *LcY* which mediates the decrease in the inhibitory effect of EGCG on biofilm formation.[33]

Streptococcus mutans and other bacteria that mediate selective bacterial aggregation and attachment to enamel use the glucan molecules as a strong adhesive of the surface binding site. GTFs and GBPs increase bacterial colonization by acting as acid producers in response to *S. mutans*' acidogenic ability. At the same time, the ability to tolerate acid in the biofilm maturation stage depends on membrane-bound F-ATPase. The *atpF* gene encodes an F-ATPase that regulates intracellular homeostasis by inducing proton pump activity and H+ transport from cells into extracellular media, which helps to maintain extracellular pH.

The maturation of the biofilm stimulates the formation of QS, which contains various enzymes such as bacteriocin (mutacin) and autolysin. *S. mutans* and other bacteria compete for adhesion sites and modify salivary pellicle protein composition. *S. mutans*' bacteriocin prevents the attachment of other bacteria so that other bacteria cannot bind to teeth, and *S. mutans* becomes the dominant species in the biofilm. Autolysin work in acidic conditions. These enzymes damage its *S. mutans* cell walls which lead to cell death. The autolysis mechanism decreases the number of cells in the biofilm community and increases antibiotic resistance. The ultimate goals is to maintain biofilm community balance. During biofilm formation, *S. mutans* also release extracellular DNA (eDNA) and become one of the components of the extracellular matrix. It maintains biofilm structural integrity, initiating adhesion to the dental surface, and facilitating horizontal gene transfer in QS.[23]

This scoping review study shows that the experimental result of reviewed articles varies. The results depend on research locations, objectives, methods, samples, and populations. All articles did not discuss the exact number associated with the increase of *Streptococcus mutans* at the beginning of caries formation. All articles discussed virulence factors of *S. mutans* causing demineralization of the hard tissues of teeth. The research on bacterial culture can be biased between the bacterial culture environment and the oral cavity environment. Future studies can systematically assess the number of additions of *Streptococcus mutans* at the beginning of

caries formation compared to the number of caries-free teeth. Furthermore, further research was carried out on the FTF factor because there was no discussion of its virulence factors in biofilms and during caries formation.

CONCLUSION

The ability of *Streptococcus mutans* to survive in the biofilm and during the formation of dental caries are: aciduric; acidogenic; quorum sensing; ability to form GTFs, GBPs, ATPase, CSP, eDNA; and the ability to produce bacteriocin (mutacin) and autolysins.

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Literature Review

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Thermographic study of the maxillofacial area: the possibilities and prospects in modern dentistry

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ABSTRACT. Thermal pattern of body especially skin can determine the changes in the human body and, the consequences it causes, and changes in normal temperature distribution are a sign of a pathological process. The objective of this essay is to review the possibilities and prospects of Infrared thermography in modern dentistry in order to support dental clinicians to develop and conduct an analysis of maxillofacial changes both the quantitative and quantitative depending on cold acclimation using infrared thermography. Literature were searched in all databases such as PubMed, Medline, and Google Search for articles published between 2019 and 2022. By means of a systematic online database search and based on the PRISMA guidelines related to word infrared thermography, dentistry, inflammation articles were identified using the search engines PubMed, Medline, and Google Scholar. After screening the abstracts and applying the eligibility criteria on those which were fully accessible, 165 articles were included in the review. Amount 145 studies were excluded due to the defined inclusion and exclusion criteria and 20 studies have finally been included in the evaluation process. This was followed by an analysis and discussion of the methodology

KEYWORDS: *Thermography, dentistry, maxillofacial area, Arctic Zone*

INTRODUCTION

Currently, body temperature is one of the most commonly used indicators of health status in humans. Thermal pattern of body especially skin can determine the changes in the human body and, the consequences it causes, and changes in normal temperature distribution are a sign of a pathological process.[1]

Physiological and pathological effects of short-term exposure to cold are well known. As known, the human body is physiologically regulated to keep it homeostatic when environmental conditions change. Humans produce or lose heat through thermoregulation to maintain the homeostasis of body temperature and protect themselves against excessive heat or cold. In the same way, environmental temperature may affect physiological responses to exercise through thermoregulation. By contrast, our body promotes heat dissipation by sweat evaporation through increased skin blood vessels when exposed to heat.[2] Exposure to cold causes various physiological responses in the human body. It has been reported that cold exposure results in increased heart rate and systolic blood pressure.[3] Cold-induced increase in heart rate may be associated with reduced vagal activation compared with sympathetic response to cold.[4]

As research reports have ever been mentioned that during inflammation, where the rate of biochemical processes will decrease but the process of separation of respiration and phosphorylation will increase. As a result, the temperature of the inflamed area will be higher than the temperature of the surrounding tissue.[5] As is known, changes in normal temperature distribution are a sign of a pathological process.[6]

One of method which working principle is by determining the thermal pattern characteristics is Infrared Thermography. This approach allow us to specify the localization of functional changes, inflammation, and the activity of the process and its prevalence. High information content and reliability of thermal imaging in some diseases is close to

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100%, and in general it is for primary examinations about 80%. Besides, Infrared thermography does not cause discomfort in patients. This technique is completely safe and quite simple in execution. The level of security is very safe, even for more frequent use. This method also allows for use in pregnant women as well as for small children.[7] In other words, thermal pattern examinations are considered an advancement in popular medical methods. Infrared Thermography also knows as an important adjunctive role in the assessment of dental-oral related illnesses, diseases, and in their clinical diagnosis.[8] This accurately method possible to examine the entire maxillofacial region.

Meanwhile, an analysis by the Centers for Disease Control and Prevention (CDC) of U.S. temperature-related deaths between 2006 and 2010 showed that 63% were attributable to cold exposure, while only 31% were attributable to heat exposure.[9] Furthermore, in 2016 number of foreign students' enrolment in Russian Federation are 244.597 students. During the year, the number of students from tropical countries in Russian universities increased by 17%. The number of students from India increased by 20% and from China increased 10%. Ministry of Education of Russian Federation also stated students from Vietnam about 3.1 thousand and from African countries were 11,000 people already received in Russian universities in 2015-2016. Meanwhile, According to World Health Organization report, it was estimated that around 60-90% of children and 100% adults suffering from dental cavities problem.[10] One of dental problem and most high prevalence rate and difficult to diagnose timely is maxillofacial diseases.[11]

According to problems above, the temperature is experienced by permanent residences and foreign students as temporary residences in Arctic zone will certainly affect the body's thermal structure, which will eventually show physiological and pathological changes. As we know, Russia Federation is one of the most important arctic zone with temperature interests in the world. Study which explained concerning maxillofacial physiological changes which indicated by cold temperatures using infrared thermography in Arctic Zones using infrared thermography approach still limited. Therefore, it is necessary to conduct study, both qualitatively and quantitatively to analyze maxillofacial physiological changes which indicated by cold temperatures using infrared thermography.

The objective of this essay is to review the possibilities and prospects of Infrared thermography in modern dentistry in order to support dental clinicians to develop and conduct an analysis of maxillofacial changes both the quantitative and quantitative depending on cold acclimation using infrared thermography.

MATERIALS AND METHODS

Literature were searched in all databases such as PubMed, Medline, and Google Search for articles published between 2019 and 2022. By means of a systematic online database search and based on the PRISMA guidelines related to word infrared thermography, dentistry, inflammation articles were identified using the search engines PubMed, Medline, and Google Scholar. After screening the abstracts and applying the eligibility criteria on those which were fully accessible, 165 articles were included in the review. Amount 145 studies were excluded due to the defined inclusion and exclusion criteria and 20 studies have finally been included in the evaluation process. This was followed by an analysis and discussion of the methodology.

DISCUSSION

Medical infrared thermography is a non-invasive and non-ionizing bidimensional imaging technique that maps the distribution of body surface thermal radiation into images. It is based on the capture and transformation of infrared radiation emitted by the human skin to form images that reflect the local vasomotor response. The mean temperature for the normal condition by using thermography was found $\cong 31^0$C and abnormal condition was $\cong 34^0$C. Approximate elevation of 3^0C was observed between the normal and diseased subjects.[12]

Infrared thermography knows as a crucial connected role within the assessment of inflammation.[13] Inflammation is classically described as a response to infection or injury. It is now increasingly appreciated that chronic inflammation is universally associated with diseases of affluence and extended lifespan such as neurodegenerative diseases and cancer. Other review also stated

response of inflammation triggered by a variety of noxious stimuli and infection.

Thermography is a sensitive clinical diagnostic tool which easy to discover signs of inflammation in a very early, pre-clinical stage, including abnormal condition with no clinical symptoms. Early detection of inflammation could help making treatment more effective and prevent crippling deformation of the joints. One of joint in facial region is Temporomandibular Joints (TMJ). Thermography has found the sites of discomfort varied; in some cases, the symptoms were experienced over and around the masseter muscle and inflammation was reported in the affected region.[14]

Meanwhile, the human body is physiologically controlled to preserve homeostatic as soon as environmental circumstances change. Humans manufacture or lose heat through thermoregulation to take care of the equilibrium of vital sign and shield themselves against excessive heat or cold. In cold weather, the body can lose heat faster than it is produced, which uses up stored energy and can lead to hypothermia, defined as a core temperature below 35°C. While, humans have excellent mechanisms to acclimatize to heat, the acclimation capabilities to cold are a topic of controversy. There are different levels of whole body cold acclimation, depending on the degree of the cold exposure. Two levels can be distinguished: 1. Severe cold exposure, leading to a drop in mean skin, tissue and body core temperature generally evoked using repeated cold water immersions, 2. Moderate cold exposure, leading to decrease in skin and tissue temperatures with no or minor drop in body core temperature, generally evoked using repeated cold air exposure. The acclimation capabilities to cold as internal factor may influence the interpretation of the thermal pictures of infrared thermography.[15]

One of the external factor that may influence both analysis and interpretation of infrared thermography is environmental factor. There are three main potential factor as environmental sub-factor which in the scope of physiology topic that may influence of infrared thermography approach in Arctic Zones. The following sub-factor are: shivering, hypothermia, and circadian rhythm.

Shivering is uncomfortable for participants especially non-indigenous arctic that could interfere the results of infrared thermography. This factor are very important because many references stated the subject is likely to shiver in lower temperature. Shivering is a protective mechanism by virtue of which heat production occurs, by vigorous involuntary muscle activity, to compensate for the decreased core temperature in a normal healthy living body. Shivering thermogenesis is main components of cold induced thermogenesis. Within this thermoregulatory continuum, humans are generally well adapted for dissipating heat in warm climates but are particularly maladapted at conserving it in the cold.[3]

The condition of shivering also possible to provoke hypothermia. Inflammation leads to hyperthermia, whereas degeneration, reduced muscular activity and poor perfusion may cause a hypothermic pattern.[18] As analysis reports have ever been mentioned that in inflammation, wherever the rate of organic chemistry processes can decrease however the method of separation of respiration and phosphorylation can increase. As a result, the temperature of the inflamed area are going to be over the temperature of the surrounding tissue.[2] As is thought, changes in normal temperature distribution are an indication of a pathological process.

It is important to take into account the circadian rhythm of the human body when conducting an experiment in Arctic Zones. Light of sufficient intensity is the main factor that maintains the 24-h period of human circadian rhythms. Consequently, living in continual daytime or nighttime can causing sleep, mood and productivity problems for people living in Arctic Zones. It has been illustrated that skin temperature varies throughout the day. In the Arctic Zones, people are deprived of natural sunlight in winter and have continuous daylight in summer. Whereas in the evening the core body temperature and proximal skin temperature rise in contrast to distal skin temperature, the opposite effect seems to take place in the morning.[19] Light of sufficient intensity, deprived of natural sunlight in winter, continuous daylight in summer, and prevailing darkness in winter are among the many challenges of making research in the Arctic Zones.

Other research also states, people living in sub-Arctic Zones may experience more seasonal variations in sleep patterns and problems than people living closer to the Equator.[20] In many situations during the winter, temporary residents are restricted in movement and causing internal desynchronize of sleep and the circadian system impairs cognitive performance. The change of circadian also related to secretion of hormone melatonin in correlate to short and long daylights. Since there is no evidence could explain the melatonin level in various people either indigenous

or non-indigenous residents in Arctic Zones, melatonin level could depend on ability to adapt to extremes of cold and day length. This clearly suggested that a change in circadian phase both permanent and temporary residents in Arctic Zones may influence the interpretation of the thermal pictures of infrared thermography.

CONCLUSION

1. The mean temperature for the normal condition by using thermography was found ≅31^{0}C and abnormal condition was ≅34^{0}C. Approximate elevation of 3^{0}C was observed between the normal and diseased subjects
2. Infrared thermography knows as a crucial connected role to inflammation which easy to discover signs of abnormal condition in a very early, pre-clinical stage such as infection, injury, neurodegenerative diseases and cancer.
3. The acclimation capabilities to cold as internal factor may influence the interpretation of the thermal pictures of infrared thermography
4. One of the external factor that may influence both analysis and interpretation of infrared thermography is environmental factor and three main potential factor as environmental sub-factor are: shivering, hypothermia, and circadian rhythm.

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