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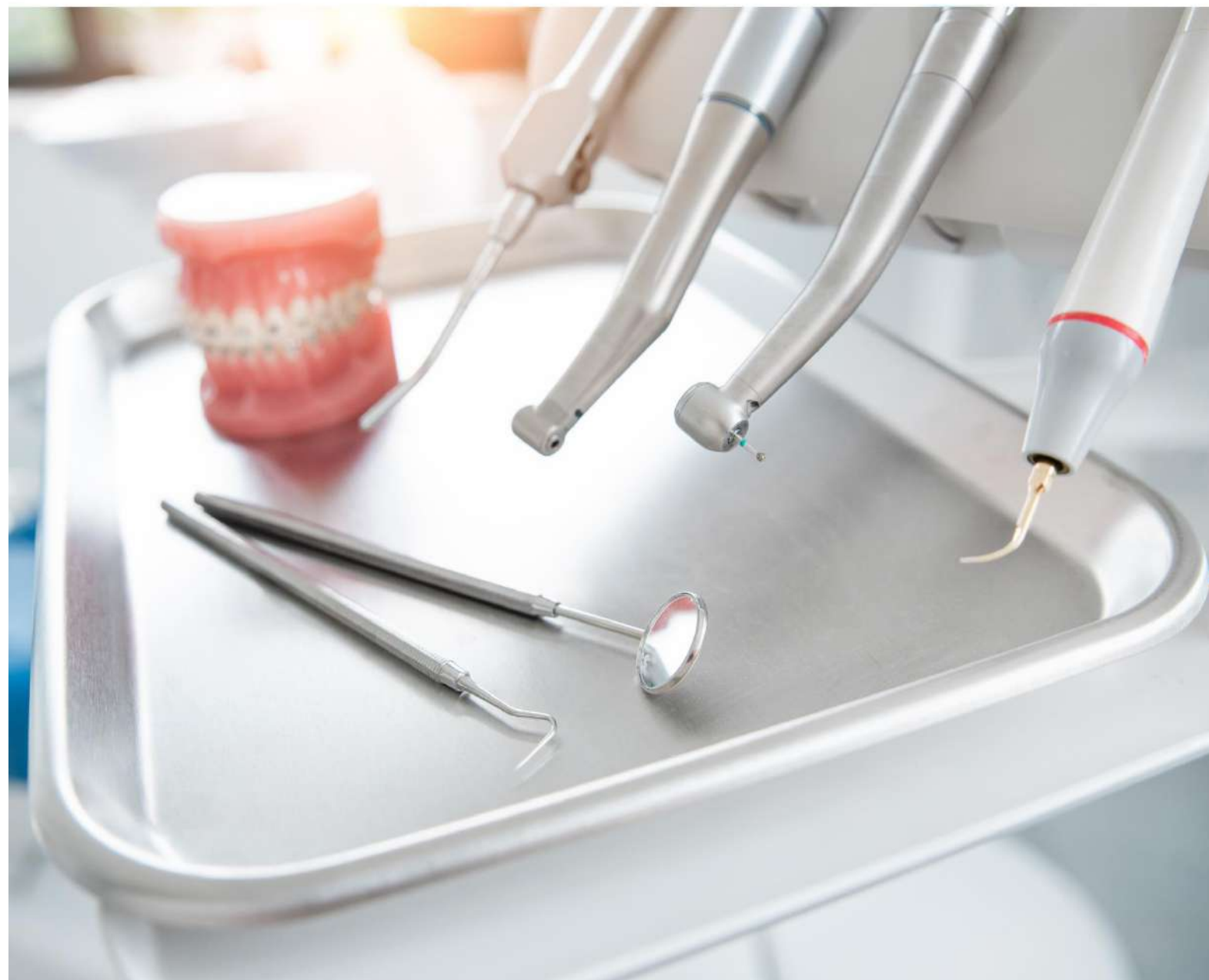
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Activity of Water and Ethanol *Syzygium myrtifolium* Walp. as an Antibacterial *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Background: One of the pathological conditions in the mouth that affects periodontal tissue is periodontitis. *Aggregatibacter actinomycetemcomitans* is a potential trigger of periodontal disease if allowed to settle, multiply, and express virulence factors. The pharmacological activity of *Syzygium myrtifolium* based on solvent extract has several functions, one of which is antibacterial. Solvents function to attract compounds or active ingredients in natural materials, and solvents are distinguished based on their polarity. Water is very polar, so it will attract compounds or active ingredients that are very polar, while ethanol is polar, so it will attract active substances/compounds that are polar. So that later it can be known how both perform. **Objective:** This study aims to determine the activity of water and ethanol extracts of *Syzygium myrtifolium* Walp. 10%, 20%, 40%, and 80% in inhibiting the growth of *Aggregatibacter actinomycetemcomitans* bacteria. **Materials and Methods:** This study is a laboratory experiment. Ethanol extract of *Syzygium myrtifolium* Walp. was partitioned to obtain a water extract of *Syzygium myrtifolium* Walp. A disc diffusion method on Mueller Hinton Agar (MHA) media was the antibacterial test method used in this study (n=4). Metronidazole 50 µg was used as a positive control and DMSO 2% as a negative control. **Results:** The average inhibition zone of ethanol extract was 9.11 mm, 11.475 mm, 13.625 mm, and 15.2875 mm, respectively, while the water extract was 10.3125 mm, 13.25 mm, 14.725 mm, and 16.725 mm, respectively. Positive control 22.975 mm and negative control 0 mm. **Conclusion:** Water and ethanol extracts of *Syzygium myrtifolium* Walp. have antibacterial activity against *Aggregatibacter actinomycetemcomitans*.

Keywords: *Aggregatibacter Actinomycetemcomitans*, *Syzygium myrtifolium* Walp., Antibacterial Activity

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INTRODUCTION

Aggregatibacter actinomycetemcomitans on the mucosa is considered a risk factor for the translocation of the organism to the gingival margin. This bacteria is a potential trigger of periodontal disease if allowed to persist, proliferate, and express virulence factors. These bacteria are early colonizers in the disease process and reject oxygen and hydrogen peroxide.¹

One of the pathological conditions that occurs in the mouth and affects the periodontal tissue or supporting teeth is periodontitis.² Periodontitis is a multifactorial disease triggered by an imbalance between environmental factors and the host defense. The environmental factors include infectious disease components, such as pathogenic bacteria in dental plaque, and lifestyle. The innate immune system in the host response may become highly reactive and result in an excessive inflammatory response.³

The prevalence of periodontal disease in the world is around 20 – 50%, and it is a continuous increase in prevalence every year.⁴ Periodontal disease in Indonesia ranks second after tooth decay, reaching a percentage of 96.58%.⁵ Based on 2018 Basic Health Research Data in Indonesia, the overall prevalence of periodontitis reached 74.1% based on certain characteristics, while only 0.4% of the population received gum or periodontal treatment.⁶ In the Central Java region, periodontal disease has a prevalence of 25.8%⁴.

Treatment and prevention of periodontal disease can be surgical and non-surgical. Various periodontal surgical procedures are used to treat chronic periodontitis, one of which is regenerative periodontal treatment using bone graft and platelet-rich fibrin (PRF).⁷ Non-surgical periodontal treatment procedures such as scaling root planing and administering antibiotics. Scaling root planing is an invasive procedure on the surface of the tooth root.⁸ The nature of the antibiotic is given for specific

pathogens in periodontal tissue, allogenic, non-toxic, and substantive.⁹

The use of synthetic drugs can cause negative impacts, both immediately and in the longer term. Adequate antibacterial administration is necessary to treat bacterial infections. However, increasing resistance to the widespread use of antibacterials has become a major public health problem worldwide. Therefore, it is necessary to search for new antibacterial agents to treat infections without causing resistance.¹⁰ Meanwhile, herbal medicines may cure diseases with minimal side effects because they are made from natural ingredients.¹¹ *Syzygium myrtifolium* Walp. has potential as a source of medicine. *Syzygium myrtifolium* Walp. belong to the Myrtaceae family.¹²

Pharmacological activity of *Syzygium myrtifolium* Walp. based on the solvent extract, it involves several functions, one of which is as an antibacterial agent.¹³ The abilities of *Syzygium myrtifolium* Walp. as an inhibitor of bacterial growth due to the content of tannins, saponins, flavonoids, and essential oils.¹⁴ In addition, the phenolic and flavonoid content is high in the ethanol and ethyl acetate extracts from *Syzygium myrtifolium* Walp. indicates that both have antioxidant properties and can inhibit the angiogenesis process.^{12,14}

Research conducted by Syafrana & Wiranti, 2022¹⁵ shows that the green and red leaves of *Syzygium myrtifolium* Walp. extract using ethanol has potential antibacterial properties against *S. mutans*. Both extracts can inhibit the growth of *S. mutans* bacteria at concentrations of 10%, 20%, 40%, and 80%. The inhibition values of the two extracts against *S. mutans* at each concentration were not significantly different, and the minimal inhibitory concentration could not be obtained.

Based on the description above, researchers are interested in investigating the antibacterial effects of extracts from *Syzygium myrtifolium* Walp. against the growth of *Aggregatibacter actinomycetemcomitans*

bacteria. This is because these bacteria are included in the type of pathogenic bacteria that is the main cause of periodontitis in the mouth. Researchers used extract solvents from water and ethanol. Solvents function to attract compounds or active ingredients present in natural materials, and solvents are differentiated based on polarity. Water is very polar and will attract compounds or active ingredients that are very polar. While ethanol is polar, it will attract active substances. So you can see later how the two of them are doing. Thus, it is expected that the water and ethanol extract of *Syzygium myrtifolium* Walp. can be an alternative for preventing and treating health problems in the oral cavity.

MATERIALS AND METHODS

This type of research was an experimental laboratory with a post-only control group design. The sample was *Aggregatibacter actinomycetemcomitans* (ATCC 43718).

Four repetitions by randomly selecting *Aggregatibacter actinomycetemcomitans* bacteria in Mueller Hinton Agar (MHA) media petri dishes then dividing them into *Aggregatibacter actinomycetemcomitans* groups with water and ethanol extract treatment of *Syzygium myrtifolium* concentrations of 10%, 20%, 40% and 80% negative control using DMSO 2% and positive control with Metronidazole 50 µg.

The inhibition zone test results that were obtained were then carried out statistical testing using *Kruskal-Wallis* test followed by *Mann Whitney*.

RESULTS

Tables 1 and 2 show the chemical content of water extract *Syzygium myrtifolium* and the chemical content of ethanol extract *Syzygium myrtifolium*

Table 1. Chemical content of water extract *Syzygium myrtifolium* Walp.

Compound Name	Alkaloid	Flavonoid	Tanin	Fenolik	Steroid	Terpenoid	Saponin
test method	ammoniacal chloroform + H ₂ SO ₄ 2N + Dragendorff	Mg powder + alcohol: HCL + amyl alcohol	+FeCl ₃ 1% 2 drops	+FeCl ₃ 5% 2 drops	H ₂ SO ₄ 2 drops + Anhydro us Acetic Acid 1 drop (Lieberman burchad (LB) test)	H ₂ SO ₄ 2 drops + Anhydro us Acetic Acid 1 drop (Lieberman burchad (LB) test)	+H ₂ O
Before Adding Reagents							
After Added Reactor							
Test results	+	-	+	+	+	-	+

Based on phytochemical testing on the water extract of *Syzygium myrtifolium* Walp., the water extract of *Syzygium myrtifolium* Walp. contains alkaloids, tannins, phenolics, steroids, and saponins (Tabel 1).

Table 2. Chemical Contents of *Syzygium myrtifolium* Walp ethanol extract.

Compound Name	Alkaloid	Flavonoid	Tanin	Fenolik	Steroid	Terpenoid	Saponin
test method	ammoniacal chloroform + H ₂ SO ₄ 2N + Dragendorff	Mg powder + alcohol: HCL + amyl alcohol	+ FeCl ₃ 1% 2 drops	+FeCl ₃ 5% 2 drops	H ₂ SO ₄ 2 drops + Anhydrous Acetic Acid 1 drop (Lieberman burchad (LB) test)	H ₂ SO ₄ 2 drops + Anhydrous Acetic Acid 1 drop (Lieberman burchad (LB) test)	+H ₂ O
Before Adding Reagents							
After Added Reactor							
Test result	+	+	+	+	+	+	+

Based on phytochemical testing on the ethanol extract of *Syzygium myrtifolium* Walp., the ethanol extract of *Syzygium myrtifolium* Walp. contains alkaloids, flavonoids, tannins, phenolics, steroids, terpenoids, and saponins (Tabel 2).

Figure 1 shows the inhibition zone results formation. Tables 3 and 4 show the mean inhibition chemical content of water extract *Syzygium myrtifolium* and the chemical content of ethanol extract *Syzygium myrtifolium*.

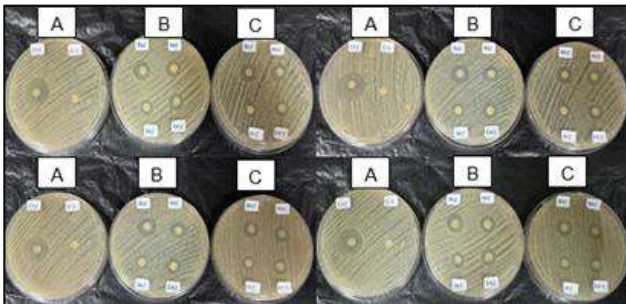


Figure 1. Bacterial Inhibition Zone Results Information:

A: Positive Control and Negative Control
B: *Syzygium myrtifolium* Walp Water Extract.
C: *Syzygium myrtifolium* Walp Ethanol Extract.

Table 3. Mean inhibition zone results of *Syzygium myrtifolium* Walp water extract.(mm)

No	K (+)	K (-)	10%	20%	40%	80%
1	22,95	-	9,15	11,60	13,80	15,20
2	23,20	-	9,05	11,35	13,75	15,40
3	22,80	-	9,20	11,40	13,40	15,35
4	22,95	-	9,05	11,55	13,55	15,20
Mean	22,975	-	9,11	11,475	13,625	15,2875
Std. Dev	0,16583	-	0,07500	0,11902	0,18484	0,10308

Table 4. Mean inhibition zone results of *Syzygium myrtifolium* Walp ethanol extract.

No	K (+)	K (-)	10%	20%	40%	80%
1	22,95	-	10,40	13,40	14,95	16,75
2	23,20	-	10,20	13,05	14,80	16,60
3	22,80	-	10,05	13,20	14,60	16,40
4	22,95	-	10,60	13,35	14,55	16,35
Mean	22,975	-	10,3125	13,25	14,725	16,525
Std. Dev	0,16583	-	0,23936	0,15811	0,18484	0,18484

Tabel 5. The statistical analysis results of *Syzygium myrtifolium* Walp water extract.

	water 80%	water 40%	water 20%	water 10%	positive control	negative control
water 80%		0,020	0,020	0,019	0,019	0,013
water 40%			0,021	0,020	0,020	0,014
water 20%				0,020	0,020	0,014
water 10%					0,019	0,013
positive control						0,013
negative control						

Tabel 6. The statistical analysis results of *Syzygium myrtifolium* Walp ethanol extract.

	etanol 80%	etanol 40%	etanol 20%	etanol 10%	positive control	negative control
etanol 80%		0,021	0,021	0,021	0,020	0,014
etanol 40%			0,021	0,021	0,020	0,014
etanol 20%				0,021	0,020	0,014
etanol 10%					0,020	0,014
positive control						0,013
negative control						

Tables 5 and 6 above show the results of the Mann-Whitney test to see the differences in inhibition zone test results between treatment groups in all group comparisons. Data can be said to have a significant difference if it has a p-value < 0.05. Based on tables 11 and 12, it can be seen that the results of the Mann-Whitney test for all group comparisons show a p-value < 0.05, so it can be interpreted that there is a significant difference between groups in the inhibition zone data for water and ethanol extracts of *Syzygium myrtifolium* Walp. on the growth of *Aggregatibacter actinomycetemcomitans* bacteria.

DISCUSSIONS

Based on the data presented, there are differences in the effectiveness of the concentrations of water extract and ethanol extract from *Syzygium myrtifolium* Walp.) with varying concentrations of 10%, 20%, 40%, and 80%. Tables 3 and 4 showed highest average diameter of the inhibition zone was recorded in the control group using Metronidazole, with a size reaching 22.9 mm. However, in the negative control group using 2% DMSO, there was no visible zone of inhibition because the result was 0.

The average growth inhibition value of *Aggregatibacter actinomycetemcomitans* bacteria by water extract of *Syzygium myrtifolium* Walp. showed variations between treatment groups. The treatment group with 10% water extract had the lowest average resistance value, namely 9.11 mm, while the treatment group with 80% water extract showed the highest average resistance value, reaching 15.28 mm. Meanwhile, in table 4, the treatment group with ethanol extract shows a similar pattern, where the 10% concentration has the lowest average resistance value, namely 10.31 mm, and the 80% ethanol concentration shows the highest average resistance value, namely 16.52 mm. From the results of this research, it can be concluded that the higher the concentration of water extract and ethanol extract from *Syzygium myrtifolium* Walp., the higher their ability as an antibacterial agent against *Aggregatibacter actinomycetemcomitans*.

The width of the inhibition zone is classified into four categories, namely: activity is weak (<5mm), moderate (6-10mm), strong (11-20mm) and very strong (>21mm). This means that bacterial growth is unable to withstand the antibacterial process produced by the extract (*Syzygium myrtifolium* Walp.) so that an inhibition zone is formed. From table 3 it can be seen that the average inhibitory power of water extract of *Syzygium myrtifolium* Walp. with a

concentration of 10% is in the medium category, while at concentrations of 20%, 40% and 80% it is in the strong category. In table 4 it can be seen that the average ethanol extract of *Syzygium myrtifolium* Walp. with a concentration of 10% is in the medium category, while at concentrations of 20%, 40% and 80% it is in the strong category.

This study used a positive control, namely 50 µg metronidazole. Metronidazole is known to have very strong antibactericidal properties. The mechanism of action of metronidazole in killing bacteria involves the penetration of the compound into microorganisms. After entering the microorganism, metronidazole is reduced and produces a polar product, namely 2-hydroxymethyl metronidazole. This compound then interacts with bacterial DNA, causing disruption of the DNA helix structure, and inhibiting nucleic acid synthesis, ultimately resulting in bacterial cell death.¹⁶

The effectiveness of an antibacterial substance in inhibiting and killing bacteria can be influenced by several factors, including the type of compound being tested, its concentration and the type of bacteria that is targeted. Phytochemical analysis in the laboratory shows that water and ethanol extracts from *Syzygium myrtifolium* Walp. contain various bioactive compounds. Water extract from *Syzygium myrtifolium* Walp. is known to contain alkaloids, tannins, saponins, phenolics, steroids and terpenoids, while ethanol extract from *Syzygium myrtifolium* Walp. contains alkaloids, flavonoids, tannins, saponins, phenolics and steroids. Based on these findings, both types of extracts show potential as antibacterial agents because they contain these bioactive compounds.

Antibacterial activity begins with terpenoid compounds, which damage and reduce the permeability of bacterial cell walls, resulting in nutritional deficiencies in the bacteria. The next involves flavonoids, which form extracellular complexes with proteins, followed by steroid compounds that damage lipid membranes, causing liposome leakage. The

saponin compound then causes leakage of proteins and enzymes in the bacterial cells. Alkaloid compounds inhibit the peptidoglycan component in bacterial cells, disrupting the formation of perfect cell walls. After that, tannins disrupt the stability of the bacterial cell membrane by removing important components such as proteins and nucleic acids from the bacterial cell. The last compound, phenol, plays a role in damaging cell walls by destroying the enzymes in bacteria. Thus, this process causes the bacteria to lack nutrition and even kills the bacteria until they die.

In this study, this significant difference was caused by variations in the concentration of water and ethanol extract from *Syzygium myrtifolium* Walp. Differences in the concentration of antimicrobial substances influence the diameter of the inhibition zone, where the higher the concentration of the substance in the extract, the larger the diameter of the bacterial inhibition zone produced.

CONCLUSION

Based on the results of research on the activity of *Syzygium myrtifolium* Walp. water and ethanol extract as antibacterial, *Aggregatibacter actinomycetemcomitans*, it can be concluded that extract of *Syzygium myrtifolium* Walp. water and ethanol fractions have antibacterial activity with concentration. 10%, 20%, 40%, 80% against *Aggregatibacter actinomycetemcomitans* bacteria.

REFERENCES

- Oscarsson J, Claesson R, Lindholm M, Åberg CH, Johansson A. Tools of aggregatibacter actinomycetemcomitans to evade the host response. J Clin Med. 2019;8(7). doi:10.3390/jcm8071079
- Pranata N. Dental Calculus as The Unique Calcified Oral Ecosystem A Review Article. Oceana Biomedicina Journal. 2019;2(2).
- Amaliya A, Pribadi S, Mukmin Akbar Y, Sitam S. Periodontal Disease : A Rise In Prevalence In Military Troops Penyakit Periodontal. Odonto Dental Journal. 2021;8(1).
- Anwar R, Aisyah LS, Lestari FP, Ilfani D, Yun YF, Prestya PD. Senyawa Steroid dari Cocor Bebek (*Kalanchoe tomentosa*) sebagai Antibakteri *Pseudomonas aeruginosa*. ALCHEMY Jurnal Penelitian Kimia. 2021;17(2):202-210. doi:10.20961/alchemy.17.2.51285.202-210
- Rohmawati N, Dyah Puspita Santik Y, Ilmu Kesehatan Masyarakat J, Ilmu Keolahragaan F, Negeri Semarang U. Status Penyakit Periodontal pada Pria Perokok Dewasa. Higeia Journal Of Public Health Research And Development. Published online 2019. doi:10.15294/higeia/v3i2/25497
- Harapan IK, Ali A, Fione VR. Gambaran Penyakit Periodontal Berdasarkan Umur Dan Jenis Kelamin Pada Pengunjung Poliklinik Gigi Puskesmas Tikala Baru Kota Manado Tahun 2017. JIGIM (Jurnal Ilmiah Gigi dan Mulut). 2020;3.
- Ilham AI, El DT, Oktawati S. Perawatan Periodontal Regeneratif Sebagai Penanganan Periodontitis Kronis: Laporan Kasus. Interdental Jurnal Kedokteran Gigi (IJKG). 2024;20(1):118-123. doi:10.46862/interdental.v20i1.8637
- Kodir AIA, Praptiningsih RS, Abdurrohman MMS. Efektivitas Debridemen Dengan Pendekatan Minimal Invasif Sebagai Terapi Awal Pada Periodontitis Kronis. 2023;(https://proceedings.ums.ac.id/index.php/densium/issue/view/57).
- Liu A. Penggunaan Antibiotik Sebagai Terapi Penyakit Periodontal. Kedokteran Gigi Terpadu. 2017;4(1):92-97.
- Anwar R, Sukmasari S, Aisyah LS, et al. Antimicrobial Activity of β -Sitosterol Isolated from *Kalanchoe tomentosa* Leaves Against *Staphylococcus aureus* and *Klebsiella pneumonia*. Pakistan Journal of Biological Sciences. 2022;25(7):602-607. doi:10.3923/pjbs.2022.602.607
- Pane MH, Ave OR, Ayudia EI. Gambaran Penggunaan Obat Herbal Pada Masyarakat Indonesia Dan Interaksinya Terhadap Obat Konvensional Tahun 2020. Journal of Medical Studies. 2021;1(1).
- Hasti S, Renita L, Santi F, Anggraini S, Sinata N, Rusnedy R. Uji Toksisitas Subkronis Ekstrak Etanol Daun Pucuk Merah (*Syzygium Myrtifolium* Walp.) Terhadap Fungsi Hati Dan



-
- Ginjal (Sub-Chronic Toxicity of Syzygium Myrtifolium Walp on Liver and Kidney Function). Jurnal Ilmu Kefarmasian Indonesia. 2022;20(1):30-37.
13. Syari JP, Djohan H, Tumpuk S. Efek Ekstrak Metanol Daun Pucuk Merah Terhadap Kadar Glukosa Darah. Jurnal Laboratorium Khatulistiwa. 2022;6(1):24-30.
14. Setiawan DA, Wakhidah AZ. Botanical, ecology, phytochemical, bioactivity, and utilization of kelat oil (Syzygium myrtifolium Walp.) in Indonesia: A Review. Jurnal Biologi Udayana. 2023;27(1):84-94. doi:10.24843/JBIOUNUD.2023.v27.i01.p09 Syafriana V, Wiranti Y. Potensi Daun Tanaman Pucuk Merah (Syzygium Myrtifolium Walp.) Sebagai Agen Antibakteri Terhadap Streptococcus mutans. Farmasains. 2022;9(2):65-75.
15. Tani PG, Wowor PM, Khoman JA. Uji Daya Hambat Daging Buah Sirsak (Annona muricata L.) Terhadap Pertumbuhan Bakteri Porphyromonas gingivalis. PHARMACON. 2017;6(3).

Antifungal Potency of *Musa paradisiaca* L. Peel Extract Against *Candida albicans* as Acrylic Denture Cleanser

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ABSTRACT

Background: Denture stomatitis is one of the diseases that can arise due to unattended denture hygiene. One of the microorganisms that cause denture stomatitis is *Candida albicans*. **Objective:** The purpose of this study is to determine the potential of *Musa paradisiaca* L. extract concentrations of 25;50;75% as antifungal against *Candida albicans* on removable denture acrylic base. **Materials and Methods:** This study is a laboratory experimental study with a post-test-only control group design using 28 samples for 4 treatment groups. *Candida albicans* (ATCC 10231) was bred on acrylic plates. The acrylic samples were immersed in aquadest, *Musa paradisiaca* L. peel extract concentrations of 25;50; and 75%, respectively. The sample was immersed for 10 minutes and the amount of *Candida albicans* was calculated using colony counter. The research data was analyzed with the One-Way Anova parametric test followed by a post-hoc LSD test. **Results:** Based on the results of statistical tests, significant differences ($p < 0.05$) were obtained in *Musa paradisiaca* L. peel extract concentrations of 25; 50; and 75% compared to the control group. There was a decrease in the amount of *Candida albicans* in the three concentrations of *Musa paradisiaca* L. peel extract, with increased effectiveness at a concentration of 75%. **Conclusion:** *Musa paradisiaca* L. peel extract 25; 50; and 75% solution, especially at 75% concentration, has antifungal activity to reduce the number of *Candida albicans* and has the potential as an acrylic denture cleaner.

Keywords: Antifungal, *Musa Paradisiaca* L. peel extract, *Candida Albicans*, Acrylic Base

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INTRODUCTION

Acrylic removable dentures are an alternative treatment to replace tooth loss because the cost is affordable, with aesthetic preference due to their similar color to the oral mucosa.^{1,2} However, acrylic material also has drawbacks, one of which is porous. This porosity property results in the absorption of fluid in the mouth and can cause the accumulation of food residues and plaque that will interfere with the health of the mucous tissue under the denture.^{1,3} Inadequacy of denture care such as poor hygiene, and long-term and night use can lead to the occurrence of *denture stomatitis* (DS).^{4,5}

*Candida-associated denture stomatitis is prevalent worldwide in the range of 20-67% of prosthesis wearers. The prevalence of DS in India is 70%, and in Indonesia, it is 57,3%. The details are 91.3% of male patients, 45.8% of patients aged 55-56, and 10% of denture stomatitis cases. The majority of the data showed a 60% prevalence rate of DS.*²⁻⁸

One of the main strain microorganisms found in DS was *Candida albicans*. It cannot only attach to the mucosa of the oral cavity but also colonize the acrylic resin base of dentures. It has several pathogenicity factors that can cause a disease called *candidiasis*.¹

Denture cleaning is needed to prevent dentures from being contaminated by bacteria and fungi so that can avoid *denture stomatitis*.⁵ Denture cleaning can usually be done using mechanical and chemical techniques; mechanical techniques are carried out using toothbrushes or ultrasonic devices, while chemical techniques are usually done by immersing dentures in disinfectant solutions with preparations that are widely circulated on the market in tablets such as *effervescent*.^{8,9} Chemical denture cleaners have drawbacks, which are abrasive and cause unwanted side effects even though the material affects oral microorganisms.^{1,5} Therefore, alternative materials are needed that are effective as

bactericides and fungicides, easy to use, and biocompatible with denture materials.⁵

Bananas are one of the plants that are widely studied for their antimicrobial effectiveness. It is known that all parts of the banana plant have a good effect on health, including the peel which has been ignored because it is considered a waste that is not useful. Banana peels contain many beneficial substances in the form of alkaloids, tannins, flavonoids, saponins, and steroids compared to other parts of the banana.^{10,11} One type of banana that can be used as an antifungal in *Candida albicans* is a type of kepok banana (*Musa paradisiaca* L.). A similar study of the effectiveness of banana peel was shown by Loyaga-Castilo et al in 2020 in Peru only examined each 10, 30 and 50% concentrate, then Sari et al in 2023 in Indonesia, compared antifungal and antimicrobial activity from *Musa paradisiaca* L. extract. The two studies revealed that banana peel extract can be effectively used as an antifungal activity at 50 and 75% concentrate, respectively.¹² This study aims to determine the potential antifungal activity of *Musa paradisiaca* L. peel extract concentrations of 25;50;75% on acrylic base.

MATERIALS AND METHODS

Extracting *Musa paradisiaca* L. peel was done by sorting out banana peels with good conditions, which are greenish-yellow with a few brown stains, no fungi or insects, and no discoloration.^{10,13} The banana peel was cleaned with *aquadest* sterile, cut into small pieces (Figure 1.A) dried in the sun, and used in an oven. The dried banana peel was mashed and macerated with 96% ethanol (Figure 1.B). The maceration result was entered in a *vacuum rotary evaporator* then a thick extract of *Musa paradisiaca* L. peel was obtained. Next, this extract was diluted to a concentration of 25;50;75% using *aquadest*.

The sample of this study was *Candida albicans* ATCC (American Type Culture

Collection) 10231, then cultured on the acrylic plate (Figure 1.C) at the Microbiology Laboratory of Andalas University. The number of acrylic plates used in each treatment was 7 plates measuring 10x10x2 mm. These plates were immersed in *aquadest* to reduce residual monomers and sterilized with an *autoclave*. The acrylic plates were then immersed in artificial saliva for 1 hour before being immersed in a container containing *Candida albicans* and incubated for 24 hours at a temperature of 37°C (Figure 1.D)

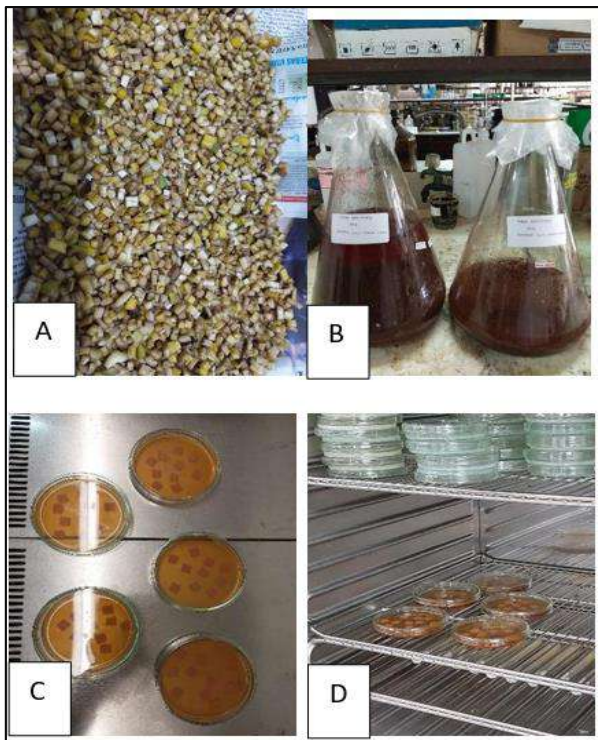


Figure 1. (A) *Musa paradisiaca* L. peel cut into small pieces for drying, (B) Extract-making procedure, (C) Acrylic plate immersed in *Candida albicans* suspension, (D) Acrylic plate and *Candida albicans* specimen incubated for 24 hours for attachment to the acrylic plate.

Antifungal testing of *Musa paradisiaca* L. peel extract as an antifungal against *Candida albicans* ATCC 10231 was performed by immersing the plate in extract (25;50;75% concentrations) and control with *aquadest*.¹¹ Acrylic plates that had been contaminated with *Candida albicans* were put into a closed test tube containing a solution of banana peel extract

of 25;50;75% concentrations and the control for 10 minutes. The plates are rinsed and the acrylic plates are put into the PDB solution for vibration using a vortex until the *Candida attachment* is loose. The *Potato Dextrose Broth* (PDB) solution from the vibration process is taken to be planted on *Potato Dextrose Agar* (PDA) media by the *Spread Plate* method and then incubated. After 48 hours of colony, *Candida albicans* will be formed and calculated on its growth using a *colony counter*. Data analysis was tested statistically using the *one-way ANOVA* followed by a *post-hoc LSD* test.

RESULTS

Data analysis was performed using the one-way ANOVA test. The results showed that the value of $p < 0.05$ means there is a significant difference in the average amount of *Candida albicans* between *aquadest*, and *Musa paradisiaca* L. peel extract concentrations of 25;50;75%.

Table 1. Colonies *Candida albicans* after 10 minutes of immersing in each group

Treatment	N	Mean $\pm$ SD (CFU/ml)	p value
Aquadest solution	7	249.14 $\pm$ 69.293	0.0001
Extract concentration 25%	7	179.43 $\pm$ 64.205	
Extract concentration 50%	7	119.57 $\pm$ 37.960	
Extract concentration 75%	7	70.14 $\pm$ 31.403	

Table 1 shows that the highest average amount of *Candida albicans* is found in the *aquadest* solution. The amount of *Candida albicans* decreased in samples immersed with *Musa paradisiaca* L. peel extract concentrations of 25;50;75%. The lowest amount of *Candida albicans* was in samples immersed with a solution of *Musa paradisiaca* L. peel extract at a concentration of 75%.

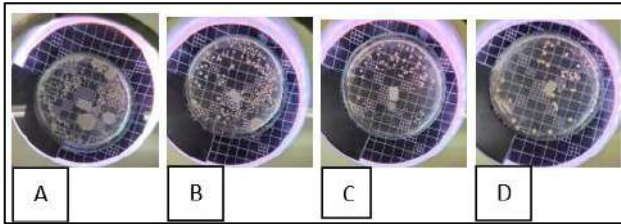


Figure 2. The growth of *Candida albicans* in the control group, a solution of extract of *Musa paradisiaca* L. peel extract concentration (A). Aquadest, (B). 25%, (C). 50% and (D). 75%

Figure 2 shows the difference between the control group and the extract solution. Most colonies were in the control group with aquadest, followed by a concentration solution of 25%; 50%; and 75%. The number of colony *Candida albicans* is counted by *Total Plate Count* (TPC).

Table 2. Results of Post Hoc Analysis of LSD *Musa paradisiaca* L. peel extract

Amount of <i>Candida albicans</i>		p-value
Extract concentration 25%	Extract concentration 50%	0.046
	Extract concentration 75%	0.001
	Aquadest solution	0.022
Extract a concentration 50%	Extract concentration 75%	0.095
	Aquadest solution	0.0001
Extract concentration 75%	Aquadest solution	0.0001

Table 2 showed a significant difference between *Musa paradisiaca* L. peel extract concentration of 25% and aquadest solution group, *Musa paradisiaca* L. peel extract concentration of 50% and 75% group. Table 2 showed a significant difference between the aquadest solution group and *Musa paradisiaca* L. peel extract concentrations of 50% and 75% group. Meanwhile, there was no significant difference between *Musa paradisiaca* L. peel extract concentrations of 50% and 75% groups.

DISCUSSION

The results of the study that was conducted on 28 acrylic plate samples for the control group and the treatment group showed that there was a significant difference in the average number of *Candida albicans* ($p < 0.05$). Increasing the concentration of *Musa paradisiaca* L. peel extract in this study may reduce the number of cells of *Candida albicans* on acrylic plates. The results of this study are in line with research conducted by Loyaga-Castillo *et al* to test the antifungal activity of banana peel extract with concentrations of 10%, 30%, and 50% against growth *Candida albicans*. In this study, it was found that the effectiveness of its inhibition of growth *Candida albicans*. The optimum concentration is 50%.¹⁰

The *Post Hoc* LSD data analysis showed a significant difference between the amount of *Candida albicans* on acrylic plates immersed with aquadest solution group and *Musa paradisiaca* L. peel extract concentrations of 25;50;75% groups. This shows that any concentration of *Musa paradisiaca* L. peel extract has potential as an antifungal against *Candida albicans*. The potential antifungal activity of *Musa paradisiaca* L. peel extract increases along with the increase in concentration from 25% to 50%, and 75%.

Based on data analysis, it is also known that there is no significant difference in the amount of *Candida albicans* from acrylic plates immersed in *Musa paradisiaca* L. peel extract with concentrations of 50% and 75%. It could be interpreted that *Musa paradisiaca* L. peel extract concentrations of 50% and 75% both have the potential to be a good antifungal among all concentrations of *Candida albicans* on removable denture acrylic plates. From the explanation above, it can be seen that in this study, *Musa paradisiaca* L. peel extract concentration of 50% is a minimal effective concentration that can be used as an antifungal against *Candida albicans* on removable denture acrylic plates.

Some other studies have suggested that *Musa paradisiaca* L. peel contains alkaloids, flavonoids, saponins, and tannins which can be used as antioxidants, analgesics, antispasmodics, and also antimicrobials.¹⁰⁻¹⁴ In Previous research conducted by Monica Loyaga-Castillo *et al*, it was found that hydroethanol extract from the peel of *Musa paradisiaca* L. peel extract on *Candida albicans*. So it can be considered an alternative therapy for *Candidiasis* and can reduce the side effects of other chemicals.¹⁰

In research conducted by Karadi *et al*, it is known that *Musa paradisiaca* L. peel extract at a concentration of 100 µg/ml has an inhibitory zone of 24±0.3 mm against fungal colony growth *Candida albicans*. The inhibitory zone is larger than the inhibitory zone in commonly used antifungal drugs such as fluconazole with an inhibitory zone value of 23 mm.¹⁴ Research by Sari *et al* showed that *Musa paradisiaca* L. peel extract has antifungal activity against *Candida albicans* and antibacterial against *Streptococcus mutans* starting from a concentration of 25%.¹⁵

Musa paradisiaca L. peel extract is proven to function as an antifungal against *Candida albicans* on acrylic plates. This happens because *Musa paradisiaca* L. peel extract can inhibit the growth of fungi (*Candida albicans*, *Candida tropicalis*, *Aspergillus niger*) with the content of compounds in the form of tannins, alkaloids, saponins, polyphenols, and flavonoids.¹⁴⁻¹⁸ These compounds are active substances in extracts that act as antifungals with different mechanisms.^{10,15,16,19,20} Based on numerous studies, *Candida* can either directly or through the plaque layer stick to acrylic resin dentures. The emergence of an infectious process can be attributed to this first phase. When food or saliva is eaten without this adhesion, the germs are released from the mouth cavity [53]. Antifungal compounds contained in the peel of *Musa paradisiaca* L. peel extract can affect the structure and function of the cell section *Candida albicans* such as cell walls and cell membranes.⁸ that prevent lipids

on the cell membrane from dissolving. Phenolic chemicals' antifungal properties can precipitate proteins, inactivate enzymes, and destroy cell walls and membranes. Then make it difficult to stick to acrylic resin.⁵

In addition to antifungal activity, other studies have also found antibacterial activity in banana peel extract against *Streptococcus mutans*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella tippy*.^{14,16} Not only limited to the peels, another study from Melisa *et al* (2023) proved antibacterial activity in a combination of Mauli Banana stem and Basil leaves against *Staphylococcus aureus*.¹⁸ Therefore, further research is needed to explore the potential of banana plants as antibacterial and antifungal optimally. Based on this research, the *Musa paradisiaca* L. peel extract, especially with a concentration of 75% has the potential to be used as an alternative to denture cleanser solution so that it can prevent the growth of *Candida albicans* exaggeration and *denture stomatitis* in acrylic removable denture users.^{19,20} However, denture-soaking by this extract has potential side effects on the acrylic denture, including tannin can lead to staining and color change. The limitation of this study is the percentage of the active compound of banana peel extract was not measured, so need advance and further study to check the rate of each active compound and side effects by using banana peel extract, especially for acrylic dentures.

CONCLUSION

Based on the results, it concluded that *Musa paradisiaca* L. peel extract especially at 75% concentration has an antifungal activity against *Candida albicans* on acrylic plate and the potential for acrylic denture cleanser. This study can be used as basic data for the next research so that the antimicrobial activity of *Musa paradisiaca* L. peel extract can be used in dentistry.

REFERENCES

- Alvianita R, Utama MD, Jubhari EH. Utilization herbal as a denture cleanser in inhibiting the growth of *Candida albicans* and *Streptococcus mutans*: a literature review Pemanfaatan herbal sebagai pembersih gigi tiruan dalam menghambat pertumbuhan *Candida albicans* dan *Streptococcus mutans*: Makassar Dent J. 2021;10(2):194–200.
- Ngena R. Gambaran Pengetahuan Tentang Penggunaan Gigi Tiruan Sebagian Lepas Terhadap Kondisi Gigi Dan Mulut Pada Masyarakat Dusun Jawa Desa Kampung Dalam Rantau Prapat. J Ilm Pann Med. 2018;11(3):173–6.
- Setyowati DI, Hamzah Z, Dewi LR. Prevalensi Oral Candidiasis pada Pasien Lanjut Usia yang Memakai Gigi Tiruan di Klinik Penyakit Mulut RSGM UNEJ Tahun 2017. In: Dent Scien Meeting of Jember 5th. 2017. p. 116–23.
- Manikandan S, Vinesh E, Selvi DT, Kannan RK, Jayakumar A, Dinakaran. Prevalence of *Candida* among Denture Wearers and Nondenture Wearers. J Pharm Bioallied Sci. 2022 Jul 13;14(Suppl 1): S702–S705. doi: 10.4103/jpbs.jpbs_781_21
- Peric M, Milicic B, Pficer JK, Zivkovic R, Arsenijevic VA. A Systematic Review of Denture Stomatitis: Predisposing Factors, Clinical Features, Etiology, and Global *Candida* spp. Distribution. J . Fungi 2024, 10(5),328; <https://doi.org/10.3390/jof10050328>
- Ratnasari D, Isnaeni RS, Putri R, Fadilah N. Kebersihan gigi tiruan lepasan pada kelompok usia 45-65 tahun. Padjadjaran J Den Res Students. 2019;3(2):87–91.
- Deepak Nallaswamy V, Anitha R, Rajeshkumar S, Lakshmi T, Ezhilarasan D, Subha M. Effervescent denture cleansing granules using clove oil and analysis of its in vitro antimicrobial activity. Indian J Public Heal Res Dev. 2019;10(11):3687–91.
- Machmud E. Effectiveness of Roselle Effervescent Tablets as Traditional Medicinal Plants in Preventing Growth of *Candida albicans* Colonies and *Streptococcus mutans*. J Contemp Dent Pr. 2018;19:1–4.
- Mansour O, Ahmad H. Materials and methods for cleaning dentures- a review. Int J Dent Clin. 2018;6(2):19–22.
- Chabuck Zainab A.G., Al-Charrakh1 Alaa H., Nada K. Hindi, Shatha K. Hindi. Antimicrobial Effect of Aqueous Banana Peel Extract, Iraq. Res Gate Pharm Sci. 2013;1(4):73–5.
- Hikal WM, Said-Al Ahl HAH, Bratovcic A, Tkachenko KG, Sharifi-Rad J, Kačániová M, et al. Banana Peels: A waste Treasure for human beings. Evidence-based Compl Alt Med. 2022;May:1–9.
- Loyaga-Castillo M, Calla-Poma RD, Calla-Poma R, Requena-Mendizabal MF, Millones-Gómez PA. Antifungal activity of Peruvian banana peel (*Musa paradisica* L.) on *Candida albicans*: An in vitro study. J Contemp Dent Pract. 2020;21(5):509–14.
- Loyaga-Castillo M, Calla-Poma RD, Calla-Poma R, Requena-Mendizabal FR. Antifungal Activity of Peruvian Banana Peel (*Musa paradisica* L.) on *Candida albicans*: An In Vitro Study. The Journal of Contemporary Dental Practice. 2020; 21 (5):509-514
- Sari DP, Rahmi E, Fitria I. Efektivitas ekstrak kulit Pisang Kepok (*Musa Paradisiaca* L) terhadap *Candida albicans* dan *Streptococcus mutans* sebagai pembersih basis akrilik gigi tiruan lepasan. Andalas Dent J.2023;11(77):35-9.
- Karadi R V, Shah A, Parekh P, Azmi P. Antimicrobial activities of *Musa paradisica* and *Cocos nucifera*. Int J Res Pharm Biomed Scien. 2011;2(1):264–7.
- Wenas DM. Kajian ulasan aktivitas farmakologi dari limbah Pisang Ambon dan Pisang Kepok. J Sainstech Farm. 2017;10(1):30–6.
- Andayani R, Afrina. Uji aktivitas antifungal ekstrak kulit Pisang Barangan (*Musa paradisica* L.) terhadap *Candida albicans*. Cakradonya Dent J. 2017;9(1):26–33.
- Asih IAR, Rita WiS, Ananta I, Wahyuni N. Aktivitas antibakteri ekstrak kuli Pisang (*Musa sp*) terhadap *Escherichiacoli* dan *Staphylococcus aureus* serta identifikasi golongan senyawa aktifnya. Cakra Kim J. 2018;6:56–63.
- Utama MD, Jubhari EH, Permatasari. The effect of dental cleanser from plant extracts inhibiting the growth of *Candida albicans* on acrylic resin plates: a systematic review. Indonesian Journal of Prosthodontics. 2022; 3(1): 30-34.
- Budipramana M, Christanti Y, Apriasari ML, Utami JP. Antibacterial activity of mixed Mauli Banana stem and Basil leaves against *Staphylococcus aureus*. Dentino J Kedokt Gigi. 2023;8(1):1.



Comparative Effectiveness of Mouthwash Containing Chlorhexidine 0.12% and Cetylpyridinium Chloride 0.1% with Chlorhexidine 0.2% against *Porphyromonas gingivalis*

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ABSTRACT

Background: Periodontitis is an inflammatory disease that affects the supporting tissue of teeth and causes damage to the periodontal ligament and alveolar bone, generally caused by microorganisms. Chronic periodontitis is often caused by gram-negative anaerobic bacteria, especially *Porphyromonas gingivalis*. Mouthwash active ingredients that frequently used such as chlorhexidine (CHX) or cetylpyridinium chloride (CPC) have an antibacterial effect and prevent plaque formation. **Objective:** This research aims to compare the effectiveness of mouthwash containing CHX 0.12% and CPC 0.1% with CPC 0.2% on the bacterium *Porphyromonas gingivalis* ATCC 33277. **Materials and Methods:** This research is an experimental laboratory by giving treatment to *Porphyromonas gingivalis* with mouthwash CHX 0.12% and CPC 0.1%, CHX 0.2%, and distilled water as the negative control, then calculating the inhibition zone for each treatment and continued with One-Way ANOVA test. **Results:** The result showed the largest average of the inhibitory zone diameter was on CHX 0.2% which was 12,7 mm. Meanwhile, mouthwash containing CHX 0.12% and CPC 0.1% showed an average diameter of inhibitory zone resulted in 10,97 mm. **Conclusion:** In conclusion, CHX 0.2% has greater ability of inhibitory than mouthwash containing CHX 0.12% and CPC 0.1% against the growth of *Porphyromonas gingivalis*.

Keywords: Antibacterial Agents, Cetylpyridinium Chloride, Chlorhexidine, Mouthwash, *Porphyromonas Gingivalis*

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INTRODUCTION

Oral health and hygiene are important things that need attention, because poor oral condition or inflammation can cause pain and discomfort.¹ Periodontal disease is one of the inflammatory condition that often found in oral cavity. The most common periodontal disease are gingivitis and periodontitis.² An inflammatory condition known as periodontitis affects the tissue that supports teeth and usually caused by certain bacteria, where there is damage to periodontal ligament and alveolar bone.³ The spread of inflammation from the epithelium to the connective tissue causes damage to the collagen fibers, followed by loss of attachment which is a sign of change from gingivitis to periodontitis. Periodontitis can be broadly classified into chronic and aggressive periodontitis.⁴ Chronic periodontitis is the most common type of periodontitis. The disease progression of chronic periodontitis is slow to moderate and related to the plaque accumulation and calculus. The development of periodontitis disease may be caused by multifactorial, such as systemic, local or environmental factors that disrupt the interaction of normal host-bacteria.³ Although it can affect people of all ages, adults are more likely to be affected from chronic periodontitis. The level of local factors is correlated with the level of disease progression. Certain bacteria cause chronic periodontitis to develop more slowly.⁴ Chronic periodontitis is often caused by gram-negative anaerobic bacteria, especially *Porphyromonas gingivalis*.⁵

Bacteria that often cause periodontal disease are *Porphyromonas gingivalis*, *Aggregibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium spp.*⁶ *Porphyromonas gingivalis* is a gram-negative bacteria that often found in the subgingival area, and sometimes found in tongue and tonsils. The characteristics of *Porphyromonas gingivalis* are gram-negative, coccobacilli, non-motile, asaccharolytic and

pleomorphic. *Porphyromonas gingivalis* grows anaerobically with dark pigmentation in media containing blood.^{6,7} *Porphyromonas gingivalis* has several virulence factors, such as gingipains, lipopolysaccharide (LPS), fimbriae, lectins (erythrocytes), capsules, collagenase, and proteases which release harmful and toxic metabolites and crucial in the early stages of periodontitis development.⁸

Periodontal disease can be prevented by maintaining oral hygiene, generally through plaque control by mechanical and chemical methods. Examples of mechanical methods are brushing teeth, using dental floss or an interdental brush. Examples of chemical methods are using toothpaste and mouthwash.⁹ The use of mouthwash can control supragingival plaque and gingivitis to clean the oral cavity mechanically.¹⁰

Various kinds of mouthwash ingredients containing antimicrobials such as chlorhexidine and cetylpyridinium chloride, have shown efficacy in decreasing plaque and preserving oral hygiene.¹¹ Chlorhexidine (CHX) is one of mouthwash with bisbiguanide ingredients that can kill microorganism by damaging their membrane cell, which damages the cytoplasm. Based on experimental studies, CHX is the gold standard for evaluating how effectively other mouthwashes work. Long-term use of CHX needs to be considered because it has the potential to cause staining on teeth and changes in taste.¹² Side effects that are often complained by the patients are stains on the teeth, mouth and buccal mucosa. There is also irritation of the oral mucosa, burning sensation and changes in taste perception.¹³ Side effects of CHX use are usually proportional to the duration of treatment.¹⁴

Chlorhexidine (CHX) is available in concentrations of 0.12% and 0.2% which affect plaque inhibition, the plaque inhibitory properties diminishing at lower concentrations.¹³ CHX 0.2% is bactericidal and CHX 0.12% is bacteriostatic. The decrease in CHX concentration is to reduce side effects while maintaining the effectiveness

of the ingredient.¹⁵ CHX 0.2% is effective for preventing plaque and gingivitis.¹⁶

Cetylpyridinium chloride (CPC) is one of the mouthwash's active ingredients, that is made up of quaternary ammonium compounds, which are known to inhibit the growth of bacteria. CPC can also be used as a treatment for halitosis. CPC with a concentration of 0.05%-0.1% effectively acts as an antimicrobial. A further approach for preventing periodontal disease is to use CPC as an antibacterial ingredient in mouthwash since it is considered to be safe, effective and has no serious adverse effects.⁸ CPC can cause extrinsic staining effects but only slightly compared to CHX mouthwash, because CPC is available in preparation alcohol-free, so the side effects that occur are less than CHX and more beneficial for all individuals.¹⁶

There is a combination of CHX and CPC mouthwash to eliminate the side effects of CHX and be more effective in inhibiting the growth of *Porphyromonas gingivalis* bacteria than using CHX mouth wash alone. This research aims to compare the effectiveness of CHX 0.12% and CPC 0.1% mouthwash with CHX 0.2% against *Porphyromonas gingivalis*.

MATERIALS AND METHODS

This field-experimental research examined the effects of different treatments on research subjects. The Randomized Controlled Clinical Trials with a pretest-posttest control group design was used.

This research took place in Tresna Werda Budi Mulia 1 Nursing Home, Jakarta in March 2022. Elderly aged 45-90 years were the population of this research, from which 30 samples were selected. This research is preliminary research done to obtain initial data regarding the effect of black tea candy in increasing saliva volume. Since this research was carried out during the COVID-19 pandemic, the researchers were restricted to including more samples and had to strictly apply safe saliva draw technique.

The inclusion criteria included the people aged 45 - 90 years, did not use drugs and therapies that affect saliva, did not suffer from diabetes, rheumatoid arthritis (RA), HIV, or Sjogren's syndrome, and were willing to sign a letter of consent as research subjects. Meanwhile, the exclusion criteria included subjects who did not consent and were not cooperative during the sampling. A simple random sampling technique was performed to select 30 samples. The independent variables of this study were the SXI score and treatments with 0.2% black tea jelly candy, jelly candy without black tea, and control without treatment, while the dependent variable was saliva volume. This research has received ethical approval from the ethics committee of YARSI University with registration number: 017/KEP-UY/BIA/I/2022.

The ingredients of black tea jelly candy were black tea, water, sucrose, distilled water, tamarind salt, and beef gelatin. These ingredients are mixed, then heated and then left at room temperature so that candy with the consistency of jelly will be formed. The procedure for collecting saliva at the baseline for the control group was to collect unstimulated saliva in a measuring tube. After 5 minutes, subjects gargled in distilled water before their saliva was collected again. As for the black tea treatment group, before consuming the jelly candy without tea or in the black tea jelly candy, subjects were instructed to slightly bow their heads during saliva collection. Saliva collection was carried out for 5 minutes with the interval of spitting into the saliva container once every 1 minute. The saliva volume in the containers was measured. Then the treatment group was instructed to chew jelly candy for approximately 5 minutes. During the saliva collection in the post-intervention examination after consuming black tea and non-black tea jelly candies, subjects were instructed to slightly bow their heads slightly. Saliva collection was carried out for 5 minutes with the interval of spitting into the saliva container once every 1 minute. The volume of saliva in the container was measured

according to the number printed on the container.

Every subject was required to answer the SXI questionnaire which consisted of 5 questions that had been validated to determine the prevalence of xerostomia. Each question is answered in a 3-point Likert scale expressing never = 1; sometimes = 2; and often = 3. All the answers were summarized with a total score ranging between 5-15. The total score was then categorized into categories of normal or no xerostomia complaints (5-7); mild (8-10); moderate (11-13); and severe complaints (greater than 13).¹¹ The data were then statistically analyzed in Shapiro Wilk normality test, dependent T-Test, Wilcoxon and Oneway ANOVA using SPSS program.

RESULTS

The results of measuring the diameter of the inhibitory zone for mouthwash containing CHX 0.12% and CPC 0.1%, CHX 0.2%, and distilled water against *Porphyromonas gingivalis* can be seen in table 1.

Table 1. Results of Measurement of Inhibitory Zone Diameter

Inhibitory Zone Diameter	Treatment		
	CHX 0.12% dan CPC 0.1% (mm)	CHX 0.2% (mm)	Negative control
1	11.57	14.55	0.00
2	10.80	12.1	0.00
3	11.05	13.42	0.00
4	11.35	12.82	0.00
5	11.15	12.65	0.00
6	11.52	12.02	0.00
7	9.7	12.05	0.00
8	10.85	13.6	0.00
9	10.72	11.05	0.00
Mean (mm)	10.97	12.7	0

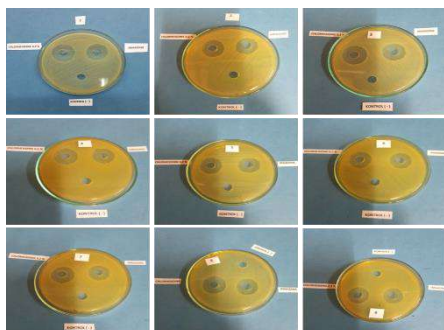


Figure 1. The results of observation of the inhibitory zone diameter of CHX 0.12% and CPC 0.1%, CHX 0.2%, and control negative against *P. gingivalis*

Based on research, the largest diameter of the inhibition zone was in the CHX 0.2% mouthwash treatment, which was 12.7 mm. The next diameter of inhibition zone is in the mouthwash treatment containing CHX 0.12% and CPC 0.1%, which was 10.97 mm. While in the negative control treatment, no inhibition zone was developed or the inhibition zone was zero. Based on the classification of inhibition, the result of measurement of the inhibitory zone diameter for CHX 0.12% and CPC 0.1% and CHX 0.2% included in the strong category (Table 2).

Table 2. Classification of inhibition zones of CHX 0.12% and CPC 0.1% and CHX 0.2% against *P. gingivalis*

Treatment	Diameter mean (mm)	Classification of Inhibition Zone
CHX 0.12% dan CPC 0.1%	10.97	Strong
CHX 0.2%	12.7	Strong

The normality test was done using *Shapiro-Wilk* because the samples were less than 50. The normality test results for CHX 0.12% and CPC 0.1% showed a significance value of 0.138 and for CHX 0.2% showed significance value of 0.905. The data results show a p-value > 0.05, it can be conclude that the data is normally distributed, then it can be continued with analysis statistic using *One Way ANOVA* test. After normality test, continued with homogeneity test using the *Levene* test to find out whether the data is homogeneous or not. The homogeneity test results show a significance value of 0.002 (p-value < 0.05) so it can be assumed that the data is not homogeneous.

The results of *One Way ANOVA* test showed a significance value of 0.000 where p-value < 0.05, means there were significant differences in the three treatments given. The non-homogeneous data was continued with *Post Hoc* test using the *T-test* to determine which treatment was the most significant. *Post Hoc* test results showed p-value < 0.05. It can be

concluded that CHX 0.12% and CPC 0.12%, CHX 0.2%, and distilled water were significantly difference in inhibiting *Porphyromonas gingivalis*.

DISCUSSION

This research used well diffusion method to see the difference in effectiveness between mouthwash containing CHX 0.12% and CPC 0.1% with CHX 0.2%. The larger the clear zone formed around the hole, the higher the inhibition. From the results of the inhibition zone measurements, the largest average of inhibitory zone's diameter is on mouthwash containing CHX 0.2%. This shows that CHX 0.2% is the most effective mouthwash in inhibiting *Porphyromonas gingivalis* compared to mouthwash containing CHX 0.12% and CPC 0.1%.

Based on the research by Betadion R, et al., chlorhexidine has the strongest antibacterial effect against *Porphyromonas gingivalis*, so CHX is used as the gold standard and often as a positive control for antibacterial examination of other materials.¹⁸ CHX is considered as gold standard for antimicrobial mouthwash due to its proven on long-term of effectiveness. However, due to the side effects of CHX such as staining/discoloration of teeth and oral mucosa, unpleasant taste, and alcohol content, certain individuals cannot use CHX and its can only be used in the short term.

The mechanism action of CHX as an antibacterial is described with the damage to bacterial cell membrane and leakage of cytoplasmic components. The positive charge (cations) in CHX attach the negative charge of bacterial molecules (anions). This will cause changes in the cell membrane which will disrupt the permeability of bacterial cell wall, then release the intercellular fluid or leakage of components resulting in cell death. The higher the concentration of CHX, the greater the damage to the bacterial cell membrane that occurs. At low and high concentrations, CHX is

bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria).¹⁸

CPC plays role in antimicrobial activity on supragingival plaque bacteria.¹⁹ Several studies examining the combination efficacy of CPC and CHX and showed a decrease result in plaque levels and numbers of bacteria, as well as bleeding on probing (BOP) scores. The mouthwash combination of CPC and CHX may be effective for long-term use, due to the lower concentrations of CHX. It is hoped that this combination of mouthwash can maintain its effectiveness by minimizing the side effects that will occur.

The mechanism action of CPC as an antibacterial by increasing interaction with bacterial cells and causing damage to the cell membrane resulting in leakage of cytoplasmic components, metabolic disorders and eventually cause death cell of bacteria. At low concentrations, CPC affects cells by disrupting osmoregulation and homeostasis. At high concentrations, CPC cause membrane damage and leakage of cytoplasmic components. CPC can also inhibit the synthesis of glucans that it can inhibit the formation of biofilms.²⁰

Based on research by Stela Lima F, et al., there are limitations to the use of CPC, where the *Porphyromonas gingivalis* only decreases with the use of CHX. Levels of periodontopathogens in CPC-treated biofilms were statistically similar to untreated biofilms. CPC only reduced 50% levels of *Porphyromonas gingivalis*.²¹ In addition, the concentration of CHX in CHX 0.2% was higher compared to the combination of CHX 0.12% and CPC 0.1%. These things allow the use of CHX 0.2% to be better than CHX 0.12% and CPC 0.1% against *Porphyromonas gingivalis*.

There are various other factors that affect the results of the differences in the inhibition zones formed. Factors that can affect the inhibition zone include the sensitivity of the organism, incubation temperature, incubation time, turbidity of the bacterial suspension, and thickness of the agar medium. The incubation

temperature must be done at 37°C and for 24 hours, temperatures less than 37°C can cause a larger inhibition zone diameter. In bacterial suspension turbidity, the diameter of inhibition zone will be larger if the suspension is not more turbid than the turbidity of standard McFarland 0.5, and will be smaller if the suspension is more turbid. Less thickness of agar media can cause the diffusion process to be faster and vice versa.²²

CONCLUSION

Based on the results of the research, it can be concluded that CHX 0.2% mouthwash has greater inhibitory zone than CHX 0.12% and CPC 0.1% mouthwash against *Porphyromonas gingivalis*. There is a significant difference between the effectiveness of mouthwash containing CHX 0.12% and CPC 0.1% with CHX 0.2% mouthwash in inhibiting the growth of *Porphyromonas gingivalis*.

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REFERENCES

- Lang NP, Bartold PM. Periodontal Health. J Periodontol. 2018; 89(1):9–16. DOI: 10.1002/JPER.16-0517.
- Achmad H, Singgih MF, Huldani, Ramdhani AF, Ramadhany YF. Inhibitory Power Test of White Rice Bran Extract (*Oryza sativa* L.) with the Solution of Ethanol and Aquades on *Porphyromonas gingivalis* (In Vitro) Bacteria. Systematic Reviews in Pharmacy. 2020; 11(6):858–63. DOI: 10.31838/srp.2020.6.123.
- Carranza FA, Newman MG, Takei HH, Klokkevold PR. Carranza's Clinical Periodontology. 13th ed. Philadelphia: Elsevier; 2019.
- Mehrotra N, Singh S. Periodontitis. StatPearls Publishing. 2023.
- Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Azodi MZ. Study of *Porphyromonas gingivalis* in periodontal diseases: A systematic review and meta-analysis. Med J Islam Repub Iran. 2017; 31(62):2–5. DOI: 10.18869/mjiri.31.62.
- Marsh PD, Lewis MA, Rogers H, Williams DW, Wilson M. Marsh and Martin's Oral Microbiology. 6th ed. Marsh and Martin's Oral Microbiology. UK: Elsevier; 2016.
- Samaranayake L. Essential Microbiology for Dentistry. 5th ed. Essential Microbiology for Dentistry. Churchill Livingstone Elsevier; 2018.
- Setiawatie EM, Valentina R, Meiliana RS. Effectiveness of Cetylpyridinium Chloride in Reducing the Growth of Bacteria that Cause Periodontal Disease. 2023; 11(2):115–20. DOI: 10.35790/eg.v11i.
- Toar AI, Posangi J, Wowor V. Daya Hambat Obat Kumur Cetylpyridinium Chloride dan Obat Kumur Daun Sirih Terhadap Pertumbuhan *Streptococcus mutans*. Jurnal Biomedik. 2013; 5(1):163–8. DOI: 10.35790/jbm.5.1.2013.2639.
- Takenaka S, Sotozono M, Ohkura N, and Noiri Y. Evidence on the Use of Mouthwash for the Control of Supragingival Biofilm and Its Potential Adverse Effects. Antibiotics. 2022; 11: 727.
- Yarahmadi N, Hashemian F, Doust HH. Clinical Effects of Chlorhexidine 0.2% and Cetylpyridinium 0.05% Combination in Comparison with Chlorhexidine, Cetylpyridinium and Persica in Reducing Oral Bacteria in Healthy Individuals. J Pharm Care. 2020; 8(3):116–22. DOI: 10.18502/jpc.v8i3.4545.
- Adkins KL. Effectiveness of cetylpyridinium chloride, chlorhexidine gluconate, chlorine dioxide, and essential oils against *F. nucleatum*, *P. gingivalis*, *S. mutans* and *S. sobrinus* - a biofilm approach. 2013; 5(1):15–23.
- Hassan Najafi M, Taheri M, Reza Mokhtari M, Forouzanfar A, Farazi F, Mirzaee M, et al. Comparative study of 0.2% and 0.12% digluconate chlorhexidine mouth rinses on the level of dental staining and gingival indices. Dent Res J (Isfahan). 2013; 9(3):305–8.
- Tartaglia GM, Tadakamadla SK, Connelly ST, Sforza C, Martín C. Adverse events associated with home use of mouthrinses: a systematic



- review. *Ther Adv Drug Saf.* 2019; 10(1):1–16. DOI: 10.1177/2042098619854881.
15. Dutt P, Kr Rathore P, Khurana D. Chlorhexidine - An antiseptic in periodontics. *IOSR Journal of Dental and Medical Sciences.* 2014; 13(9):85–8. DOI: 10.4103/jpbs.JPBS_162_20.
16. Sari D Novita, Cholil, Sukmana B Indra. Perbandingan Efektifitas Obat Kumur Bebas Alkohol Yang Mengandung Cetylpyridinium Chloride Dengan Chlorhexidine Terhadap Penurunan Plak. *Dentino.* 2014; 2(2):179–83.
17. Tille PM. Bailey & Scott's Diagnostic Microbiology. 13th ed. St. Louis, Missouri: Elsevier; 2014.
18. Sinaredi BR, Pradopo S, Wibowo TB. Daya antibakteri obat kumur chlorhexidine, povidone iodine, fluoride suplementasi zinc terhadap *Streptococcus mutans* dan *Porphyromonas gingivalis*. *Dent J.* 2014; 47(4):211–4. DOI: 10.20473/j.djmk.v47.i4.p211-214.
19. Setiawatie EM, Valentina R, and Meiliana RS. Effectiveness of Cetylpyridinium Chloride in Reducing the Growth of Bacteria that Cause Periodontal Disease. *e-GiGi.* 2023; 11 (2): 115-120 DOI: <https://doi.org/10.35790/eg.v11i2.44510>.
20. Mao X, Auer DL, Buchalla W, Hiller KA, Maisch T, Hellwig E, et al. Cetylpyridinium Chloride: Mechanism of Action, Antimicrobial Efficacy in Biofilms, and Potential Risks of Resistance. *Antimicrob Agents Chemother.* 2020; 64(8):2–4. DOI: 10.1128/AAC.00576-20.
21. De Miranda SLF, Damaceno JT, Faveri M, Figueiredo LC, Soares GMS, Bueno-Silva B. In Vitro Antimicrobial Effect of Cetylpyridinium Chloride on Complex Multispecies Subgingival Biofilm. *Braz Dent J.* 2020; 31(2):103–8. DOI: 10.1590/0103-6440202002630.
22. Zeniusa P, Ricky Ramadhian M, Hamidi Nasution S, Karima N. Uji Daya Hambat Ekstrak Etanol Teh Hijau Terhadap *Escherichia coli* Secara In Vitro. *Majority.* 2019; 8(2):136–43.



Correlation Between Cariogenic Diets and Oral Hygiene Habits on Early Childhood Caries in Klungkung, Bali

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ABSTRACT

Background: Early Childhood Caries (ECC) is one of the most common dental and oral diseases occurring in children aged 48-71 months. Several factors can influence the occurrence of dental caries in children, one of which is the consumption of cariogenic foods that easily stick to teeth. These foods are fermented by certain bacteria to form acids, which can lead to plaque formation and damage tooth structures if left unchecked for an extended period. **Objective:** To examine the influence of cariogenic dietary patterns and dental and oral hygiene habits on ECC in children aged 48-71 months at Kumara Bhuana Saraswati Kindergarten, Klungkung.

Materials and Methods: This research employs a quantitative study design with an analytical cross-sectional approach. The sampling technique used was non-probability sampling. The study involved 67 pairs of parents and their children (aged 48-71 months) from Kumara Bhuana Saraswati Kindergarten, Klungkung. **Results:** This study indicate a correlation with a p-value of <0.05 between the consumption of cariogenic foods with dental and oral hygiene maintenance behavior, and between the consumption of cariogenic foods with incidence of ECC in children aged 48-71 months at Kumara Bhuana Saraswati Kindergarten, Klungkung.

Conclusion: There is a significant influence of cariogenic dietary patterns and dental and oral hygiene maintenance behavior on the incidence of Early Childhood Caries (ECC) in children aged 48-71 months at Kumara Bhuana Saraswati Kindergarten, Klungkung. Efforts to reduce ECC cases should focus on promoting healthier dietary choices and improving dental and oral hygiene practices among children and their caregivers.

Keywords: Cariogenic Diet, Early Childhood Caries, Oral Hygiene Habits

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INTRODUCTION

Child dental caries often occurs in the maxillary deciduous teeth and first permanent molars.¹ This pattern of caries is commonly referred to as Early Childhood Caries (ECC).² ECC is a complex disease that involves the maxillary deciduous incisors, which can develop within a month after the eruption of these teeth and spread rapidly to other deciduous teeth.^{3,4} ECC not only affects the oral health of infants and preschool-aged children but can also impact their overall health. ECC leads not only to pain in the oral cavity and enamel defects but also causes other issues, such as difficulties with eating and speaking, as well as an increased risk of caries in permanent teeth.⁵

Dental caries remains one of the most common oral health problems faced by children worldwide. The prevalence of dental caries in children aged 3-5 years varies across continents and countries.⁶ Data from the United States show a higher prevalence compared to countries in Europe, with 40% of children experiencing dental caries by preschool age, while in the UK, 12% of 3-year-old children show visible signs of dental caries.⁷ Studies in other countries indicate higher caries prevalence among children, with 36-85% in Asia, 38-45% in Africa, and 22-61% in the Middle East.⁸ Surveys in Cambodia and Indonesia show high prevalence and severity of ECC, with 90% of children aged 3-5 years having a dmft index > 6.⁹ The 2018 Basic Health Research (Riskesdas) survey indicated that the proportion of the population with oral health problems, based on Indonesian characteristics, was 41.1% for children aged 3-4 years, 67.3% for those aged 5-9 years, and 55.6% for those aged 10-14 years, with a national percentage of 57.6% of the population having oral health issues.¹⁰

Children are vulnerable to dental caries because they typically have behaviors or habits that do not support good oral health. One such behavior is the consumption of cariogenic foods, which are foods that can lead to dental caries.

Cariogenic foods tend to be sticky, break down easily, and are high in carbohydrates, especially refined carbohydrates, such as candies, chocolate, bread, and biscuits. Excessive consumption of cariogenic foods can increase acid production by bacteria, making the oral cavity more acidic and damaging the tooth enamel.^{8,9}

In addition, oral hygiene habits are also identified as a risk factor for dental caries. There is a strong association between oral hygiene behaviors and the prevalence of dental caries.^{11,12} The quality of children's oral hygiene practices and parents' ability to limit the consumption of cariogenic foods are factors that can influence dental caries in children.¹³ Oral hygiene behaviors, such as the method of brushing teeth, brushing from an early age, brushing with parental supervision, and using fluoride toothpaste daily, have been proven to significantly reduce the prevalence of ECC.¹⁴ A study by Chu et al. in 2012 showed that tooth brushing is one of the factors that influences dental caries in preschool-aged children. The survey in this study revealed that children who started brushing their teeth at an early age had fewer dental caries. Brushing teeth helps keep erupted teeth clean and can deliver fluoride to the tooth surface when using fluoride toothpaste.¹⁵ Parental assistance and guidance are crucial in reducing the risk of developing dental caries. Children's tooth brushing with the help of parents or caregivers is more effective in removing dental plaque and maintaining the oral cavity environment optimally, thus reducing the risk of dental caries in children.¹⁶ Based on the previous explanation, the objective of this study is to determine the impact of cariogenic dietary patterns and oral hygiene habits on ECC in children aged 48-71 months at Kumara Bhuna Saraswati Kindergarten in Klungkung.

MATERIALS AND METHODS

The type of research used is a quantitative research method with an analytic

cross-sectional approach. The research was conducted from April to May 2022 at Kumara Bhuana Saraswati Kindergarten, Klungkung District, Klungkung Regency. The sampling technique used in this study is non-probability sampling. The sampling type used is purposive sampling. The sample size in this study is 67 pairs of parents and children aged 48 - 71 months who meet the inclusion criteria and do not have exclusion criteria. The ECC assessment is conducted by asking parents to send photos of their child's teeth via the available google form. The photo-taking procedure follows the instructions and examples provided, which include front view, right and left side views, and occlusal views of maxilla and mandible. The photos submitted will be analyzed to determine the ECC classification. Cariogenic food consumption and oral hygiene habits are evaluated using a questionnaire. The questionnaire has been tested for validity and reliability using SPSS. The data obtained were processed using SPSS. Univariate analysis was presented in the form of frequency distribution tables and interpreted narratively, while bivariate analysis to examine the relationship between cariogenic food consumption and oral hygiene habits was conducted using Fisher's Exact Test.

RESULTS

Respondents in this study were parents (father/mother) and the sample in this study were children who attended Kindergarten Kumara Bhuana Saraswati Klungkung who met the inclusion and exclusion criteria and who were willing to be the research sample. The total number of respondents and samples in this study were 67 pairs of parents and children. The following are the characteristics of the respondents, namely parents' education and the characteristics of the sample, namely the sex of the child and the age of the child.

Table 1. Sociodemographic characteristics

Sociodemographic Characteristics	Frequency (n)	Percentage (%)
Parents Education:		
Did not finish elementary school	0	0
Graduated from elementary school/middle school	4	6
Graduated from high school/equivalent	22	32.8
Graduated from college S1/S2/S3	41	61.2
Total	67	100
Gender		
Man	30	44.8
Woman	37	55.2
Total	67	100
Sample Age		
48-59 months	20	29.9
60-71 months	46	70.1
Total	67	100

Based on parents' education, the highest number of respondents were in the S1, S2, S3 tertiary graduate group, namely 41 people (61.2%) and the least were in the elementary school/middle school graduate group, namely 4 people (6%). In the gender of the sample, there were more women, namely 37 children (55.2%), while there were fewer male samples, namely 30 children (4.8%). In the sample age, there were 46 children (68.7%) aged 60-71 months compared to those aged 48-59 months, namely 21 children (31.3%).

Table 2. Distribution of consumption of cariogenic diets

Cariogenic Diets	Frequency (n)	Percentage (%)
Low	30	44.8
Moderate	28	41.8
High	9	13.4
Total	67	100

Table 3. Cross-tabulation of cariogenic diets by genders

Cariogenic Diets	Male		Female		Total	
	n	%	n	%	n	%
Low	12	17.9	18	26.9	30	44.8
Moderate	11	16.4	17	25.4	28	41.8
High	7	10.4	2	3.0	9	13.4
Total	30	44.8	37	55.2	67	100

Table 2 shows the highest number of samples with a cariogenic diet in the low criteria, namely 30 samples (44.8%) and the least in the high criteria, namely 9 samples (13.4%). Table 3 shows that the male is mostly in the low criteria, namely 12 samples (17.9%) and the least in the high criteria, namely 7 samples (10.4%). In the female, the most were in the low criteria, namely 18 samples (26.9%) and the least in the high criteria, namely 2 samples (3.0%).

Table 4. Cross-tabulation of cariogenic diets by age

Cariogenic Diets	48-59 months		60-71 months		Total	
	n	%	n	%	n	%
Low	9	13.4	21	31.3	30	44.8
Moderate	8	12.0	20	29.9	28	41.8
High	3	4.5	6	8.9	9	13.4
Total	20	29.9	47	70.1	67	100

Table 5. Distribution of dental & oral hygiene behavior

Dental & Oral Hygiene Behavior	Frequency (n)	Percentage (%)
Poor	28	41.8
Fair	21	31.3
Good	18	26.9
Total	67	100

Table 4 shows that at the age of 48-59 months most of the low criteria are 9 samples (13.4%) and the least are high criteria, namely 3 samples (4.5%). At the age of 60-71 months the most were in the low criteria, namely 21 samples (31.3%) and the least in the high criteria, namely 6 samples (8.9%). Table 5 shows that the behavior of maintaining dental & oral hygiene is most common in the poor criteria, namely 28 samples (41.8%) and at least in the good criteria, 18 samples (26.9%).

Table 6. Cross-tabulation of dental & oral hygiene behavior by genders

Dental & Oral Hygiene Behavior	Male		Female		Total	
	n	%	n	%	n	%
Poor	17	25.4	11	16.4	28	41.8
Fair	9	13.4	12	17.9	21	31.3
Good	4	6.0	14	20.9	18	26.9
Total	30	44.8	37	55.2	67	100

Table 7. Cross-tabulation of dental & oral hygiene behavior by age

Dental & Oral Hygiene Behavior	48-59 months		60-71 months		Total	
	n	%	n	%	n	%
Poor	8	11.9	20	29.9	28	41.8
Fair	6	9.0	15	22.3	21	31.3
Good	6	9.0	12	17.9	18	26.9
Total	20	29.9	47	70.1	67	100

Table 6 shows that the male is mostly in the less criteria, namely 17 samples (25.4%) and the most slightly on good criteria, namely 4 samples (6.0%). In the female, the most were in good criteria, namely 14 samples (20.9%) and the least were in poor criteria, namely 11 samples (16.4%). Table 7 shows that at the age of 48-59 months the most are in the less criteria, namely 8 samples (11.9%) and the least in the sufficient and good criteria, namely 6 samples (9.0%). At the age of 60-71 months, the most were in the less criteria, namely 20 samples (29.9%) and the least in the good criteria, namely 12 samples (17.9%).

Table 8. ECC incidence

ECC	Frequency (n)	Percentage (%)
ECC 0	17	25.4
ECC 1	4	6.0
ECC 2	16	23.8
ECC 3	30	44.8
Total	67	100

Table 9. Cross-tabulation of ECC by genders

ECC	Male		Female		Total	
	n	%	n	%	n	%
ECC 0	3	4.5	14	20.9	17	25.4
ECC 1	2	3.0	2	3.0	4	6.0
ECC 2	6	9.0	10	14.9	16	23.9
ECC 3	19	28.4	11	16.4	30	44.8
Total	30	44.8	37	55.2	67	100

Table 8 shows that the highest number of samples is in the ECC 3 criteria of 30 samples (44.8%) and the least number of samples is in the ECC 1 criteria, namely 4 samples (6.0%). Table 9 shows that male is mostly at ECC 3 of 19 samples (44.8%) and the least in ECC 1, namely 2 samples (3.0%). Female is the most common in ECC 0 criteria, namely 14 samples (20.9%) and the least in ECC 1 criteria, namely 2 samples (3.0%).

Table 10. Cross-tabulation of ECC by age

ECC	48-59 months		60-71 months		Total	
	n	%	n	%	n	%
	n	%	n	%	n	%
ECC 0	6	9.0	11	16.4	17	25.4
ECC 1	1	1.5	3	4.5	4	6.0
ECC 2	5	7.5	11	16.4	16	23.9
ECC 3	8	11.9	22	32.8	30	44.8
Total	20	29.9	47	70.1	67	100

Table 10 shows that the age of 48-59 months is the most in ECC 3 criteria, namely 8 samples (11.9%) and the least in ECC 1 criteria, namely 1 sample (1.5%). At the age of 60-71 months the most on ECC criteria 3, namely 22 samples (32.8%) and the least on ECC criteria 1, namely 3 samples (4.5%). Bivariate analysis in this study used the chi-square test to determine the effect between the two variables. However, the results of the chi square test did not meet the requirements because the expectation value was more than 20%, so the Fisher's Exact Test was used as an alternative test.

Table 11. The effect of consuming cariogenic diets on ECC incidence

Cariogenic Diets	ECC 0		ECC 1		ECC 2		ECC 3		Total		p-value
	n	%	n	%	n	%	n	%	n	%	
Low	8	11.9	2	3.0	7	10.4	13	19.4	30	44.8	0.029
Moderate	9	13.4	2	3.0	9	13.4	8	11.9	28	41.8	
High	0	0	0	0	0	0	9	13.4	9	13.4	
Total	17	25.3	4	6.0	16	23.9	30	44.8	67	100	

The p-value of the cariogenic food consumption variable with ECC is 0.029 (<0.05), so that the null hypothesis (H_0) is rejected, and the alternative hypothesis (H_a) is accepted. It can be concluded that there is an effect of a cariogenic diet on the incidence of ECC in children aged 48-71 months at Kumara Bhuana Saraswati Klungkung Kindergarten.

Table 12. The influence of dental & oral hygiene behavior with ECC incidence

Dental & Oral Hygiene Behavior	ECC 0		ECC 1		ECC 2		ECC 3		Total		p-value
	n	%	n	%	n	%	n	%	n	%	
Poor	2	3.0	1	1.5	9	13.4	16	23.9	28	41.8	0.000
Fair	3	4.4	2	3.0	6	9.0	10	14.9	21	31.3	
Good	12	17.9	1	1.5	1	1.5	4	6.0	18	26.9	
Total	17	25.3	4	6.0	16	23.9	30	44.8	67	100	

The p value of the dental & oral hygiene maintenance behavior variable with the incidence of ECC is 0.000 (<0.05) so the null hypothesis (H_0) is rejected, and the alternative hypothesis (H_a) is accepted. It can be concluded that there is an influence of dental & oral hygiene maintenance behavior on the incidence of ECC in children aged 48-71 months at TK Kumara Bhuana Saraswati Klungkung

DISCUSSIONS

The results of the study show that there is an effect of a cariogenic diet on the incidence of ECC (p-value 0.029). Based on these results, it can be concluded that children who consume cariogenic foods are more at risk of developing dental caries compared to children who do not consume cariogenic foods. Cariogenic foods are those that contain high amounts of processed carbohydrates, which can lead to dental caries.¹⁷

Dental caries is mainly caused by the frequent consumption of refined carbohydrates. Refined carbohydrates, often referred to as simple carbohydrates, are carbohydrates that have had their nutrients removed. Sucrose is the most cariogenic simple carbohydrate because it has a unique ability to support the synthesis of extracellular glucan by *Streptococcus mutans*. All fermentable carbohydrates are metabolized by bacteria in various ways, producing acids as byproducts.¹⁸ This can lower the pH of the oral cavity to as low as 4.5. After consuming cariogenic foods, the plaque pH decreases and remains low until the sugar is cleared from the mouth and the acid produced is dissolved or neutralized by saliva. Persistent acidic conditions can lead to the dissolution (demineralization) of minerals in the hard dental tissue underneath.¹⁹

Caries tends to develop relatively slowly, over months or years. In the early stages, lesions under the surface can be arrested or reversed. When the plaque pH returns to neutral, mineral ions in the plaque contribute to the remineralization of carious lesions. The process of dental caries is caused by an imbalance between demineralization and remineralization.²⁰ Thus, a high consumption of cariogenic foods can lower plaque pH, disrupting the balance between demineralization and remineralization, which leads to dental caries. The results of this study align with research conducted by Rekawati and Frisca on children in grades 1–3 at SD Negeri 3 Fajar Mataram, which showed a significant statistical relationship between the consumption of cariogenic foods and dental caries.²¹ A study by Winahyu et al on children in grades 1 and 2 at SDN 1 Sepatan, Tangerang, also reported a significant relationship between the consumption of cariogenic foods and the incidence of dental caries.²²

This study also found an effect between oral hygiene habits and the incidence of ECC (p-value 0.000). The results of this study are consistent with research conducted by Maulidta

et al, which stated that there is a relationship between tooth brushing habits and the incidence of dental caries in preschool-aged children.²³ A study by Percival et al also showed a link between children with ECC and poor oral hygiene in children aged 3-5 years.²⁴ As we know, dental caries is a multifactorial disease. Several interactions between the host, diet, and microorganisms on the tooth surface over time result in the dissolution of tooth tissue, which causes dental caries.²⁵ Caries will not occur if children have good oral hygiene, such as regular tooth brushing habits. Regular tooth brushing helps remove food particles stuck to teeth after consuming cariogenic foods, thereby reducing the risk of dental caries.²⁶

Behaviors related to dental and oral health, such as cleaning teeth, dietary practices, and using preventive dental services when visiting the dentist, are linked to the incidence of ECC. Oral hygiene behaviors such as brushing techniques, starting tooth brushing at an early age, brushing with parental supervision, and using fluoride toothpaste daily can reduce the prevalence of ECC.²⁷ Brushing regularly with fluoride toothpaste can decrease *Streptococcus mutans* colonization, helping to reduce the risk of ECC. Brushing teeth can help remove plaque from teeth and reduce the number of bacteria, while fluoride in toothpaste aids in remineralizing carious lesions. Parents are advised to start brushing their children's teeth with a soft-bristled toothbrush and fluoride toothpaste as soon as the first tooth erupts to prevent ECC.²⁸

The causes of early childhood caries are more complex than dental caries that occur in adulthood due to the strong influence of infant feeding practices and parental oral hygiene behaviors.²⁹ The etiology of ECC also includes other risk factors such as genetics, saliva, oral hygiene, family, child behavior and habits, as well as the environment. Physical characteristics of teeth, such as deep and narrow occlusal fissures, deep buccal or lingual pits, and enamel hypoplasia, will affect the development of dental caries. The shape, size, and arrangement of

teeth will also influence the cleaning effects of saliva.³⁰

CONCLUSION

There is a relationship between cariogenic food consumption and oral hygiene habits with Early Childhood Caries (ECC) in children aged 48-71 months at Kumara Bhuana Saraswati Klungkung Kindergarten.

REFERENCES

1. Khulwani QW, Nasia AA, Nugraheni A. Hubungan pengetahuan, sikap, dan perilaku kesehatan gigi dan mulut terhadap status karies siswa SMP Negeri 1 Selogiri, Wonogiri. *e-GiGi* 2021;9(1):41–4. Doi: <https://doi.org/10.35790/eg.9.1.2021.32570>.
2. Daud S, Said H. Cariogenic foods as the cause of dental caries in children. *e-GiGi*. 2022;10(1):38–45. Doi: <https://doi.org/10.35790/eg.v10i1.37435>.
3. Evans MH. Gambaran Early Childhood Caries (ECC) Pada Anak Prasekolah Berdasarkan Faktor Risiko Biopsikosial. *Andalas Dental Journal*. 2019; 9(1): 44–50.
4. Apro V, Susi S, Sari DP. Dampak karies gigi terhadap kualitas hidup anak. *Andalas Dental Journal*. 2020;8(2):89–97. Doi: <https://doi.org/10.25077/adj.v8i2.204..>
5. Anil S, Anand PS. Early Childhood Caries: Prevalence, Risk Factors, and Prevention. *Frontiers in Pediatrics*. 2021; 5: 1–13. doi:10.3389/fped.2017.00157.
6. Maramis JL, Yuliana NM. Peran orang tua dalam memelihara kesehatan gigi mulut dengan karies gigi pada anak Sekolah Dasar kelas 1-3 di Desa Wori Kecamatan Wori Kabupaten Minahasa Utara. *Jurnal Ilmiah Gigi dan Mulut*. 2019;2(1):26–31. Doi: <https://doi.org/10.47718/jgm.v2i1.1411>.
7. Rehena Z. Hubungan Jenis dan Frekuensi Konsumsi Makanan Kariogenik Dengan Kejadian Karies Gigi Pada Anak SD Negeri 5 Waal Kabupaten Maluku Tengah. *Maluccas Helath Journal*. 2020; 2(1): 41–48.
8. Wirata IN, Arini NW, Supariani NN. Gambaran karies gigi serta kebiasaan makan makanan kariogenik pada siswa SDN 5 Abiansemal tahun 2018. *Jurnal Kesehatan Gigi (Dental Health Journal)* 2021;8(1):1–6. Doi: <https://doi.org/10.33992/jkg.v8i1.1350..>
9. Paramytha AN, Jeddy. Hubungan Antara Pola Konsumsi Diet Kariogenik Dengan Tingkat Keparahan Karies Pada Anak Usia 3-5 Tahun. *JKGT*. 2021; 3: 58–62.
10. Kementerian Kesehatan Republik Indonesia. Potret sehat Indonesia dari riskesdas 2018 [Internet]. 2018 [cited 2024 Oct. 28]. Available from: <https://www.kemkes.go.id/article/print/18110200003/potret-sehat-indonesiadari-riskesdas-2018.htm>
11. Saputri MD. Perilaku Pemeliharaan Kesehatan Gigi Mulut dan Kualitas Hidup Siswa MTS AL Islamiyah di Desa Karanganyar Kecamatan Beringin Kabupaten Deli Serdang. 2021. Thesis. Universitas Sumatra Utara.
12. Saptiwi B, Hanafi M, Purwitasari D. Perilaku Pemeliharaan Kesehatan Gigi dan Mulut Terhadap Status Kebersihan Gigi dan Mulut (OHI-S) Warga Samin Surosentiko Kabupaten Blora. *Jurnal Kesehatan Gigi*. 2019; 6(1): 68–71. doi:10.31983/jkg.v6i1.4436.
13. Featherstone J, Crystal Y, Gomez FR. CAMBRA: A Comprehensive Caries Management Guide for Dental Professionals. *Journal of the California Dental Association*, 2019; 3–42.
14. Anita MM. Brushing Your Teeth. *The Journal of the American Dental Association*. 2019; 152 (10): 876.
15. Rachmawati A. Perbedaan Efektivitas Menyikat Gigi Menggunakan Kayu Siwak Dengan Sikat Gigi Konvensional Terhadap Penurunan Debris Indeks Siswa Mi Ma'arif Candran Yogyakarta. 2019. Thesis. Politeknik Kesehatan Kementerian Kesehatan Yogyakarta.
16. Dean JA. McDonald and Avery's Dentistry For The Child and Adolescent. 11th edn. elsevier. 2022.
17. Zhou N, Wong HM, McGrath C. Toothbrush Deterioration and Parents' Suggestions to Improve The Design Of Toothbrushes Used By Children With Special Care Needs," *BMC Pediatrics*. 2020; 20(1): 1–9. doi:10.1186/s12887-020-02347-8.
18. Musa N. The relationship between oral hygiene status and dental caries was assessed using



- ICDAS II criteria. *Journal of Dental Medical Public Health*. 2021;1(1):23–33..
19. Rahman E, Ilmi MB, Anam K. Kebiasaan mengonsumsi jajanan kariogenik sebagai penyebab karies gigi pada anak di Madrasah Diniyah Islamiyah Muhammadiyah Kindaung Banjarmasin. *Jurnal Kesehatan Indonesia*. 2019;9(1):34–7.
 20. Monita W. Gambaran Mengonsumsi Makanan Manis Dengan Jumlah Karies Gigi Pada Anak Pra Sekolah Di Paud Bima Giripeni Wates Kulon Progo. *Politeknik Kesehatan Kementerian Kesehatan*. 2019. Thesis. Poltekkes Jogja.
 21. Rekawati A, Frisca F. Hubungan kebiasaan konsumsi makanan kariogenik terhadap prevalensi karies gigi pada anak SD Negeri 3 Fajar Mataram. *Tarumanagara Med J*. 2020;2(2):205–10. Doi: <https://doi.org/10.24912/tmj.v3i1.9719>
 22. Achmad H, Ramadhany S, Mudjari S, Adam AM. Determinant factors of dental caries in Indonesian children age 8-12 years. *Pesqui Bras Odontopediatria Clin Integr*. 2019;18(1):1–7. Doi: 10.4034/PBOCI.2019.181.64
 23. Maulidta, K. W., Wahyuningsih, & Hastuti, S. Hubungan Kebiasaan Menggosok Gigi dan Konsumsi Makanan Jajanan Kariogenik dengan Kejadian Karies Gigi pada Anak Usia Prasekolah di Taman Kanak-Kanak Pohon Beringin Semarang. *JITK*. 2020; 1 (1): 1-7.
 24. Percival, T., Edwards, J., Barclay, S., Sa, B., & Majumder, M. A. Early Childhood Caries in 3 to 5 Year Old Children in Trinidad and Tobago . *Dentistry Journal*. 2019; 7(1):16. doi: 10.3390/dj7010016.
 25. Ambarawati IGD, Sukrama IDM, Yasa IWPS. Deteksi gen Gtf-B Streptococcus mutans dalam plak dengan gigi karies pada siswa di SD N 29 Dangin Puri. 2020; 11(3): 1049–55. Doi: <https://doi.org/10.15562/ism.v11i3.337>.
 26. Andayasari L, Wibowo W. Status Kesehatan Gigi dan Tindakan Menyikat Gigi Pada Murid Taman Kanak-Kanak. *Padjadjaran Journal of Dental Researchers and Students*. 2020; 4(1): 62–67. doi:10.24198/pjdrs.v4i1.25720.
 27. Laksmiastuti SR, Astoeti TE, Sutadi H, Budiardjo SB. Caries risk factors among children aged 3–5 years old in Indonesia. *Contemp Clin Dent*. 2019;10(3):507–11. Doi: 10.4103/ccd.ccd_877_18.
 28. Annissa S, Nurchandra F. Pola Konsumsi Makanan Kariogenik, Kebiasaan Menggosok Gigi, dan Karies pada Anak Usia Sekolah di SDN Cipedak 02 Jakarta Selatan. *Jurnal Ilmiah Kesehatan Masyarakat*. 2019; 11(2): 159–165.
 29. Ambarawati IGD, Sukrama IDM, Yasa IWPS. Deteksi gen Gtf-B Streptococcus mutans dalam plak dengan gigi karies pada siswa di SD N 29 Dangin Puri. 2020;11(3):1049–55. Doi: <https://doi.org/10.15562/ism.v11i3.337>.
 30. Nasia AA, Rosyidah AN, Ibrahim N. Hubungan Perilaku Kesehatan Orang Tua dan Kualitas Hidup Terkait Kesehatan Gigi dan Mulut Pada Anak Prasekolah. *e-GiGi*, 2022; 10(1): 135. doi:10.35790/eg.v10i1.39126.



The Difference in Blood Pressure Using the Open Extraction Method Before and After Local Anesthesia with Lidocaine + Adrenaline 1:80,000 at RSGM Nala Husada

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ABSTRACT

Background: Local anesthesia is the first step before open method extraction. The local anesthesia contains 2% lidocaine and adrenaline in a 1:80,000 ratio to extend its duration and reduce the risk of toxicity. However, the use of vasoconstrictors may lead to a spike in blood pressure. **Objective:** To determine the difference in blood pressure before and after the administration of lidocaine + adrenaline in a 1:80,000 ratio for open method extraction at RSGM Nala Husada Surabaya. **Materials and Methods:** Blood pressure was measured before and after the administration of local anesthesia containing lidocaine + adrenaline in a 1:80,000 ratio for open method extraction, following the inclusion criteria with a sample size of 21. Blood pressure was measured using an "Omron HEM-FL31" digital sphygmomanometer. Data analysis was performed using a paired t-test. **Results:** The paired t-test results showed a significant difference in systolic blood pressure ($p < 0.05$), but no significant difference in diastolic blood pressure ($p > 0.05$) before and after the administration of local anesthesia for open method extraction patients. The systolic blood pressure showed the highest value after the administration of local anesthesia (122.95 ± 14.59). **Conclusion:** Blood pressure showed a significant difference before and after the administration of local anesthesia containing lidocaine + adrenaline in a 1:80,000 ratio for open method extraction at RSGM Nala Husada Surabaya.

Keywords: Blood Pressure, Lidocaine + Adrenaline 1:80,000, Open Method Extraction

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INTRODUCTION

One of the most common dental treatments performed in oral surgery is tooth extraction. Tooth extraction is the process of removing a tooth from its socket when it cannot be treated with endodontics or other dental procedures.¹ Tooth extraction can also be defined as a procedure for removing a tooth from its alveolar bone socket. This procedure is performed using forceps, a dental elevator, and, in some cases, surgical techniques. There are two types of tooth extractions: the closed method (simple technique) and the open method (surgical technique). Surgical extraction involves removing a tooth through a surgical process, which may include cutting the tooth or bone.² Before performing a tooth extraction using the open method (surgical technique), local anesthesia must be administered to the patient.

The local anesthesia used in dental procedures is typically a combination of lidocaine 2% and adrenaline 1:80,000. Local anesthesia is one of the most commonly used materials in dentistry. Dentists use local anesthesia to eliminate pain during dental treatments. Local anesthesia is divided into two groups: esters and amides. Examples of ester-based local anesthetics include cocaine, procaine, 2-chloroprocaine, tetracaine, and benzocaine. Amide-based local anesthetics include lidocaine, etidocaine, mepivacaine, bupivacaine, prilocaine, and articaine.³ Amide-based local anesthetics are the most commonly used materials in dentistry. Lidocaine, an amide-based local anesthetic, easily diffuses through lipid-rich interstitial tissues and nerves. It has a short onset time of approximately 2 to 5 minutes and a moderate duration of action.⁴ Local anesthesia is often combined with adrenaline, which acts as a vasoconstrictor. Vasoconstrictive agents are used to narrow blood vessels, counteract the vasodilatory effects of local anesthesia, prolong its duration, reduce systemic absorption and toxicity, and provide a blood-free surgical field.⁵

Understanding the pharmacokinetics and potential adverse effects of local anesthesia is crucial for preventing complications and achieving the desired outcomes. However, due to its short onset time, the toxic symptoms that arise are temporary and typically resolve quickly, returning to normal.⁴ The addition of adrenaline to lidocaine may lead to undesirable side effects, such as increased blood pressure, hypotension, chest pain, arrhythmia, tachycardia, and even heart attack. The cardiovascular side effects of local anesthesia are closely related to the concentration of the anesthetic agent in the bloodstream and are dose-dependent.⁵

High blood pressure or High blood pressure, or hypertension, is a common disease with a high prevalence in Indonesia. According to data from the World Health Organization (WHO), hypertension affects approximately 22% of the global population. The results of the 2018 Riskesdas in Indonesia revealed that 34.1% of the population aged 18 years and older suffered from hypertension. The prevalence of hypertension increases significantly among individuals aged 60 years and older.⁶ Hypertension is characterized by an increase in both systolic and diastolic blood pressure. Systolic blood pressure refers to the pressure exerted on the arteries when the left ventricle of the heart contracts, representing the top value in a blood pressure reading. Diastolic blood pressure, on the other hand, is the pressure when the heart relaxes, representing the bottom value.⁷

The condition of a patient who remains conscious during dental treatment can lead to anxiety and pain. The rise in blood pressure during the open-method extraction process may be triggered by the pain stimulus during anesthesia administration and the effects of the vasoconstrictor present in the anesthetic agent.² Blood pressure can increase due to several factors, such as an imbalanced diet, lack of physical activity, physiological stress, anxiety, or pain. Dental treatments, in particular, can induce anxiety and pain, which may contribute to

elevated blood pressure.² It is crucial for dentists to exercise caution when selecting and administering local anesthesia and to ensure the appropriate use of lidocaine. Administering local anesthesia with an incorrect dose or concentration of the anesthetic solution can lead to systemic complications. Additionally, the use of adrenaline is contraindicated in cardiovascular patients, as it can elevate both blood pressure and heart rate.⁸

Previous research has shown that the administration of lidocaine mixed with adrenaline 1:80,000 as a local anesthetic during the closed-method extraction process did not significantly alter blood pressure.⁹ According to Ketabi's (2012) study, the administration of a single cartridge of local anesthetic containing lidocaine + adrenaline 1:80,000 slightly increased blood pressure and pulse rate. However, this minor increase was neither clinically nor medically significant. Nevertheless, the use of lidocaine + adrenaline 1:80,000 may be contraindicated in patients who have undergone heart transplants. Uchiyama's (2018) study found that the use of 2% lidocaine with adrenaline 1:80,000 for inferior alveolar nerve blocks is safe for patients without systemic diseases. However, further investigation is required for patients with cardiovascular issues.

Based on the background above, this study aims to determine the effect of 2% lidocaine+adrenaline 1:80,000 as a local anesthetic on patients undergoing open-method extraction.

MATERIALS AND METHODS

This study is an analytical observational study employing a cross-sectional method. The population of this study consists of all patients who underwent open method extraction with local anesthesia using lidocaine 2% + adrenaline 1:80,000 at RSGM Nala Husada Surabaya during the period of September to October 2022. The sample size for this study is 21 participants, selected using a simple random sampling

technique based on predetermined inclusion and exclusion criteria.

Sample Criteria

The sample criteria for this study included patients aged 18-50 years who were scheduled to undergo open-method tooth extraction with local anesthesia using lidocaine+adrenaline 1:80,000. Participants were required to have no immunocompromised conditions and to exhibit either normotension or prehypertension.

The collected data will be processed and analyzed using SPSS version 27. Initially, the data will be analyzed descriptively, followed by a normality test using the Shapiro-Wilk method. Subsequently, a homogeneity test will be conducted using Levene's Test. The data were found to be normally distributed and homogeneous.

For normally distributed and homogeneous data, a paired t-test will be performed to compare blood pressure before and after the administration of local anesthesia in patients undergoing open-method tooth extraction. The results of the study will then be derived and interpreted.

RESULTS

After measuring blood pressure, a descriptive analysis of the data from 21 samples was conducted.

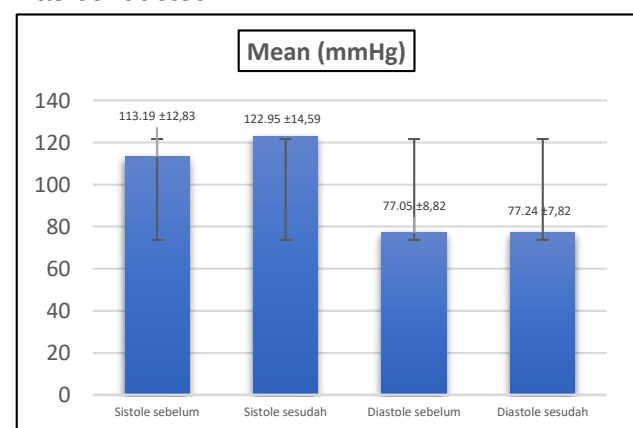


Figure 1. Graph of Descriptive Test Results for Blood Pressure

The lowest systolic blood pressure recorded before anesthesia during open-method extraction was 89 mmHg, while after anesthesia was 97 mmHg. The lowest diastolic blood pressure before anesthesia was 60 mmHg, whereas after anesthesia was 63 mmHg. The highest systolic blood pressure values observed were 134 mmHg before anesthesia and 149 mmHg after anesthesia. Similarly, the highest diastolic blood pressure values were 92 mmHg before anesthesia and 88 mmHg after anesthesia. Figure 1 showed the mean and standard deviation values for systolic blood pressure before anesthesia (113.19 ± 12.83 mmHg), and after anesthesia (122.95 ± 14.59 mmHg). The mean and standard deviation values for diastolic blood pressure before anesthesia were 77.05 ± 8.82 mmHg and 77.24 ± 7.82 mmHg after anesthesia.

Table 1. Results of Normality Test of Blood Pressure

	Sig.
Systole before	.281
Systole after	.214
Diastole before	.775
Diastole after	.264

* $p > 0,05$

Based on Table 1, the normality test results before and after the administration of local anesthesia in open-method tooth extraction show significance values greater than 0.05. Therefore, it can be concluded that the data are normally distributed, and the homogeneity test, specifically the Levene Test, is subsequently conducted.

Table 2. Results of Blood Pressure Homogeneity Test

	Sig.
Systolic Blood Pressure	.445
Diastolic Blood Pressure	.482

* $p < 0,05$

Based on Table 2, the significance value obtained is greater than 0.05 for the data collected before and after the administration of local anesthesia in open-method tooth extraction. This indicates that the data is homogeneous, meaning that the variations in the data are consistent. Following this, a paired t-test will be conducted to examine potential differences.

Table 3. Paired T-test Hypothesis Test

	Sig.
Systolic Blood Pressure	.000
Diastolic Blood Pressure	.882

* $p < 0,05$

Based on Table 3, the results of the paired t-test for systolic blood pressure revealed a significance value of 0.000 ($p < 0.05$), indicating a statistically significant difference. In contrast, the results for diastolic blood pressure showed a value of 0.882 ($p > 0.05$), suggesting no significant difference before and after the administration of lidocaine + adrenaline in a 1:80,000 ratio for open method extraction.

DISCUSSION

The open method of tooth extraction involves a surgical approach that requires cutting either the tooth or the bone. This technique follows several key principles, including flap preparation, bone removal, tooth sectioning, tooth extraction, bone leveling, curettage, and suturing.¹⁰ Before performing the open method extraction, local anesthesia is administered, typically using lidocaine, a type of local anesthetic from the amide group. To enhance its effectiveness, a vasoconstrictor such as adrenaline is often added to the local anesthetic. This combination helps constrict blood vessels, counteracting the vasodilation effects of the anesthetic. As a result, it creates a blood-free surgical field, reduces systemic

absorption of the anesthetic, and minimizes the risk of toxicity.⁵

However, despite these precautions, this study observed an increase in blood pressure, which contrasts with the findings of previous studies. Anxiety experienced by patients due to the open method extraction is one factor that can elevate blood pressure. This occurs because when patients are aware that open method extraction will be performed, endogenous catecholamines (epinephrine/norepinephrine) may be released due to the psychological stress they experience, leading to fear. The presence of catecholamines can trigger a rise in blood pressure.¹¹

This study was conducted to determine the increase in blood pressure in patients undergoing open method extraction before and after using local anesthesia lidocaine + adrenaline 1:80,000. Blood pressure measurements can be utilized if there is an excessive increase that can endanger the open method extraction process. Blood pressure measurements were taken before the administration of local anesthesia and three to five minutes after the administration, as lidocaine has a short onset time of 2-5 minutes. The researcher conducted an observational analytic study with a sample size of 21 data to determine changes in blood pressure in patients undergoing open method extraction before and after using local anesthesia lidocaine + adrenaline 1:80,000.

The change in blood pressure in patients undergoing open-method extraction before and after the administration of local anesthesia (lidocaine + adrenaline 1:80,000) at RSGM Nala Husada was analyzed. The results of a paired t-test statistical analysis showed that the p-value for systolic blood pressure was 0.000 ($p < 0.05$), while the p-value for diastolic blood pressure was 0.882 ($p > 0.05$). Based on these findings, it can be concluded that there was a significant change in systolic blood pressure before and after the administration of local anesthesia, but no significant change in diastolic blood pressure.

The increase in blood pressure can be attributed to the effects of adrenaline, which acts on α and β adrenergic receptors. The narrowing of blood vessels, caused by the action of anesthesia on alpha adrenergic receptors in peripheral blood vessels, contributes to this rise. Additionally, local anesthesia affects beta adrenergic receptors, which can lead to an increase in heart rate and contractile force, further resulting in elevated blood pressure.¹²

Figure 1 presents the results of the study conducted on 21 samples, indicating an overall increase in the average systolic blood pressure following the administration of local anesthesia to patients, with an average rise of 9.76 mmHg. Systolic blood pressure showed a significant increase before and after the administration of local anesthesia. This increase was attributed to the effect of adrenaline on the β_1 receptors, which strengthens and accelerates the contraction of the heart muscle, thereby increasing cardiac output. Additionally, the activation of α receptors causes vasoconstriction and the contraction of blood vessel muscles, further contributing to the rise in blood pressure. The surge in adrenaline levels leads to more forceful contractions of the cardiac muscle.¹³

The inclusion of adrenaline in the local anesthetic agent resulted in a negligible increase in diastolic blood pressure, as it binds to β_2 receptors, inducing vasodilation and relaxation of the blood vessel muscles. Diastolic blood pressure is associated with the pressure in the arteries during the heart's relaxation phase between two heartbeats, and therefore, it does not increase significantly.¹⁵

Diastolic blood pressure can be influenced by various factors, including age, gender, and the consumption of carbohydrates, saturated fats, and unsaturated fats. Diastolic blood pressure tends to remain stable between the ages of 55 and 60, as blood vessels become stiffer with increasing age.¹⁶ Both medications and age can affect the mechanisms regulating diastolic blood pressure. Certain types of drugs, such as alpha blockers, can influence diastolic

blood pressure by inhibiting alpha receptors. Alpha blockers, including doxazosin, indoramin, and terazosin, are commonly used as antihypertensive medications. Additionally, the use of local anesthetics containing adrenaline is another example of a treatment that works by inhibiting alpha receptors.¹⁷

In this study, it remains unclear whether the increase in blood pressure in patients is caused by the effects of vasoconstrictor substances or psychological stress. Psychological stress can be assessed by measuring the patient's pulse rate and respiration rate before administering local anesthesia. Patients who experience stress or fear typically exhibit an elevated pulse rate. This measurement can serve as an indicator to determine whether an individual is experiencing psychological stress before undergoing the open method procedure. Several previous studies have demonstrated that pre-anesthesia can help reduce psychological stress in patients. Factors that may alleviate stress include social support, a healthy diet, relaxation techniques, spiritual practices, and humor.¹⁸ Patients are advised to avoid factors that could contribute to an increase in blood pressure, such as smoking, alcohol consumption, and excessive salt intake.¹⁶

One of the reasons why the patient did not experience a significant increase in blood pressure is that they remained calm. Psychologically, the patient had prepared their mind and emotions before undergoing the open-method extraction procedure. The calmness experienced by the patient originates from the amygdala in the brain. The amygdala is a brain structure that regulates thoughts and emotions, such as joy, sadness, and fear. Due to its role in fear conditioning, the amygdala can regulate the autonomic nervous system, thereby reducing the release of adrenaline from the sympathetic nerves.^{19,20}

CONCLUSIONS

There was a significant increase in systolic blood pressure and a non-significant increase in diastolic blood pressure. Based on the study results, the rise in blood pressure before and after the administration of lidocaine + adrenaline 1:80,000 is considered safe for performing the open method extraction procedure.

REFERENCES

1. Distribusi Pencabutan Gigi Akibat Karies Berdasarkan Karakteristik Sosiodemografi Pada Pasien Poli Gigi Puskesmas Tanah Grogot. Tahun 2017-2019. Jurnal Sains Dan Kesehatan. 2024; 4(2), 112-119.
2. Z Qin, C Zhou, Y Zhu, Y Wang , H Cao , W Li , Z Huangvirtual Reality For Hypertension In Tooth Extraction: A Randomized Trial
3. Nikola Janev, Aviv Ouanounou. Local Anesthetics In Oral And Maxillofacial Medicine: Pharmacology, Adverse Effects, Drug Interactions And Clinical Manifestation. Journal Of Oral And Maxillofacial Anesthesia. Vol 3 March 30, 2024
4. Lini Deng, Yi Wang, Xianwang Xiang, Chuanjun Chen. Bone Absorption And Deformity In Mandible As Initial Symptom And Manifestation Of Neurofibromatosis Type 1: A Case Report And Literature Review. Oral Oncology Report. 2025. Vol.13.
5. Justin W. Walker, Theodore J. Cios. Local Anesthetic Choice For Regional Techniques In Cardiac Surgery: The Sauce Matters. Journal Of Cardiothoracic And Vascular Anesthesia. 2025. 39 (1).
6. Dhea Natashia, Pricelia Alifa Rahma, Dian Fitria, Syamikar Baridwan Syamsir, Diana Irawati. Exploring The Relationships Between Psychological Changes, Sleep Quality, And Hypertension Among Community-Dwelling Elderly. Jurnal Persatuan Perawat Indonesia. 2024. 9 (3)
7. Áine M De Bhailis , Philip A Kalra. Hypertension And The Kidneys. J Hosp Med. 2022; 83(5): 1-11. Doi:10.12968/Hmed.2021.0440. Epub 2022 May 27.



8. Zainab Al Ghareeb , Kawther Al Haji, Bayan Alhaddad, Balgis Gaffar. Assessment Of Dental Anxiety And Hemodynamic Changes During Different Dental Procedures: A Report From Eastern Saudi Arabia. *Eur J Dent.* 2022; 16(4): 833–840. Doi: 10.1055/S-0041-1740222
9. Ahyar Riza, Indra B. Siregar, Abdullah Oes, Tilagamalar. Differences In Blood Pressure Before And After Administration Of Local Anesthetic Among Obese Adult Female Patients. *Journal Of Dentomaxillofacial Science (J Dentomaxillofac Sci).* 2021;6 (1): 17-21.
10. Makki KIF, AKMA Abbas, YAA Alhadi, HA Al-Shamahy. Clinical Effects Of Platelets Rich Fibrin (Prf) Following Surgical Extraction Of Impacted Lower Third Molars Among A Sample Of Yemeni Adults. *Universal Journal Of Pharmaceutical Research.* 2023; 7 (6). Doi:10.22270/Ujpr.V7i6.864.
11. Lingdi Niu, Mingchun Gao, Shanshan Wen, Fang Wang, Haikun Shangguan, Zhiyuan Guo, Runxiang Zhang, Junwei Ge. Effects Of Catecholamine Stress Hormones Norepinephrine And Epinephrine On Growth, Antimicrobial Susceptibility, Biofilm Formation, And Gene Expressions Of Enterotoxigenic Escherichia Coli. *Int. J. Mol. Sci.* 2023, 24(21), 15646; <https://doi.org/10.3390/ijms242115646>
12. Abhijith George, Mandeep Sharma, Prasanna Kumar, Sneha Kulkarni, Vinay Patil Y, Jacob John Plackal. Hemodynamic Changes During Exodontia In Hypertensive And Normotensive Patients Following Injection Of Local Anesthetics With And Without Epinephrine: A Prospective Comparative Study. *Rev Esp Cirug Oral Y Maxilofac [Online].* 2022; 44 (1): 9-15.
13. Kenji Nakamura , Takeshi Fujiwara , Satoshi Hoshide , Yusuke Ishiyama , Mizuri Taki , Seiji Ozawa , Kazuomi Kario. Differences In Exercise-Induced Blood Pressure Changes Between Young Trained And Untrained Individuals. *J Clin Hypertens (Greenwich).* 2021; 23 (4): 843–848. Doi: 10.1111/Jch.14177.
14. Abdumanobov Mashhurbek Mansur O'g'li. Local Anesthetics: Review of Pharmacological Considerations. *Texas Journal of Medical Science.* 2023; 27: 60-63.
15. Komang Krisna Dewi, Lukas Kusparmanto, DKPS. The Effect of Vasoconstrictor Substances in Local Anesthetic Solutions on Blood Pressure in Tooth Extraction Patients. *MDERJ.* 2022; 2 (1): 8–16.
16. Mohammed Alfaqeeh, Sofa D Alfian, Rizky Abdula. Factors Associated with Hypertension Among Adults: A Cross-Sectional Analysis of The Indonesian Family Life Survey Vascular Health and Risk Management. 2023; 19: 827–836.
17. Craighead DH, Heinbockel TC, Freeberg KA, Rossman MJ, Jackman RA, Jankowski LR, Et Al. Time-Efficient Inspiratory Muscle Strength Training Lowers Blood Pressure And Improves Endothelial Function, No Bioavailability, And Oxidative Stress in Midlife/Older Adults With Above-Normal Blood Pressure. *J Am Heart Assoc.* 2021; 10(13).
18. Rulin Wang , Xin Huang , Yuan Wang , Masod Akbari . Non-Pharmacologic Approaches in Preoperative Anxiety, A Comprehensive Review. *Front Public Health.* 2022; 10: 854673. Doi: 10.3389/Fpubh.2022.854673
19. David G. Weissman, Wendy Berry Mendes. Correlation of Sympathetic and Parasympathetic Nervous System Activity During Rest and Acute Stress Tasks. *International Journal of Psychophysiology.* 2021: 60-68.
20. Ibrahim Nourwali. The Effects of Platelet-Rich Fibrin on Post-Surgical Complications Following Removal of Impacted Wisdom Teeth: A Pilot Study. *Journal of Taibah University Medical Sciences.* 2021; 16 (4): 521e528.



The Influence of Entrepreneurship and Career Planning in the Regeneration of Dentist Family Businesses in Indonesia

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ABSTRACT

Background: Many children of parents working in the healthcare industry tend to pursue the same profession as their parents or family members. Dentistry is one of the medical professions often passed down to the next generation, as children grow up observing their parents, close relatives, or role models. This study explores the role of Entrepreneurship and Self Efficacy in influencing career selection among second or later-generation dentists. Career selection motivation is shaped by both internal and external factors, including the entrepreneurial drive to manage and sustain a family-owned dental practice. **Objective:** This research aims to provide insights into the motivations underlying career choices in dentistry. **Materials and Methods:** A purposive snowball sampling technique was used to collect data from 50 dentists across various cities in Java, Indonesia, all of whom are at least second-generation dentists actively working in family-owned or independent practices. **Results:** The findings highlight that motivation and Career-Related Modeling play a significant role in shaping the career preferences of second-generation dentists, particularly in family-owned businesses. This study contributes to understanding how entrepreneurial influences and career role models impact career decisions in the field of dentistry. **Conclusion:** The results of this research highlighted the dominant influence of entrepreneurial drive in second and later generation dentist in choosing dentist as a profession.

Keywords: Entrepreneurship, Career Modelling, Dentistry, Family Business, Self-efficacy

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INTRODUCTION

Career choice is a big decision in the lives of young people. Becoming a dentist is a choice that will require time and large resources, so the supporting factors that shape motivation need to be understood so that career choices are correct.⁷ Many parents who work as dentists will pass on their interests and arouse the desire of their children, grandchildren, and future generations to choose the same profession and how to develop strategies and environments so that the younger generation is interested and interested in becoming dentists. For dentists who come from families of entrepreneurs or clinic owners, entrepreneurial drive can be the key to successfully opening and managing their clinics.²⁴ However, if they do not have enough interest in entrepreneurship, the potential for success as independent clinic owners will be reduced.

Although motivation is formed from the environment, sometimes a personal desire to become a dentist arises from an entrepreneurial spirit, not only continuing but developing oneself or a business that has been built by parents or future generations. Many clinics or small hospitals are owned and managed by families. If the younger generation in the family is not interested in continuing the family business, there is a risk that the business will not be sustainable. Family businesses that can survive across generations often require innovation by the next generation.⁸ If the interest of the younger generation in entrepreneurship is low, family businesses in the dental health sector may not thrive. This research is urgently needed to understand why the younger generation may be less interested in continuing the family business in the dental sector, and what can be done to strengthen their interest, especially in the aspect of entrepreneurship.

According to data from the Ministry of Health Annual Report, in 2023 the number of dentists in Indonesia with active registration papers by the 31st of December 2023 is 43,641

people.⁴ Of that number, 38,542 people are general dentists, while 5,099 of them are specialist dentists. Many second-generation dentists and beyond work in private practices, either private, clinics continuing the business or practice of the previous generation or private hospitals, which exceeds the number of dentists working for the government.⁵

The phenomenon of intergenerational career transmission by dentists is common in Indonesia. Families transmit or pass on knowledge, resources, positions, and even workplaces to the next generation so that there is intergenerational transmission and also encouragement that can be in the form of recommendations to continue or form internal motivation from the next generation to take up the profession of dentistry.⁶

Previous research states that as many as 30% of doctors are currently working as doctors or dentists because of encouragement from their families or will inherit the family business. Medical families are families that already have many multi-generational family members forming their successors to work in the same or similar fields.⁶ Although it is something that is considered common and considered inevitable, research has never been conducted that explores the encouragement, motivation, and career-related modelling of the figure who is the catalyst for the next generation to choose the profession of dentist and the figure used as a role model, especially when working to continue the family business or working independently.⁵

One of the problems often encountered in a family business is the failure of regeneration or succession, resulting in the family business not continuing due to various reasons, one of which is loss of motivation or disinterest in running the family business.³ One phenomenon that is seen in the health sector, especially second or generation dentists, chooses to become dentists because of role models or designs their careers based on parents or family members who work as dentists. Dentistry is one

of the professions that has succeeded in shaping the mindset of the next generation to take over this profession so that intergenerational transmission occurs.⁶

Entrepreneurship is the ability to recognize opportunities, opportunities, the desire to have abilities, gain knowledge and resources, carry out a plan, leadership skills, and readiness to accept risks and tolerance. The concept of entrepreneurship in dentistry includes starting, continuing, developing a dental practice, managing human and financial resources, and subsequent accepting the risks and rewards obtained in running the business.²⁴ Dentistry today is not only about the health and healing of patients but has developed into a business that takes into account profit and loss, efficiency, and sustainability.

Therefore, creating and transferring knowledge in the succession process is critical to the family's success.¹² If not effectively managed through the succession process, this can lead to business failure immediately or over time. Therefore, the creation and transfer of knowledge used in the succession process have potentially important implications for effective business succession. Continuing their parents' practice usually offers financial stability and the establishment of an established professional network. However, some second-generation dentists may show a tendency towards Entrepreneurial Creativity, such as developing new business models or expanding their clinical practice with modern technology.

This study was conducted to see the influence of parents, immediate family, role models in influencing the next generation in choosing a profession as a dentist or doctor through interviews with second-generation dentists or beyond. It is hoped that this study can more accurately determine the triggers, origins of motivation, entrepreneurial drive, and also career planning used by second-generation dentists who currently work in family businesses or other health facilities.

MATERIALS AND METHODS

Methods

This study was an observational analytic study with a cross-sectional research design. Participants were 52 actively working dentist who are second generation or later in Indonesia. The study data was collected from an online questionnaire conducted from September 2024 to June January 2025. The online questionnaire consisted of a series of question based on Self Efficacy Theory and modified with Entrepreneurial Drive as dependent variable.

The online questionnaire has a brief cover letter in written form, describing the research purpose of the collected information and the confidential guarantee policy. Before starting the questionnaire, the ethics statement was disclosed to all respondents and verbal informed consents were obtained from all respondents. All responses were voluntary and anonymous.

Questionnaire

The questionnaire adopted an established scale to measure the key concepts of Self-Efficacy Theory: Career Modelling, Instrumental Assistance, Emotional Support, Verbal Encouragement, and Entrepreneurial Drive as the added dependent variable. Self-Efficacy is the mediator used for career selection.

Sampling

A Snowball sampling method was adopted. Two or three second or later generation dentists in major cities in Surabaya, Jakarta, Yogyakarta were first randomly sampled, then each of them gave multiple referrals to other second or later generation dentists in their cities. This pattern was repeated until a sufficient number of dentists became available for the sample. The sampling and survey process was performed during the ten months between September 2024 and January 2025. A total of 100 questionnaires were distributed in electronic

forms using google forms through Whatsapp (the dominant texting and social media App in Indonesia), depending on the respondents' preference and convenience. Eventually, 88 responses were received in the initial distribution and trimmed to 52 valid responses. All questionnaires were self-administrated. All data collected through the questionnaires were entered and coded.

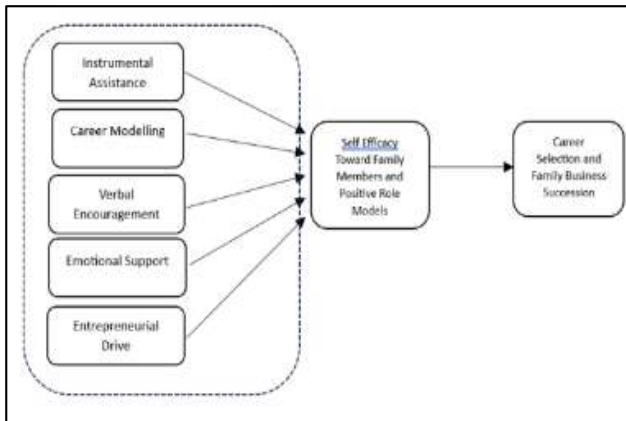


Figure 1. Working theory of self-efficacy theory modified from Albert Bandura added entrepreneurial drive as a dependent variable

Self-efficacy

Self-efficacy, as conceptualized by Albert Bandura¹, can be reframed through the lens of career modeling, emotional support, verbal encouragement, and instrumental assistance, which are crucial elements in fostering an individual's belief in their capabilities to achieve career-related goals. Career modeling involves the observation of role models who successfully navigate their professional paths, providing a template for individuals to emulate. This process enhances self-efficacy by demonstrating the attainability of success and helping individuals visualize their potential career trajectories.¹³ Emotional support encompasses the provision of empathy, understanding, and reassurance from peers, mentors, or family members, which can significantly bolster an individual's confidence during challenging times. Such support helps individuals manage stress and anxiety, thereby enhancing their belief in their ability to succeed in their chosen careers.¹⁴

Verbal encouragement and instrumental assistance further contribute to the development of self-efficacy in career contexts. Verbal encouragement involves positive affirmations and constructive feedback that reinforce an individual's belief in their skills and potential. This encouragement can come from mentors, colleagues, or family members who recognize and validate an individual's efforts and achievements.¹⁵ Instrumental assistance refers to the tangible support provided to individuals, such as resources, training, or networking opportunities that facilitate career advancement. This type of support enhances an individual's capabilities and reinforces their belief in their ability to navigate career challenges effectively.¹⁶ Together, these elements create a supportive environment that fosters self-efficacy, ultimately leading to greater motivation, persistence, and success in professional endeavors.³⁶

Self-efficacy in this research is assessed by using 7 items of 35 questions that combines Bandura's self-efficacy theory with the theory of Entrepreneurship. Self-efficacy is an individual's belief in his abilities which influences the way an individual reacts to certain situations and conditions.

Higher scores are in line with the amount of individual confidence in his abilities. Career selection is the consequences or results obtained from the parameters of self-efficacy. In the career context, the outcome expectation is a belief in the consequences that will be obtained if someone does career related behavior. Respondents will respond to statements given such as "I am confident that I can deliver adequate dental treatment " on a scale of 1-5 (1 = strongly disagree and 5 = strongly agree). Higher scores relate to the amount of confidence they have towards their career selection.

Entrepreneurial Drive

This variable reflects personalities and skills associated with identifying and exploring business opportunities, taking risks and being

engaged in career selection as a dentist and working in various locations that they are currently are. Entrepreneurial drive and intent among dentists, particularly those in family-owned practices, are influenced by various factors, including family background, education, and the use of structured assessment tools like questionnaires. Family-owned practices often foster a unique entrepreneurial environment where dentists are motivated by the desire to continue family legacies and leverage familial support systems.

Research indicates that dentists with entrepreneurial family backgrounds exhibit higher entrepreneurial intentions, as these familial ties instill values and expectations that encourage self-employment and business ownership.^{17,18} Furthermore, the integration of entrepreneurship education into dental curriculum has been shown to enhance students' entrepreneurial aspirations, equipping them with essential skills and confidence necessary for navigating the complexities of running a dental practice.^{19,20} The application of structured questionnaires, particularly those utilizing Likert scales, can effectively measure entrepreneurial intent among dental students and practitioners, providing valuable insights into their motivations and aspirations.^{22,23}

The use of questionnaires with Likert scales allows for a nuanced understanding of the factors influencing entrepreneurial intent in the dental field. Studies have employed such instruments to assess various dimensions of entrepreneurial intention, including personal values, perceived feasibility, and the influence of subjective norms.^{21,22} For instance, a recent study utilized a questionnaire to analyse the entrepreneurial intentions of dentists in public hospitals, revealing that personal characteristics and prior entrepreneurial exposure significantly impacted their intent to transition to private practice.^{10,24} This method not only quantifies the level of entrepreneurial intent but also identifies specific areas for improvement in educational programs aimed at fostering entrepreneurship

among dental students.^{23,25} By systematically evaluating these factors, stakeholders in dental education can better tailor curricula and support systems to enhance the entrepreneurial capabilities of future dentists, ultimately contributing to a more dynamic and innovative dental practice landscape.^{17,20,22} All measurements are based on a 5-point Likert scale from 1 (disagree strongly) to 5 (agree strongly).

Statistical analysis

The baseline model was a multivariate logistic regression model analyzing the major factors associated with the dependent variable the dentist's Career Modelling, Instrumental Assistance, Emotional Support, Verbal Encouragement, and Entrepreneurial Drive. The study employed frequency distribution analysis to summarize the demographic and professional characteristics of second or later-generation dentists. This method allows researchers to present the data in an organized manner, showing how variables such as gender distribution, age range, educational background, years of practice, and family entrepreneurial background are distributed within the sample. The frequency distribution analysis helps identify patterns and dominant characteristics, such as the fact that a majority of respondents (67.31%) were male, and 59.62% fell within the 30-35 age range, as observed in the dataset. This step is crucial in research as it provides a clear overview of sample characteristics before performing more complex statistical tests.

Moreover, frequency distribution was used to analyse the prevalence of entrepreneurial drive and self-efficacy among respondents. For example, the study found that 75% of the sample had family members involved in entrepreneurship, which might indicate an intergenerational influence on career choices. This statistical approach helps quantify how common certain traits are in the population, allowing researchers to draw preliminary conclusions about how entrepreneurial

exposure in families influences the self-efficacy and career decisions of second-generation dentists.

To ensure that the measurement instruments used in the study were consistent and dependable, a reliability analysis was conducted using Cronbach's Alpha. This statistical test measures the internal consistency of items within a construct—meaning that if multiple questions in the questionnaire were designed to assess the same concept (e.g., self-efficacy or entrepreneurial drive), their responses should be highly correlated. A Cronbach's Alpha value above 0.7 is considered acceptable, while values above 0.8 or 0.9 indicate excellent reliability. By achieving high internal consistency, the study ensured that its questionnaire items effectively measured the intended psychological and career-related variables.

Reliability was tested through Composite Reliability (CR), another measure in Structural Equation Modelling (SEM-PLS) that assesses whether all items in a construct collectively provide stable and consistent results. A CR value above 0.7 indicates that the construct is reliable and can be confidently used in statistical modelling. This process ensures that the responses are not arbitrary but instead reflect genuine patterns in self-efficacy, entrepreneurial motivation, and career selection behaviors among second-generation dentists.

This study also conducted validity testing to ensure that the constructs measured what they were supposed to measure. Two primary types of validity were assessed: (1) Convergent Validity: This measures how well multiple indicators of the same concept correlate with each other. In SEM-PLS analysis, convergent validity is assessed by examining factor loadings, which should ideally be above 0.5 to indicate a strong relationship between the questionnaire items and the construct being measured. For instance, all self-efficacy items (SE1-SE4) had factor loadings ranging from 0.838 to 0.905, suggesting that the survey items

were strongly correlated with the overall self-efficacy construct. (2) Discriminant Validity: This type of validity ensures that each construct in the study is distinct from others. If two constructs are too highly correlated, it suggests redundancy, meaning that the study might not be measuring separate concepts. The Fornell-Larcker Criterion and Heterotrait-Monotrait (HTMT) ratio were used to confirm that the self-efficacy construct was statistically distinct from the entrepreneurial drive, instrumental assistance, career modelling, and emotional support. By confirming discriminant validity, the study ensured that self-efficacy was measured independently from other influencing factors in career selection.

This study utilized Structural Equation Modelling (SEM-PLS 4.0) to analyse the relationships between variables. SEM-PLS is particularly useful for studies involving multiple latent variables (such as self-efficacy, entrepreneurial drive, and career selection), as it allows researchers to assess the direct and indirect effects between constructs. This approach was necessary given that self-efficacy was treated as a mediator between various factors (entrepreneurial drive, career modelling, emotional support, etc.) and career selection. The analysis included path coefficients that measured the strength of relationships between constructs. The results showed that entrepreneurial drive had the strongest positive effect on self-efficacy (0.528), followed by instrumental assistance (0.411), while emotional support had a negligible impact (-0.015). This confirms that self-efficacy in career selection is more influenced by practical entrepreneurial exposure and financial mentorship rather than emotional encouragement alone.

By using frequency distribution, reliability, and validity testing, this study ensured that its findings were both statistically sound and meaningful. The frequency analysis provided insights into the demographic and professional characteristics of the sample, while reliability testing (Cronbach's Alpha, Composite

Reliability) ensured that the questionnaire items were consistent. Validity testing (Convergent and Discriminant Validity) further confirmed that the measured constructs were distinct and relevant. Lastly, SEM-PLS modelling allowed for an in-depth examination of how entrepreneurial drive and self-efficacy influence career selection among second-generation dentists. These rigorous analytical methods helped strengthen the study's conclusions regarding the intergenerational transmission of entrepreneurship in dentistry and its impact on career decisions.

RESULTS

The survey included 52 respondents seen in table 1, with a higher representation of male participants (67.31%) compared to female participants (32.69%). The majority of the respondents fall within the 30-35 years age range (59.62%), followed by those above 35 years old (26.92%). A smaller percentage belongs to the 26-30 years category (11.54%), while only 1.92% of respondents are aged 20-25 years. The majority of respondents hold a Specialist Degree (69.23%), reflecting advanced education in their field. Bachelor's Degree holders account for 17.31%, while Doctoral Degree holders represent 7.69%. A smaller proportion of respondents hold a Master's Degree (5.77%). Regarding professional experience, the highest proportion of respondents have been practicing for 5-10 years (46.15%), followed by those with 10-15 years of experience (25%). A smaller percentage of respondents have more than 15 years (15.38%), while 13.46% have 0-5 years of experience. A significant 75% of respondents reported having family members involved in entrepreneurship, while 25% do not have any entrepreneurial family background. This suggests a potential influence of family business culture in shaping their career decisions.

Table 1. Reports the demographic and professional characteristics of the sample.

Variables	N	N(%)
Gender		
Male	35	67.31
Female	17	32.69
Age (Years)		
20-25	1	1.92
26-30	6	11.54
30-35	31	59.62
>35	14	26.92
Educational Level		
Bachelor's Degree	9	17.31
Master's Degree	3	5.77
Specialist Degree	36	69.23
Doctoral Degree	4	7.69
Years of Practice		
0-5	7	13.46
5-10	24	46.15
10-15	13	25.0
>15	8	15.38
Entrepreneurial Family Member		
Yes	39	75.0
No	13	25.0

Table 2 shows different self-efficacy (SE) parameters contributing to career selection (CS). Self-efficacy (SE) acts as the mediator between several independent variables and career selection. The independent variables include: Instrumental Assistance (IA), Career Modelling (CM), Emotional Support (ES), Entrepreneurial Drive (ED). The Entrepreneurial Drive (ED) parameter has the strongest positive relationship with SE (0.528). This suggests that individuals with strong entrepreneurial motivation tend to have higher self-efficacy in making career-related decisions.

Instrumental Assistance (IA) contributes positively to SE (0.411), indicating that practical help (such as financial or mentorship support)

significantly influences confidence in career choices. Career Modelling (CM) also contributes positively to SE (0.261), though weaker than IA and ED. This suggests that observing role models alone is not as strong as active entrepreneurial engagement in fostering self-efficacy. The Emotional Support (ES) variable has a weak negative relationship with SE (-0.015). This suggests that emotional encouragement alone does not significantly impact self-efficacy. While support from family and mentors can help reduce stress, it does not necessarily translate into higher career confidence unless accompanied by practical career exposure.

Self-Efficacy (SE) has a strong direct effect on Career Selection (CS) (0.666). This confirms that individuals with higher belief in their abilities are more likely to confidently pursue a career path in dentistry. The SE indicators (SE1, SE2, SE3, SE4) are all strongly linked to SE, with values ranging from 0.838 to 0.905. This suggests that the measured aspects of self-efficacy are reliable and significantly contribute to the final career decision. SE strongly predicts CS (0.666), and CS itself is further supported by high factor loadings from CS2 (0.843) and CS3 (0.925). This means that individuals with high self-efficacy are highly likely to make confident career choices in dentistry. Entrepreneurial Drive (ED) and Instrumental Assistance (IA) are the most crucial contributors to self-efficacy. Career Modelling (CM) is useful but less impactful on SE than active entrepreneurial experiences. Emotional Support (ES) does not significantly influence SE, suggesting that motivation alone is insufficient without practical skill development. Ultimately, higher self-efficacy (SE) leads to a stronger and more confident career selection (CS) process.

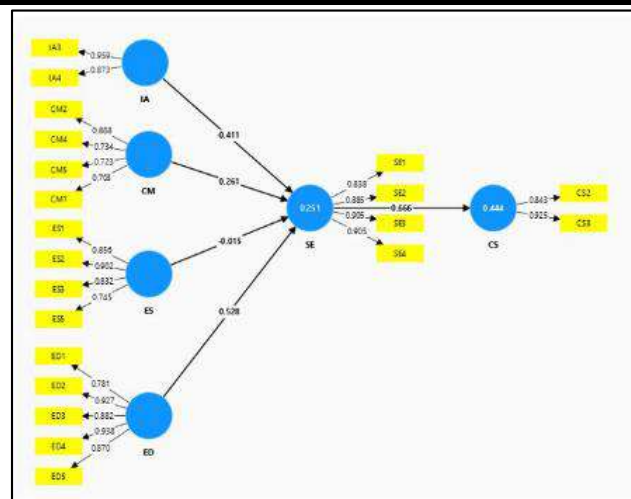


Figure 2. Diagram of degree of importance from the relevant parameters used in the correlation between elements of self-efficacy and Career Selection of 2nd and later generation dentists.

DISCUSSIONS

The influence of entrepreneurship mindset or entrepreneurial drive on the careers of second or later generation dentists is underscored by the significant correlation between an entrepreneurial family background and enhanced self-efficacy. Previous research indicates that individuals with such backgrounds report increased confidence in their professional capabilities, primarily due to early exposure to business ownership and management practices.²⁷ This exposure equips them with essential skills in patient management and operational strategies, facilitating a smoother transition into their professional roles compared to their peers who do not have similar familial experiences.³⁴

Moreover, the study suggests that entrepreneurial drive is a more potent factor in fostering career confidence than traditional parameters such as career modeling, instrumental assistance, or verbal encouragement.²⁹ Dentists raised in entrepreneurial environments are more adept at

identifying opportunities and making informed, risk-based decisions, which are critical skills for navigating the complexities of private practice.³³ In contrast, those lacking this entrepreneurial motivation may find themselves ill-prepared for the challenges of independent practice management, ultimately limiting their career autonomy and growth potential.³⁰

While career modeling can provide a foundational understanding of practice management, it often falls short in instilling the proactive mindset necessary for entrepreneurial success.³² Many second-generation dentists may observe their parents' practices but may not fully embrace the entrepreneurial mindset required to take ownership of their career trajectories.²⁸ This highlights the importance of integrating entrepreneurial education into dental training programs, as traditional curricula often emphasize clinical skills at the expense of business acumen.³⁴

Instrumental assistance, such as financial support from family, can facilitate entry into the profession but does not inherently build self-efficacy.³⁵ Over-reliance on such support can hinder the development of critical decision-making skills necessary for independent practice.²⁷ The study emphasizes that dentists who cultivate an entrepreneurial mindset are more likely to make strategic business choices, even when initial support is available.²⁷

Verbal encouragement, while beneficial for psychological support, does not equate to professional competence.²⁶ The findings suggest that self-efficacy is more robustly developed through active participation in decision-making processes and risk management rather than through passive affirmations.³³ Emotional support from family can alleviate stress but does not substitute for the practical skills needed for business success.³¹

The study also notes that academic excellence alone does not guarantee high self-efficacy among dentists. Many dental schools prioritize clinical training over business management education, creating a gap that can

hinder long-term career success.²⁷ Years of practice do not necessarily correlate with self-efficacy; dentists may possess extensive experience yet lack confidence in managing the financial and operational aspects of their practices if they have not been exposed to these elements.³⁴

In conclusion, the research underscores the critical role of entrepreneurial drive in shaping the self-efficacy of second-generation dentists. This drive not only fosters self-reliance and innovation but also encourages adaptability in exploring new treatment technologies and business models.³⁰ While the study provides valuable insights, it acknowledges limitations, including its cross-sectional design and potential response bias, suggesting the need for further research to explore the long-term effects of entrepreneurial exposure on self-efficacy.³⁴

CONCLUSIONS

Analysing the survey results of 52 second generation or later dentists, this study showed that many factors influence the career choice of second or later generation dentists. Accumulation of practical skills and knowledge during their career, as implied by age, professional qualifications help to promote Entrepreneurial Drive. This research showed that entrepreneurial drive is the most dominant aspect of the choice of becoming a dentist coming from a family that has a previous generation who practiced dentistry. Based on the results and discussion of this study, it was concluded that entrepreneurial drive influenced self-efficacy and mediated career selection of second or later generation dentists significantly both directly and indirectly.

The research findings indicate that while career modelling, verbal encouragement, instrumental assistance, and emotional support contribute to career selection, they do not necessarily build self-efficacy in the same way that entrepreneurial drive does. A strong entrepreneurial mindset equips second-

generation dentists with the confidence to navigate business challenges, make independent decisions, and expand their professional reach. To enhance self-efficacy in future generations of dentists, families, educators, and institutions should prioritize entrepreneurial education and exposure alongside clinical training. The finding that dentists in Indonesia are more likely to report Entrepreneurial Drive is dominant even though the previous generation encouraged their choices but in the end the drive to become purely internal.

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REFERENCES

1. Bandura, A. Self-efficacy: The Exercise of Control. New York: Freeman; 1997
2. Abdul Hamid, N. F., Jaafar, A., Asming, N. N., Suria, N. S., Ho, T. K., Lim, Z. Y. J., & Lim, T. W. (2024). Factors Influencing the Selection of Dentistry as a Career Among Prospective Candidates: A Multicentre Cross-Sectional Study. *European Journal of Dental Education*. <https://doi.org/10.1111/eje.13044>
3. Ahmad, Z., Najam, U., & Mustamil, N. (2024). Uncovering the research trends of family-owned business succession: past, present and the future. *Journal of Family Business Management*, ahead-of-print(ahead-of-print). <https://doi.org/10.1108/JFBM-04-2024-0084>
4. Kemenkes RI. Data dan Informasi Profil Kesehatan Indonesia 2023. 2018 (Accessed on September 28th 2024 on <http://www.depkes.go.id/>)
5. Caesarani, F. P., Astria, F., Syakurah, R. A., Aulia, B., & Siburian, R. (n.d.). MODELING OF CAREER CHOICE BEHAVIORS IN INDONESIAN DENTISTRY STUDENTS. *Jurnal Kedokteran Gigi Universitas Baiturrahmah*, 7(2), 114–122.
6. Cheng, F. C., Wang, L. H., Wang, Y. C., & Chiang, C. P. (2024). The influence of dentist parents on their children's career decision-making for dentistry or medicine. In *Journal of Dental Sciences* (Vol. 19, Issue 1, pp. 678–681). Association for Dental Sciences of the Republic of China. <https://doi.org/10.1016/j.jds.2023.10.003>
7. Davavilana, F. A., Putri, F. M., & Suryanti, N. (2024). Career choice and the influencing factors of bachelor and dental profession students: an observational study. *Padjadjaran Journal of Dentistry*, 36(1), 77. <https://doi.org/10.24198/pjd.vol36no1.48690>
8. Hastenteufel, J., & Staub, M. (2020). Current and future challenges of family businesses. *Managerial Economics*, 20(2), 119. <https://doi.org/10.7494/manage.2019.20.2.119>
9. Yeheskiel Pane, Y., & Christanti, R. (2023). Exploring Family Businesses Succession in Indonesia: The Knowledge Management Lenses. *Jurnal Ilmiah Manajemen*, 11(1), 52–67. <https://creativecommons.org/licenses/by-nc/4.0/>
10. Peng, B., Li, J., & Liu, D. (2023). Entrepreneurial intention of public hospital dentists and its importance factors: a study based on machine learning and binary logistic regression. <https://doi.org/10.21203/rs.3.rs-3285589/v1>
11. Redmond, P. (2017). Talking about my generation: dentistry in a multi-generational world. *Faculty Dental Journal*, 8(4), 156–159. <https://doi.org/10.1308/rcsfdj.2017.156>
12. Brinkerink, Jasper & Rondi, Emanuela & Benedetti, Carlotta & Arzubaga, Unai. (2020). Family business or business family? Organizational identity elasticity and strategic responses to disruptive innovation. *Journal of Family Business Strategy*. 10.1016/j.jfbs.2020.100360.
13. Sinring, A. and Fadhillah, N. (2023). Other people in career choice influence career self-efficacy and individual career self-management. *European Journal of Educational Research*, volume-12-2023(volume-12-issue-2-april-2023), 1045-1057. <https://doi.org/10.12973/eu-jer.12.2.1045>
14. Ramasamy, R. and Nithyanandan, D. (2016). Positive psychological factors of career development among undergraduate students. *International Journal of Indian Psychology*, 3(4). <https://doi.org/10.25215/0304.057>
15. Caprara, G., Fida, R., Vecchione, M., Bove, G., Vecchio, G., Barbaranelli, C., ... & Bandura, A. (2008). Longitudinal analysis of the role of



- perceived self-efficacy for self-regulated learning in academic continuance and achievement.. *Journal of Educational Psychology*, 100(3), 525-534. <https://doi.org/10.1037/0022-0663.100.3.525>
16. Mu, F., Liu, J., Lou, H., Zhu, W., Wang, Z., & Li, B. (2024). How breaking a sweat affects mood: the mediating role of self-efficacy between physical exercise and emotion regulation ability. *Plos One*, 19(6), e0303694. <https://doi.org/10.1371/journal.pone.0303694>
 17. Li, J., Nair, S., & Wider, W. (2022). Effects of family background and entrepreneurship competition on students' entrepreneurial intention in china. *Humanities and Social Sciences Letters*, 10(4), 557-568. <https://doi.org/10.18488/73.v10i4.3185>
 18. Georgescu, M. and Herman, E. (2020). The impact of the family background on students' entrepreneurial intentions: an empirical analysis. *Sustainability*, 12(11), 4775. <https://doi.org/10.3390/su12114775>
 19. i, M., Kodali, U., Gadicherla, S., Smriti, K., Singh, A., & Khurshid, Z. (2024). The role of soft skills in dentaKodall education: challenges and importance. *European Journal of Dentistry*. <https://doi.org/10.1055/s-0044-1791938>
 20. Brijlal, P. and Brijlal, P. (2013). Entrepreneurial knowledge and aspirations of dentistry students in south africa. *Industry and Higher Education*, 27(5), 389-398. <https://doi.org/10.5367/ihe.2013.0171>
 21. Sahputri, R., Mawardi, M., Yumarni, T., & Sujarwoto, S. (2023). Entrepreneurship education, family entrepreneurial orientation and entrepreneurial intention among students in indonesia. *Journal of International Education in Business*, 16(3), 295-311. <https://doi.org/10.1108/jieb-02-2022-0010>
 22. Kurjono, Kurjono. "Entrepreneurial Intentions: Between Entrepreneurial Knowledge, Entrepreneurial Skills and Perceived Control Behavior." *Dinamika Pendidikan* 17.2 (2022): 146-163.
 23. Mollica, A., Cain, K., & Callan, R. (2017). Using assessments of dental students' entrepreneurial self-efficacy to aid practice management education. *Journal of Dental Education*, 81(6), 726-731. <https://doi.org/10.21815/jde.016.036>
 24. Jia-bi, W., Peng, B., Zhou, H., & Zhang, J. (2020). Dentists' entrepreneurial intention and associated factors in public hospitals in major cities in guangdong (south china): a cross-sectional study.. <https://doi.org/10.21203/rs.3.rs-52297/v2>
 25. Roberts, B., Roberts, E., Brachvogel, W., & Epstein, J. (2020). Practice management curricular changes may lead to enhanced preparedness for practice. *Journal of Dental Education*, 84(8), 887-894. <https://doi.org/10.1002/jdd.12136>
 26. AlQahtani, S., Murshid, E., Fadel, H., & Kassim, S. (2017). Practicing dentists' self-efficacy and associated factors in managing the treatment of adults with mental and physical disabilities: an exploratory cross-sectional study in riyadh, saudi arabia. *International Journal of Environmental Research and Public Health*, 14(12), 1549. <https://doi.org/10.3390/ijerph14121549>
 27. Barber, M., Wiesen, R., Arnold, S., Taichman, R., & Taichman, L. (2011). Perceptions of business skill development by graduates of the university of michigan dental school. *Journal of Dental Education*, 75(4), 505-517. <https://doi.org/10.1002/j.0022-0337.2011.75.4.tb05074.x>
 28. Chaker, H. and Dellagi, H. (2022). Combining teaching methods and developing students' entrepreneurial skills and entrepreneurial intention: the case of students in the faculty of economics and management of tunis. *Industry and Higher Education*, 37(4), 551-573. <https://doi.org/10.1177/09504222221146426>
 29. Christina & Widjojo, H. (2023). A comprehensive entrepreneurship education model based on social cognitive theory. *Jurnal Manajemen Teori Dan Terapan | Journal of Theory and Applied Management*, 16(2), 339-355. <https://doi.org/10.20473/jmtt.v16i2.44034>
 30. Dyantyi, N. and Faleni, N. (2023). Entrepreneurship education to stimulate entrepreneurial mindset in chemistry students. *International Journal of Research in Business and Social Science* (2147-4478), 12(10), 209-216. <https://doi.org/10.20525/ijrbs.v12i10.3110>
 31. Hakim, N., Ode, H., Rahmadhani, M., & Naim, S. (2024). How entrepreneurial mindset, gender stereotypes, and innovation practices influence the sustainability of women-owned businesses in bogor city, indonesia. *International Journal of Business Law and Education*, 5(2), 2433-2444. <https://doi.org/10.56442/ijble.v5i2.891>



32. Neck, H. and Greene, P. (2010). Entrepreneurship education: known worlds and new frontiers. *Journal of Small Business Management*, 49(1), 55-70. <https://doi.org/10.1111/j.1540-627x.2010.00314.x>
33. Nowiński, W., Haddoud, M., Lančarič, D., Egerová, D., & Czeglédi, C. (2017). The impact of entrepreneurship education, entrepreneurial self-efficacy and gender on entrepreneurial intentions of university students in the visegrad countries. *Studies in Higher Education*, 44(2), 361-379. <https://doi.org/10.1080/03075079.2017.1365359>
34. Saleem, Z., Sethi, A., Hassan, M., & Wajahat, M. (2019). Assessments of dental students' entrepreneurial self-efficacy to aid practice management. *Health Professions Educator Journal*, 2(2), 54-59. <https://doi.org/10.53708/hpej.v2i2.105>
35. Wardana, L., Narmaditya, B., Wibowo, A., Mahendra, A., Wibowo, N., Harwida, G., ... & Rohman, A. (2020). The impact of entrepreneurship education and students' entrepreneurial mindset: the mediating role of attitude and self-efficacy. *Heliyon*, 6(9), e04922. <https://doi.org/10.1016/j.heliyon.2020.e04922>
36. Frenzel, A. C., Goetz, T., Lüdtke, O., & Pekrun, R. (2022). "The interplay of students' emotions and self-efficacy in academic achievement: A longitudinal study." **Learning and Instruction**, 78, 101536. [doi:10.1016/j.learninstruc.2021.101536](https://doi.org/10.1016/j.learninstruc.2021.101536).



The Toxicity Assessment of Hydroxyapatite with Theobromine Formulation on Human Dental Pulp Stem Cells (hDPSC)

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ABSTRACT

Background: Deep caries not treated immediately can cause more severe damage and loss of tooth vitality. Ca(OH)₂ and MTA are often used as vital pulp treatment materials, but both have limitations. Therefore, alternative materials are needed. Hydroxyapatite is a major component of bones and teeth forms reparative dentin without tunnel defects, while theobromine has been shown to have a better anti-cariogenic effect than fluoride. The combination of both can be a choice for vital pulp treatment. Therefore, conducting cytotoxicity and cell proliferation tests is important to ensure safety and determine whether the material can induce cell proliferation. **Objective:** To determine the toxicity and proliferation of hydroxyapatite theobromine on human dental pulp stem cells. **Materials and Methods:** The toxicity assessment of hydroxyapatite with theobromine formulation on human dental pulp stem cells (hDPSC) was a laboratory experimental study (in vitro) carried out using a posttest control group design with the method used being the MTT assay. **Results:** The highest cell viability value was Ca(OH)₂ 122.08%, control cells 113.70%, HA/TB 2:1 108.03%, HA/TB 1:1 103.23%, HA/TB 1:2 102.09%, and the lowest was negative control 100%. The highest proliferation value was in control cells 138.00%, HA/TB 2:1 135.00%, HA/TB 1:2 129.64%, Ca(OH)₂ 128.38%, and HA/TB 1:1 122.11%, and the lowest cell proliferation value was in the negative control at 100%. This shows that the higher the hydroxyapatite value compared to theobromine, the greater the percentage value. **Conclusion:** Theobromine hydroxyapatite formulation is non-toxic, so it has potential as a vital pulp treatment ingredient, but it is less effective in inducing cell proliferation.

Keywords: Cytotoxicity, Proliferation, Hydroxyapatite, Theobromine, MTT Assay.

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INTRODUCTION

Caries is a disease of the teeth and mouth experienced by almost the entire population of the world.¹ If not treated immediately, it will worsen caries and cause loss of tooth vitality.² To maintain the vitality of the pulp in young permanent teeth, treatments can be carried out, including pulpotomy and pulp capping.³

Ca(OH)₂ and MTA are materials used in pulp capping and pulpotomy treatments.⁴ These materials are used in vital pulp therapy treatments.⁵ One of the gold standard materials in pulp capping treatments is Ca(OH)₂. This material has good antibacterial properties because it has a pH of around 12, but this can also be a limitation because it causes necrosis in tissues that come into direct contact with the material.⁶ Previous studies have found that Ca(OH)₂ causes damage to tunnel defects, causing the potential for bacterial infection and necrosis in the superficial part of the pulp layer.⁷ Compared to Ca(OH)₂, MTA is more effective and superior as a pulp coating material in pulp capping and pulpotomy treatments, showing a higher success rate with good results in maintaining tooth vitality in the long term.⁸ There are limitations to MTA, including requiring a long setting time and not being easy to manipulate as a direct pulp capping material.⁹

Other alternative materials for vital pulp treatment are formulations of hydroxyapatite and theobromine. Hydroxyapatite (HA) Ca₁₀(PO₄)₆(OH)₂ is a biomineral material of hard tissue in bones and teeth containing phosphate and calcium.¹⁰ Hydroxyapatite is synthesized from calcium-rich materials such as mollusk shells, chicken eggshells, and cow and goat bones.¹¹ In vital pulp therapy, HA forms reparative dentin,¹² without tunnel defects with the inflammatory response caused more effectively reduced compared to Ca(OH)₂.¹³ Theobromine (TB) C₇H₈N₄O₂ is an alkaloid compound of the methylxanthine group contained in the cocoa plant. Theobromine is

often used in dental health care, including vital pulp therapy.

HA-TB comes from a natural composition, so it has high biocompatibility and can increase tissue regeneration. Apart from that, hydroxyapatite can support bone remineralization such as pulp capping,¹² theobromine has antibacterial properties and stimulates the formation of HA crystals,¹⁴ this formulation of HA-TB can potentially become a pulp capping material by combining the remineralization ability of HA and the antibacterial properties and structural strengthening of theobromine. This formulation can provide better results in pulp and dentin regeneration, so it can potentially be an alternative material for vital pulp therapy treatments.

Research on HA-TB formulation is still rare because it is a new material, so toxicity and cell proliferation testing is needed to ensure the safety of the material if it enters the oral cavity and to determine whether the formulation of the material can induce cell proliferation.

This study discusses the toxicity and proliferation tests of HA formulations derived from chicken eggshells and theobromine from cocoa on hDPSC cells with ratios of 1:1, 1:2 and 2:1 for both test materials, to see whether hydroxyapatite or theobromine is more dominant in the results of the toxicity and proliferation tests, so that it has the potential as an alternative vital pulp treatment.

MATERIALS AND METHODS

This is a laboratory experimental study with a posttest-only control group design divided into three groups with HA/TB ratios of 1:1, HA/TB 1:2, HA/TB 2:1, and a control group with Ca(OH)₂ (n=6). The samples of this study were hDPSC cells that were first cultured at the ProSTEM Laboratory.

Culture Cell of hDPSC Procedure

hDPSC cells were cultured in 50 mm cell culture dishes (5-10 ml of complete medium) and incubated for 2-7 days until confluence. The cells were then harvested by discarding the old medium and washing with fetal bovine serum, adding trypsin to release the cells, and then incubating in a CO₂ 5% incubator at 37°C for 7 minutes. Next, PBS (1:1) was added to wash the cells, then the cells were taken and collected in a tube. Furthermore, centrifugation was carried out at 300g for 5 minutes to separate the cells from the supernatant. After that, the supernatant was discarded, and 1 ml of cell medium was added to stabilize the cell condition. The number of cells was counted using an automated cell counter, then 5000 cells were put into each well of a 96-well plate. Furthermore, the cells were incubated for 3-4 days.

HA-TB Formulation Procedure

The Hydroxyapatite and theobromine were dissolved in 100 mL of deionized water with ratios of 1:1, 1:2, and 2:1.

The formulation was made by mixing hydroxyapatite and theobromine that had been prepared in a dish and then dissolved with 0.5 mL of Dulbecco Modified Eagle Medium (DMEM). The HA-TB formulation that had been dissolved was transferred to a 96-well microplate and incubated for 24 hours.

Toxicity Test and Proliferation Cell Test

Toxicity test was conducted by MTT method, where 100 µL of MTT solution was added to each well of 96-well microplate using micropipette. The microplate was then incubated at 37°C for 24 hours. After incubation, the MTT reaction was stopped by adding 100 µL of solubilizer to each well. Absorbance was measured using an Elisa reader at 450 nm wavelength, and cell viability at various concentrations was calculated using viability formula.

Cell proliferation measurements were performed by calculating cell viability after 48

hours using the MTT method. The hDPSC cells were grown on 96-well microplates at a density of 5x10³ cells per well and incubated for 24 hours. Then, the culture medium was replaced, and test solutions with various concentrations were added and then incubated for 24 hours. Next, 100 µL of culture medium and 100 µL of MTT solution were added to each well and incubated for 4-6 hours in a CO₂ 5% incubator at 37°C. Absorbance was measured using a microplate reader at a wavelength of 450 nm.

RESULTS

Table 1 and Figure 1 show the results of the toxicity test. Table 1 shows the mean, standard deviation, and significance of the effects of the various treatment groups on human dental pulp stem cells (hDPSC). Statistical analysis was performed using *One-Way ANOVA*. There were significant differences between the test groups ($p < 0.05$).

Table 1. Mean value, standard deviation, and significance of the effect between groups of test materials on hDPSC cells

Group Test	Average $\pm$ SD	P
HA/TB 1:1	0,141517 $\pm$ 0,0036108	<0,001
HA/TB 1:2	0,139700 $\pm$ 0,0024706	
HA/TB 2:1	0,147833 $\pm$ 0,0033916	
Control (+)	0,151733 $\pm$ 0,0044107	

**One-way ANOVA* ($p < 0,05$)

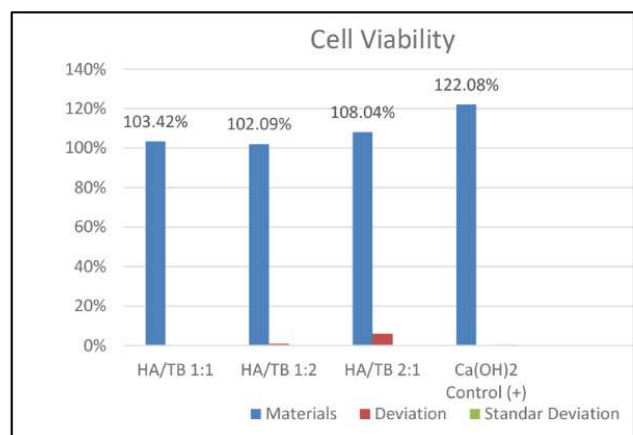


Figure 1. The human dental pulp stem cell viability chart (%).

Figure 1 shows the results of the toxicity test calculation in percentages with values above 100 percent, indicating that the test material is not toxic. The highest value was the control (+) 122.08, HA/TB 2:1 108.04%, HA/TB 1:1 103.42%, and the lowest being HA/TB 1:2 102.09%.

Table 2. The significance value between groups

	HA/TB 1:1	HA/TB 1:2	HA/TB 2:1	Control (+)
HA/TB 1:1		0,978	0,155	0,004
HA/TB 1:2			0,033	<0,001*
HA/TB 2:1				0,639
Control (+)				

*Uji Tukey ($p < 0,05$)

Table 2 shows the significance value between groups. There is no significant difference on HA/TB 1:1 and HA/TB 2:1, but on HA/TB 2:1, there is a significant difference from HA/TB 1:2.

Furthermore, proliferation tests were carried out to determine whether HA/TB formulations with various ratios could induce cell proliferation; the test results are in table 3 and figure 2.

Table 3. Mean, standard deviation, and significance of the test materials against hDPSC cells

Group Test	Average $\pm$ SD	P
HA/TB 1:1	0,141517 $\pm$ 0,0036108	
HA/TB 1:2	0,139700 $\pm$ 0,0024706	<0,001
HA/TB 2:1	0,147833 $\pm$ 0,0033916	
Control (+)	0,151733 $\pm$ 0,0044107	

*One-way ANOVA ($p < 0,05$)

In Table 3, the results obtained from the One-way ANOVA test show significant differences within the tested groups ($p < 0.05$).

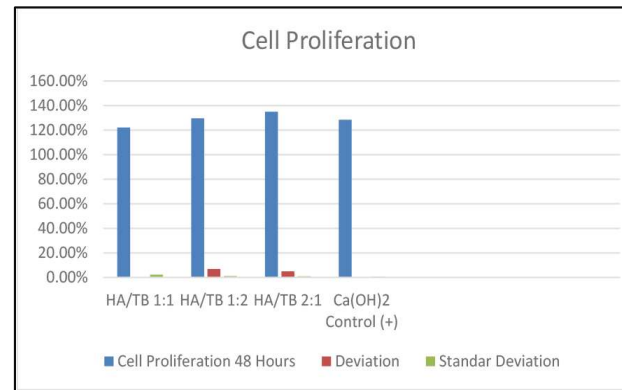


Figure 2. The Human dental pulp stem cell viability chart (%) showing hydroxyapatite formulation on hDPSC cell proliferation

Figure 2 Based on the test results, HA/TB 1:2 and 2:1 had a higher proliferation value than the positive control, but HA/TB 1:1 had a lower proliferation value than the positive control. It can be concluded that HA-TB formulation can induce proliferation but not significantly.

Table 4. Significance value between proliferation test groups

	HA/TB 1:1	HA/TB 1:2	HA/TB 2:1	Control (+)
HA/TB 1:1		0,561	0,074	0,731
HA/TB 1:2			0,838	1,000
HA/TB 2:1				0,685
Control (+)				

*Tukey test ($p < 0,05$)

Based on the cell proliferation test, there is no significant difference between HA/TB 1:2, HA/TB 2:1, and HA/TB 1:1 test materials (Table 4). Compared with the positive control, there was a significant difference in HA/TB 1:1. It can be concluded that HA-TB formulation can induce cell proliferation but not significantly.

DISCUSSIONS

Untreated dental caries can lead to the opening of the pulp chamber,¹⁵ which leads to inflammation of the pulp tissue and loss of tooth vitality.² To prevent this, vital pulp treatments such as pulpotomy and pulpcapping can be performed to maintain pulp vitality.³ Common materials used in these treatments are $\text{Ca}(\text{OH})_2$ and MTA, although $\text{Ca}(\text{OH})_2$ has some limitations,⁴ so alternative materials are needed.

This aims to evaluate whether hydroxyapatite and theobromine formulations can be an alternative treatment for vital pulp therapy in human dental pulp stem cells (hDPSC) through toxicity and cell proliferation tests. The study was conducted with an hDPSC cell culture and cultured *in vitro*.¹⁶ Primary cell culture was chosen because it has a lower risk of genetic mutation and ease of use. The hDPSC cells have an important role in pulp tissue regeneration and the ability to repair damage to dental pulp.¹⁷

Cytotoxicity and proliferation tests were conducted to evaluate the safety of the material and the increase in cell number. In this study, the test was conducted with the MTT method using several HA/TB treatment groups with a ratio of 1:1, 1:2, 2:1, and positive control $\text{Ca}(\text{OH})_2$. The cytotoxicity test was conducted for 24 hours, while the proliferation test was conducted for 48 hours, with 6 repetitions each.

The MTT method was used in this study because the time required is relatively short and can be measured accurately. The working principle of the MTT method is a colorimetric measurement by determining the functional state of mitochondria and indicating cell viability with a mechanism in the form of tetrazolium salts that are soluble in water by producing a yellow solution, which will be reduced in cells that have metabolic activity.¹⁸ The results of the MTT test can be measured by absorbance of light at 450 nm wavelength. In living cells, it will react with MTT to form purple formazan crystals, while in dead cells, no color change occurs.

Based on the ISO 10993-5 standard, in measuring the cell viability value, if the value results in a test material above 90%, the material is biocompatible. In this study, all samples tested showed cell viability above 90%, indicating that the test material is biocompatible.¹⁹ These results also indicate a relationship between cell viability and the amount of HA exposure. HA with a large concentration will increase the viability value. No significant difference was found between the viability values at HA/TB 1:1, 1:2, 2:1 ($P > 0.05$), indicating that the test samples will not produce different effects in clinical application.

HA/TB may have potential as an alternative material for vital pulp treatment, as it is non-toxic but not significant in inducing cell proliferation, so further studies with other characterizations are needed.

In conducting a study on test materials, the pH value is important to note because it can play a role in maintaining pulp vitality and regeneration. In previous studies, HA had a lower pH value than $\text{Ca}(\text{OH})_2$. The high pH value of calcium hydroxide can cause tunnel defects that create the potential for bacterial infection and result in necrosis in the superficial part of the pulp layer.⁷ In this study, $\text{Ca}(\text{OH})_2$ can support cell viability because its high calcium concentration may induce cell proliferation.²⁰ Besides pH, the amount of hydroxyapatite used in the HA-TB formulation also has an effect, as from the results of this study the ratio of HA/TB 1:1 with the positive control the results obtained were higher values for the positive control compared to the results of the value of HA/TB 1:1, so that the amount of hydroxyapatite used if it was greater than theobromine, this could be effective in inducing cell proliferation. In previous studies, cell proliferation tests with 24 hours and 48 hours were shown to induce cell proliferation.²² In this study, there are limitations, namely that the study only used 3 concentrations of HA / TB ratio and only used 1 time parameter in the research observation, namely 48 hours in the cell proliferation test, so that it has not shown

the actual conditions in the cell life cycle, which are measured using several time parameters and several characterizations of materials that have potential as vital pulp therapy treatment materials. Further research needs to be done to identify other characteristics of the HAP and theobromine as alternative materials in vital pulp treatment.

CONCLUSIONS

Based on the results of the study, the formulation of HAP Theobromine to hDPSC with a ratio of 1:1, 1:2, 2:1, is non-toxic because it has a viability value above 100, but is not significant in inducing cell proliferation. Nevertheless, HAP and Theobromine have potential as alternative materials in vital pulp treatment.

REFERENCES

- Sardi, I, Putra, KAD. Gambaran Kejadian Karies Gigi Dan Tingkat Konsumsi Makanan Kariogenik Pada Anak Usia Prasekolah Di Asrama Kompilasi Senapan B Yonmek 741/Gn Masceti Gianyar. Bali Health Published Journal. 2022; 4(1): 39-46.
- Ricucci D, Jr José FS, Li Y, Tay FR. Vital Pulp Therapy: Histopathology And Histobacteriology-Based Guidelines To Treat Teeth With Deep Caries And Pulp Exposure. Journal of Dentistry. 2019; 86: 41-52.
- Islam R, Islam Md RR, Tanaka T, Alam MK, Ahmed HMA, Sano H. Direct pulp capping procedures – Evidence and practice. Japanese Dental Science Review. 2023; 59: 48-61.
- HA William N, Kahler B, Walsh LJ. Dental Material Choices for Pulp Therapy in Paediatric Dentistry. European Endodontic Journal. 2017; 2(1): 2-6.
- Taneja S, Singh A. Evaluation of effectiveness of calcium hydroxide and MTA as pulpotomy agents in permanent teeth: A meta-analysis. *Pediatric Dental Journal*. 2019; 29(2): 90-96.
- Hanafi MGS, Izham A, Harismanto, Bahtiar EW. Biokompatibilitas Bahan Kaping Pulpa. Cakradonya Dent J. 2021; 13(1): 14-21.
- Aprilia, Kunarti S, Budhy TI, Sari RP. Biocompatibility of Beta-Tricalcium Phosphate Nanoencapsulation from Synthesis of Anadara granosa Shells on Fibroblast Cell Line BHK-21 Cell Culture. International Journal of Integrated Engineering. 2022; 14(2): 7-12.
- Mostafa NM, Moussa SA. Mineral Trioxide Aggregate (MTA) vs Calcium Hydroxide in Direct Pulp Capping. On J Dent & Oral Health. 2018; 1(2): 1-6.
- Fagundez TC, Barata TJ. Indirect Pulp Treatment in a Permanent Molar: Case Report of 4 year Follow-Up. Journal of Endodontics. 2009; 32: 50-52.
- Ulian G, Moro D, Valdrè G. Hydroxylapatite and Related Minerals in Bone and Dental Tissues: Structural, Spectroscopic and Mechanical Properties from a Computational Perspective. Biomolecules. 2021; 11(5): 1-34.
- Akbar AF, Cahyaningrum SE. Characterization and Anti-Bacterial Activity Testing of Nano Hydroxyapatite Clove (*Eugenia Caryophyllus*) Against *Streptococcus Mutans* Bacteria. Indonesian Journal of Chemical Science. 2022; 11(1): 1-8.
- Mozartha M. Hidroksiapatit Dan Aplikasinya Di Bidang Kedokteran Gigi. Cakradonya Dent J. 2015; 7(2): 807-868.
- Amanda HG, Elline, Fibryanto E. Synthesis and Physical Characterization of Nano-Hydroxyapatite-Collagen Epigallocatechin-3-Gallate Hydrogel Composite. Journal of Indonesian Dental Association. 2022; 5(1): 7-13.
- Lestari U, Syamsurizal TY. The Antiplaque Efficacy and Effectiveness of Activated Charcoal Toothpaste of *Elaeis Guineensis* in Smokers. Indonesian Journal of Pharmaceutical Science and Technology. 2021; 1(1): 75-87.
- Kambaya PP, Jumiaty, Masyhudi. Uji Efek Antibakteri Ekstrak Etanol Buah Belimbing Wuluh (*Averrhoa Bilimbi* L) Sebagai Kandidat Bahan Medikamen Saluran Akar Gigi Terhadap Pertumbuhan *Staphylococcus Aureus* Secara In Vitro. Mulawarman Dental Journal. 2021; 1(1): 1-9.
- Aljauhari MM, Asri MT, Trimulyono G. Media Alternatif untuk Pertumbuhan Sel Midgut *Spodoptera litura*. Lentera Bio. 2013; 2(2): 161–165.
- Kwack KH, Lee HW. Clinical Potential of Dental Pulp Stem Cells in Pulp Regeneration: Current Endodontic Progress and Future Perspectives. Journal Frontiers in Cell and Developmental Biology. 2022; 10: 1-18.



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18. Sirait M, Sinulingga K, N Siregar, Doloksaribu ME, Amelia. Synthesis And Characterizations Of Natural Limestone-Derived Nano-Hydroxyapatite (Hap): A Comparison Study Of Different Metals Doped Haps On Antibacterial Activity. *Journal of Physics Conference Series*. 2021; 11(26): 15896–1590.
 19. López-García J, Lehocký M, Humpolíček P, Sáha P. HaCaT Keratinocytes Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell Viability and Proliferation. *Journal Of Functional Biomaterials*. 2014; 5(2): 43-57.
 20. Chen L, Zheng L, Jiang J, Gui J, Zhang L, Huang Y, et al. Calcium Hydroxide–induced Proliferation, Migration, Osteogenic Differentiation, and Mineralization via the Mitogen-activated Protein Kinase Pathway in Human Dental Pulp Stem Cells. *J Endod*. 2016; 42(9):1355–61.
 21. Rahayu YC, Triwahyuni IE, Sari DY, Kusumawardhani B. The Cytotoxic And Proliferative Activity Of Cocoa Pod Husk Extract (*Theobroma Cacao* L.) On Periodontal Ligament Fibroblasts. *Odonto Dental Journal*. 2022; 9(1): 46-52.

Toxicity Test of HA-TCP Variation from Synthesis of Blood Shell with Fluoride Addition as Dental Remineralization Material

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ABSTRACT

Background: Caries is the most found problem in teeth. Caries begins with fermentation process of carbohydrates. The preventive and curative actions of white spot lesions aim to initiate and improve the process of tooth remineralization. HA TCP from the synthesis of blood shells with fluoride added is expected to be a dental remineralization agent. **Objective:** To determine the cytotoxicity of HA-TCP variation from the synthesis of blood clam shells (*Anadara granosa*) with fluoride added as a dental remineralization material. **Materials and Methods:** This type of research is true experimental with a post test only control design. The data from the research results were then carried out a descriptive test and viability test followed by the Kruskal wallis test and significance test using the Chi Square Test. The number of samples was 15 which were divided into 4 groups, namely the KS (control group of cells, without the addition of scaffolds, P1 (HA-TCP 15% with fluoride addition), P2 (HA TCP 20% with fluoride addition) and P3 (HA-TCP 25% with fluoride addition). The cytotoxicity test was carried out using MTT Assay on BHK-21 cells. **Results:** The results of cell viability percentages in the KS, P1, P2, and P3 groups were 100%, 61.16%, 79.12% and 88.83% respectively. **Conclusion:** The cytotoxicity test results of HA-TCP variation from the synthesis of blood clam shells (*Anadara granosa*) with fluoride added as a dental remineralization material showed non-toxic results.

Keywords: *Anadara Granosa* Shell, Cytotoxicity, HA-TCP, Remineralization

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INTRODUCTION

Caries is a dental problem, begins with the fermentation process of carbohydrates by bacteria such as *Streptococcus mutans* which produces acid products in the hard tissues of the teeth and decreasing pH of the oral cavity.¹ The condition of continuous pH decrease causes demineralization or loss of mineral structure, especially calcium, phosphate, carbonate and the main mineral hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) which forms an early lesion of white caries called white spot lesion.² Remineralization is the process of reforming lost minerals, especially calcium and phosphate ions that form hydroxyapatite crystals in demineralized enamels.³ Saliva played a role in affecting the remineralization. Saliva a natural source of calcium and phosphate in the oral cavity.⁴ The use of calcium from blood shell powder has been widely studied as remineralization agent. The powder of blood shell can be synthesized into hydroxyapatite (HA) and tricalcium phosphate (TCP) (HA-TCP) which can be used as a tooth remineralization agent

Hydroxyapatite (HA) ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is a type of ceramic known for its excellent biocompatibility due to its chemical and physical composition being identical to that of human bone and tooth minerals. HA powder can be derived from two primary sources: synthetic chemical processes and natural biological materials, such as *Anadara granosa* (blood cockle shells). However, HA has a notable drawback, as it exhibits poor toughness, low wear resistance, and is challenging to degrade.⁵ To reduce the degradation process, the combination of hydroxyapatite (HA) and tricalcium phosphate (TCP) is assumed to have higher quality and more stable.⁶ This research using HA-TCP 22:78 was due to previous research that hydrothermal method of 18 hours sintering 4 hours will produce HA-TCP 22:78 based on the XRD results.⁷

The addition of fluor in Tri Calcium Phosphate (fTCP) can controls the delivery of calcium and phosphate ions into tooth. TCP formulations are designed with fluorine to increase remineralization and build acid-resistant minerals without requiring high levels of calcium and will produce great benefits when in neutral pH.⁸ Topical application of fluoride in post eruptive tooth promotes enamel remineralization and inhibit demineralization, reduces glycolysis, and exerts antibacterial effects. The addition of fluoride into restorative materials can reduce the incidence of caries, particularly in high risk individuals.^{9,10} In this reasearch, the selection of 900ppm of fluoride because tooth paste with 1000ppm of fluoride can prevent the caries in primary and permanent tooth.¹¹

The cytotoxicity test is a component of the biocompatibility assessment aimed at evaluating the biological impact of a material. Its purpose is to ensure that the material does not induce toxic effects that could interfere with biological functions, thereby preventing rejection by the host. Based on this context, this study was carried out to assess the cytotoxicity of cream HA-TCP variations synthesized from blood cockle shells with added fluoride, intended for use as a dental remineralization agent, utilizing the MT-Assay method. The choice of percentages of 15%, 20% and 25% was based on research before which states that a 20% suspension of shell calcium is the optimal concentration for remineralization of enamel that has experienced initial caries.¹²

MATERIALS AND METHODS

This study is a true experimental laboratory study using a post test only control group design. The experimental unit of this research is HA TCP 22-78 cream added with 900ppm fluoride. The subjects in this study were divided into 4 groups, namely cell control (KS), HA TCP 15% (P1) variant, HA TCP 20% (P2) variant and HA TCP 25% (P3) variant.

The produce of HA-TCP cream with fluor addition, started with a clear gel mass was produce with the process of mixing aquabidestilata at a temperature of 80°C with Na CMC and the process of re-adding aquabidestilata at room temperature and then continuing with the process of adding *Anadara granosa* shell material composition of 15%, 20% and 25% and 900 ppm fluoride mixed until homogeneous.

The cytotoxicity test was carried out on BHK 21 cell culture and 96-well microplate. Wells are filled with control media, control cells and research samples with concentrations of 15%, 20% and 25%. Then the microplate is incubated with 5% CO₂ at a temperature of 37°C for 20 hours. Then, the microplate is removed from the incubation tool, the culture media and scaffold in the well are taken using a syringe, BHK-21 cells will be left in the well. Then incubated again for 3 hours. Total incubation time in an incubator at 37°C for 24 hours. After the incubation period is complete, MTT and culture media are taken using a syringe The optical density value of formazan was read with an Elisa reader with a wavelength of 620 nm. To determine the percentage of live BHK-21 cells, we use the cell viability formula. Cell viability is defined as the ability of cells to survive. The formula for calculating cell viability is (number of living cells/total number of cells) x 100%.

Data from each examination was analyzed statistically with a significance level ($\alpha = 0,05$). Normality test in this research was using *Shapiro-Wilk* test, and followed by *Kruskal-Wallis* test.

RESULT

The results of toxicity test showed that the percentage of cell viability is above 50%, which means more and more living cells. The percentage of cell viability from the synthesis of blood clam shells (*Anadara granosa*) measured by ELISA reader can be seen in table 1. The data

from the study results then carried out a normality test using *Saphiro-Wilk*.

Table 1. Average and Standard Deviation of live cell and percentage of cell viability in HA-TCP 22-78 with the Addition of 900ppm Fluoride.

Group	Average+Standard Deviation	Percentage of cell viability (%)
P1	0.324 ± 0.219	61.16
P2	0.439 ± 0.308	79.12
P3	22.36 ± 72.514	88.83

Information:

P1 = HA TCP 22-78 with a concentration of 15%

P2 = HA TCP 22-78 with a concentration of 20%

P3 = HA TCP 22-78 with a concentration of 25%

Table 2. Normality Test Results with Shapiro-Wilk

Shapiro-Wilk	
Groups	Sig.
P1	.098
P2	.045
P3	.000

Table 1 shows that the P3 group have the highest result of the average value of living cells, continued with P2 group, and P1 group. Based on table 2, the significance value (sig.) of normality test showed normal undistributed data ($p < 0.05$), in the 15%, 25%, and 25% of HA-TCP micro group. These results mean that the normality test is not normally distributed because the significance value (Sig.) is less than ($p < 0.05$). The test is continued with a non-parametric test using the *Kruskal Wallis* Test.

Table 3. Hypothesis Test with Kruskal Wallis Test

	Viabilitas
Chi-Square	1.882
Df	2
Asymp. Sig.	.390

The test results using *Kruskal Wallis* test showed that the significance value was 0.390 greater than 0.05 ($p > 0.05$), which means that there was no significant difference in cytotoxicity between treatment groups.

DISCUSSIONS

This study aims to determine the cytotoxicity of the HA-TCP 22-78 from the synthesis of blood clam shells added with fluoride to BHK-21 cells. The selection of fluoride as one of the active ingredients in this study is through the process of forming fluorapatite which plays a role in preventing demineralization and triggering remineralization.⁸ The cytotoxicity test on BHK-21 cell culture was chosen because it is stable, easy to grow, easy to culture, and does not show mutations. This cell culture has structural similarities to human fibroblast cells which are often found in the oral cavity.^{13,14}

In this study, direct contact has been carried out between HA-TCP synthesis of blood clam shells (*Anadara granosa*) in cell culture. The purple color change caused by the addition of formazan crystals was then read the absorption using an ELISA reader. The more intense the color shown, the higher the absorption value and the more cells live. The absorbance value obtained was then included in the cell viability formula to get the percentage of the number of viable cells. Actually, in the cytotoxicity test after obtaining the percentage of cell life/death, the data on the percentage of the number of dead cells can be further calculated by measuring LC_{50} (*lethal concentration*) which shows the concentration value that results in cell death of 50% or IC_{50} (*inhibitory concentration*). It indicates that the concentration value results in the inhibition of cell proliferation by 50% and shows the potential toxicity of a compound to cells.¹⁶ However, this study was only carried out until the calculation stage of the percentage of cell viability was achieved. The results of the calculation are said to be non-toxic if the percentage of cell viability is more than 50%, and it is said to be toxic if the percentage of viability cell is less than 50%.^{15,16}

The sample used in this study is a cream preparation created by initially mixing aquabidestillata at a temperature of 80°C with sodium carboxymethyl cellulose (Na CMC) to

form a clear and homogeneous gel mass. After this process, additional aquabidestillata is added at room temperature. Next, we incorporate HA-TCP materials at percentages of 15%, 20%, and 25%, along with 900 ppm of fluoride. This mixture is blended until it becomes homogeneous, resulting in a cream preparation that serves as a candidate for dental remineralization materials. The selection of cream preparations as candidates for remineralization materials is based on several factors. Firstly, they are easy to apply on tooth enamel. Additionally, these preparations effectively deliver important ingredients that can diffuse into the microporosity of the enamel. The cream formulations also adhere well to the enamel layer, facilitating a more efficient absorption process for the medicinal properties of the cream.¹⁷

Based on the calculation of the data, the results of this study showed that the percentage of cell viability in the P1 group (HA-TCP 15%), has a value of 61.16% while the P2 group (HA-TCP 20%) is 79.12% and the P3 group (HA-TCP 25%) is 88.83%. In the KS group (cell control), the percentage of cell viability was 100% because the wells in the microplate only contained media cultures without the administration of HA-TCP. Cell control was used for the normalization of the viability percentage of the other treatment groups. The cell viability percentages in the P1, P2, and P3 groups are all above 50%, indicating that the materials are non-toxic. This conclusion is supported by the toxicity test of the HA-TCP micro scaffold synthesized from blood cockle shells, which yielded non-toxic results.¹⁸

The addition of fluoride in HA-TCP cream does not affect the results of toxicity tests. This is because the amount of fluoride used is 900 ppm, which is still below the daily dose of topical fluoride (1000-1500ppm).^{11,19} Meanwhile, the toxic dose of fluoride in humans is 5,0-10,0 F/kg in weight.²⁰

CONCLUSION

HA-TCP synthesis results of blood clam shells given the addition of 900ppm fluoride showed non-toxic results with cell viability percentages of 61.16%, 79.12% and 88.83%, respectively.

REFERENCES

1. Marwah N. 2019. Textbook of : Introduction to Pediatric Dentistry. 3rd Ed. 1–1004. Jaypee Pub. P: 1-18.
2. Jeanny KH, Lunardhi CGJ, Subiyanto A. Kemampuan Bioaktif Glass (Novamin) dan Casein Peptide Amorphous Calcium Phosphate (CPP-ACP) terhadap Demineralisasi Enamel The Potential of Bioactive Glass (Novamin) and Casein Peptide Amorphous Calcium Phosphate (CPP-ACP) on Enamel Demineralization. 2017; 7. Conservative Dentistry Journal 7(2); 2017: 111-119.
3. Vo Truong Nhu N, Pham Thi Hong T, Le AQ, Minh ST, Nguyen Thi Thu P. The Effect of Casein Phosphopeptide-amorphous Calcium Fluoride Phosphate on the Remineralization of Artificial Caries Lesions: An In Vitro Study. Journal of Dentistry Indonesia. 2017;24(2): 45-49. doi:10.14693/jdi.v24i2.1044
4. Talaat DM, Abdelrahman AAM, Abdelaziz RH, Nagy D. Effect of Two Remineralizing Agents On Initial Caries-Like Lesions in Young Permanent Teeth: An in vitro study. Journal of Contemporary Dental Practice. 2018; 19 (10): 1181–8.
5. Neel EAA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization – Remineralization Dynamics in Teeth And Bone. International Journal of Nanomedicine. 11(1); 2016: 4743-4763.
6. Ferdynanto RA, Dharmayanti PES, Dewi PTK, Prananingrum W. The effect of various concentrations of HA-TCP derived from cockle shell synthesis on scaffold porosity. Dent J. 2018; 51 (3): 114–8.
7. Sari RP, Kurniawan H. Effectiveness of Anadara granosa shell-Stichopus hermanni granules at accelerating woven bone formation fourteen days after tooth extraction. Dent J. 2019; 52 (4): 177–82.
8. Tiffany AS, Wahyuni S. Pengaruh Pengolesan Bahan Remineralisasi FTCP (Clinpro®) Terhadap pH Saliva. Jurnal Kesehatan Gigi dan Mulut (JGKM). 2020; 2(1): 14.
9. Mankar N, Kumbhare S, Nikhade P, Mahapatra J, Agrawal P. Role of Fluoride in Dentistry: A Narrative Review. J Cureus. 2023; 15 (12): 12. DOI: 10.7759/cureus.50884
10. Zirk M, Schievelkamp S, Kemnitz E, Lausch J, Wierichs RJ, Esteves-Oliveira M, et al. Evaluation of Novel Nanoscaled Metal Fluorides On Their Ability to Remineralize Enamel Caries Lesions. Sci Rep. 2019; 9(1): 1-8.
11. Walsh T, Worthington HV, Glenny AM et al. Fluoride Toothpastes of Different Concentrations For Preventing Dental Caries. Cochrane Database Syst Rev. 2019; 3(3): 1-13.
12. Abdelnabi A, Hamza NK, El-Borady OM, Hamdy TM. Effect of Different Formulations and Application Methods of Coral Calcium on Its Remineralization Ability on Carious Enamel. Open Access Maced J Med Sci. 2020; 8: 94–9.
13. Adnyana VC. Uji Sitotoksitas Periodontal Dressing Yang Mengandung Ekstrak Kulit Buah Kakao 15% Terhadap Kultur Sel Fibroblas Baby Hamster Kidney-21 (BHK-21). Skripsi. Jember: Fakultas Kedokteran Gigi, Universitas Jember; 2017. P:19.
14. Emilda Y, Budipramana E, Kuntari DS. Uji Toksisitas Ekstrak Bawang Putih (Allium Sativum) Terhadap Kultur Sel Fibroblast (Garlic Allium Sativum) Extract Toxicity Test On Fibroblast Cell Culture). Dent. J. (Maj. Ked. Gigi) 2014; 47(4) : 215–219.
15. Michael Josef Kridanto Kamadjaja, Bambang Agustono, Innocencio Kresna Pratama. Biocompatibility of Crab Shell-Derived Graft Sterilized by Ultraviolet Light Towards Human Gingival Fibroblast Cell Culture. World Journal of Advanced Research and Reviews. 2023; 17 (3): 728–35.
16. Arquitectura EY, Introducci TI, 赫晓霞, Iv T, Teatinas LAS, Conclusiones TVII, et al. No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis [Internet]. 2015; 53 (9): 1689–99. Available from: <http://publications.lib.chalmers.se/records/fulltext/245180/245180.pdf%0Ahttps://hdl.handle.net>



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- /20.500.12380/245180%0Ahttp://dx.doi.org/10.1016/j.jsames.2011.03.003%0Ahttps://doi.org/10.1016/j.gr.2017.08.001%0Ahttp://dx.doi.org/10.1016/j.precamres.2014.12
17. Effendi MD, Suhendra N. Pengaruh Rasio HA/TCP terhadap Karakteristik Komposit BCP-Gelatin-CMC sebagai Bahan Injectable Bone Substitute. *Jurnal Inovasi dan Teknologi Material*. 2020; 1 (2): 15–20.
18. Subagiyo S, Margino S, Triyanto T, Ari Setyati WA. Effects of pH, Temperature and Salinity In Growth and Organic Acid Production of Lactic Acid Bacteria Isolated From Penaeid Shrimp Intestine. *Ilmu Kelaut*. 2015; 20 (4) :187.
19. Clark MB, Keels MA, Slayton RL. Fluoride Use in Caries Prevention in the Primary Care Setting. *Pediatrics*. 2020; 146 (6): 1-11. DOI: <https://doi.org/10.1542/peds.2020-034637>
20. Susanto A, Padjadjaran U. The Effect of the Application of HA-TCP Bone Graft towards Gingival Crevicular Fluid Osteocalcin in the Treatment of Periodontal Infrabony Defects. *Journal of International Dental and Medical Research*. 2020; 13 (4): 12–7.