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SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE-NANOSILVER-CLOVE OIL (*Eugenia Caryophyllus*) AS ANTIBACTERIAL IN TOOTHPASTE PREPARATIONS AGAINST STREPTOCOCCUS MUTANS BACTERIA

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Abstract: The formation of dental caries results from the activity of Streptococcus mutans bacteria. One of the efforts to prevent dental caries is using toothpaste containing antibacterial compounds such as hydroxyapatite-nanosilver-clove oil. This study aims to synthesize and characterize hydroxyapatite-nanosilver-cloves oil as an antibacterial that causes dental caries. In this study, physical properties were tested, namely, particle size with PSA, spreadability, stickiness, homogeneity, and chemical properties tested in functional groups with FTIR, pH, and antibacterial activity with the disc diffusion method. This study used 2% hydroxyapatite, ten ppm nanosilver, tween 80, xanthan gum, 70% sorbitol, sodium benzoate, sodium lauryl sulfate, sodium saccharin, calcium carbonate, peppermint, distilled water, clove oil with variations in the addition of concentrations of 1%, 2%, 3%, and 4%. The synthesis results produced a toothpaste preparation made from hydroxyapatite-nanosilver-clove oil, as evidenced by the results of FTIR characterization showing the presence of OH^-, CO_3^{2-}, PO_4^{3-}, C-H, C-O, and C=O functional groups. PSA results show hydroxyapatite-nanosilver-clove oil has an average size of 4464 nm which is not included in the nanoparticle category (<100 nm). The toothpaste spreadability test resulted in a spread diameter of 5-6 cm, which met the requirements for paste spreadability (5-7 cm), while the adhesion test resulted in adhesion (7-12 seconds) which has not met the ideal requirements (1-6 seconds). The pH measurement resulted in a toothpaste pH of 8.8-9.0 per SNI (4.5-10.5). The homogeneity test of the toothpaste has met the requirements according to SNI, namely the absence of air bubbles, clumps, and separated particles. The antibacterial activity test showed that the hydroxyapatite-nanosilver-clove oil toothpaste formulation was able to inhibit the growth of S.mutans bacteria with the highest inhibition diameter in the variation of adding 4% concentration of clove oil, namely 14.99 mm with normally distributed data ($p>0.05$). One Way ANOVA statistical analysis showed that the treatment variation had a significantly different effect ($p<0.05$). These results indicate that adding clove oil into the formulation of hydroxyapatite-nanosilver-clove oil toothpaste can affect its chemical, physical, and antibacterial activity.

Keywords: *Hydroxyapatite, Nanosilver, Clove Oil, Streptococcus mutants, Antibacterial*

INTRODUCTION

Teeth are hard organs found in the mouth which have the function of tearing, chewing, and grinding food before it enters the esophagus. Therefore, there is a need for dental care so that the teeth are maintained intact that they do not cause diseases that can damage the layers of the teeth, namely dental caries [1]. Data according to the World Health Organization there is half the human population on Earth suffered from dental and oral diseases in 2019, with the most common disease based on Global Burden Disease research, namely dental caries, with around 2 billion cases, making it a disease that dominates and affects human life [2].

Dental caries is an infectious disease that damages the tooth structure; this disease causes cavities, pain, sleep disturbances, tooth loss, infection, various dangerous cases, and even death [3]. Dental caries is a disease caused by *Streptococcus mutants* bacteria that metabolize carbohydrates into acid; in this acidic state, the *S. mutants* bacteria cause dental caries [4].

Bacteria have been shown to play an important role in damaging tooth tissue, especially by creating acidic conditions that support demineralizing teeth so that teeth lose their minerals [5]. Thus the degree of acidity (pH) of saliva is an important part of increasing tooth mineral (remineralization), so a decrease in salivary pH can cause tooth demineralization [6]. Remineralization can occur with the help of saliva, which contains the minerals needed for the formation of enamel and will be optimal if the saliva has enough calcium and phosphorus and the pH conditions in the mouth are supportive. With the ongoing remineralization process, the condition of dental caries can actually be prevented and managed [5].

Zulsantrius et al. [7] show that fluoridated and non-fluoridated toothpaste ingredients can increase tooth remineralization. Fluoridated toothpaste poses a dilemma because it can cause side effects in fluorosis or tooth demineralization when used in concentrations that are not recommended. Indonesia has determined that fluoride compounds in toothpaste cannot be more than 0.15% or 1500 ppm calculated from the total F (fluor) level [8].

New materials have been developed to inhibit the development of dental caries, including those containing hydroxyapatite (HAp). Hydroxyapatite (HAp) is the main constituent of the inorganic matrix of teeth and bones, and this mineral will be able to

provide calcium and phosphate ions to overcome tooth demineralization and remineralization [9]. HAp is included in bioceramics which have recently been studied intensively for its application in the medical field [10]. HAp has been shown to be biocompatible and very well tolerated by human oral tissues [11] and also has bioactivity, osteoconductivity, non-toxic and non-immunogenic properties [12].

HAp can be synthesized by various methods, one of which is calcination. The calcination method can remove organic compounds and water from beef bones to produce HAp and increase the yield of HAp produced. The advantage of using the calcination method is that the organic components in the bone can be decomposed thermally, and any signs of disease genomes can be removed, thereby providing a high biosafety factor [13].

In addition, one of the substances that is safe and proven to have antimicrobial activity is silver. Silver has significant and effective antimicrobial activity against *S. mutants* in the human oral cavity and periodontal pathogens. It is useful as an antibacterial agent incorporated into dental restorations, especially when applied in nanometer sizes [14].

Using HAp and Nanosilver will help increase the effectiveness in reducing the number of bacteria, addressing caries and plaque problems on teeth, and increasing the effectiveness of tooth enamel remineralization so that the process will take place more quickly and effectively. The materials used will be optimally utilized to maintain oral and dental health [15]. Synthesis of silver nanoparticles can be carried out by several methods, such as electrochemical methods, chemical reduction, ultrasonic, irradiation, photochemistry, and sonochemistry. The method of chemical reduction of silver salts by sodium citrate or sodium borohydride is the most frequently used because it is easy and simple [16].

One of the substances commonly added to toothpaste is herbal ingredients because they can inhibit plaque growth as the initial formation of dental caries and are also safe because they come from plants [17]. Many studies confirm spice plants' antibacterial, antifungal, antiviral, and anticarcinogenic properties. Cloves, in particular, have attracted attention due to their strong antioxidant and antimicrobial activity that stand out among other spices. Cloves produce about 14-21% essential oil, with the main component being 95% eugenol [18]. The HAp-Clove combination showed that the antibacterial activity inhibited S. mutants bacteria with an inhibition diameter of 17.6 mm at a concentration of 4 ml of clove oil [19].

The synthesis and characterization of hydroxyapatite-nanosilver-clove flower extract (*Syzygium Aromaticum L.*) gel as a toothpaste-forming gel showed that the toothpaste gel formulation with hydroxyapatite-nanosilver-clove oil had met the test eligibility standards, such as homogeneity, pH level, performance spread, and adhesion [20].

Based on this description, this study examines toothpaste with the basic ingredients HAp which comes from beef bone (*Bos taurus*), nanosilver, and clove oil with various concentrations of clove oil. Then it characterizes it with several tests, including homogeneity, pH, scattering, adhesion, functional group identification using a Fourier Transform Infrared (FTIR) spectrophotometer, and particle distribution testing using a Particle Size Analyzer (PSA) instrument.

RESEARCH METHODS

This research uses the true experimental method. Some of the tools used in this study are test tubes, 4-digit analytical balance, spatula, beaker, measuring cup, watch glass, dropper, oven, furnace, FTIR Instrument, PSA Instrument, thermometer, magnetic stirrer, Spin bar, mortar pestle, 100 mesh sieve, volume pipette, stopwatch, and aluminum foil.

The materials used in this study were distilled water, distilled water, hydrogen peroxide, acetic acid, cloves, Tween 80, Hydroxyapatite, phosphoric acid, silver nitrate solids, 1% citric acid, clove oil, xanthan gum, sodium benzoate, 70% sorbitol, sodium lauryl sulfate, sodium saccharin, calcium carbonate, and peppermint.

This research was conducted in stages. The first stage is the synthesis of hydroxyapatite and nanosilver, and the second stage is the manufacture of toothpaste.

The first stage, HAp 2% m/v, was prepared by mixing 0.4 g HAp with 20 mL phosphoric acid [19]. Nanosilver synthesis was carried out by chemical reduction technique using reducing sodium, namely sodium citrate. 5 mL of 1% sodium citrate solution is put in the burette. Next, a beaker containing 40 mL of 0.001 M $AgNO_3$ solution was stirred and heated to boiling. Then drip with 5 mL of sodium citrate solution in the burette. The stirrer process and heating were stopped when the solution started to turn yellow [15].

Table 1. Formulation of Hydroxyapatite-Nanosilver-Clove Oil toothpaste

	Formulation % m/m			
Material	F_1	F_2	F_3	F_4
Tween 80	4	4	4	4
HAp 2%	2	2	2	2
Nanosilver 10	5	5	5	5
ppm	1	2	3	4
Clove Oil	2	2	2	2
Xanthan Gum	20	20	20	20
Sorbitol 70%	0.5	0.5	0.5	0.5
Na-Benzoate	1.5	1.5	1.5	1.5
SLS	1	1	1	1
Na-Saccharin	40	40	40	40
$CaCO_3$				
Peppermint	1	1	1	1
Ad Aquades	100	100	100	100

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The second stage, toothpaste, is made by adding the active ingredient, which has been added with peppermint oil, into a beaker containing xanthan gum dispersion and then stirring with a magnetic stirrer until homogeneous. Then a solution containing sodium benzoate-saccharin and sodium lauryl sulfate was added and stirred at low speed using a magnetic stirrer until homogeneous. Furthermore, the addition of calcium carbonate, little by little, with the help of an electric mixer, is carried out until the sample is homogeneous. It produced white toothpaste. Below is presented data in tabular form regarding the toothpaste formulation used.

The synthesized toothpaste was then characterized physically and chemically. Physical characterization was carried out by testing with a PSA, spreadability test, adhesion test, and homogeneity. Chemical characterization was done by testing with an FTIR instrument to determine the functional groups and the pH test of the resulting toothpaste preparation.

Toothpaste made from hydroxyapatite-nanosilver-clove oil was tested using a PSA instrument to determine the particle size distribution. The spreadability test was carried out by smearing 1 gram of toothpaste on the glass, covering it again with transparent glass, giving it a weight of 200 g, and then measuring the diameter of the spread [21].

The adhesion test was carried out by weighing 0.25 g of the toothpaste preparation, placing it on the object glass, and then covering it with another glass object until it was completely closed. A weight of 1 kg is placed on the object glass that covers the preparation for 5 minutes. Then a load of 80 g removes the glass object from the toothpaste attachment. The time required to remove the two slides was then measured using a stopwatch [22].

The homogeneity test was conducted by smearing the toothpaste preparation on a transparent glass and then visually observing it in an inverted position [21].

The FTIR test was carried out on toothpaste made from hydroxyapatite-nanosilver-clove oil using an FTIR spectrophotometer to determine the success of the reaction. FTIR spectra were recorded at room temperature in the 4000–400 cm-1 range.

This antibacterial test was used to determine the antibacterial activity of the paste using the disc diffusion method by measuring the diameter of the growth inhibition of S. mutans. The antibacterial test was carried out with the stages of making Nutrien Agar (NA) and Muller Hinton Agar (MHA) media cultures. Then the bacterial suspension as inoculum. Suspension of S. mutans test colonies was prepared by taking one ose colony from solid NA medium to a test tube containing 9 mL NaCl 0.9%. Turbidity in the test colony suspension was standardized to the 0.5 McFarland standard (approx. 1.5 x 10 8 CFU/mL). When ready, put the paper disc into the petri dish and add 25 µl of toothpaste. Then incubated for 24 hours at 37ºC then observed the clear zone around the disc paper [23].

RESULTS AND DISCUSSION

The results showed that the synthesis of toothpaste had been successfully made and characterized. In addition, the resulting toothpaste is proven to have an antibacterial effect and is included in the strong category.

PSA Test Result

The HAp-nanosilver-clove oil sample was tested with five repetitions to analyze the particle size distribution using PSA so that an average of 13139 nm was obtained with the smallest size found in the 5th repetition, namely 2350 nm.

The results of measurements using PSA (in the form of particle size) and the potential zeta value from nanosilver synthesis tested with five repetitions obtained an average particle size of 32.64nm with the smallest size obtained on the 1st repetition, namely 30.47 nm as shown in Figure 1.

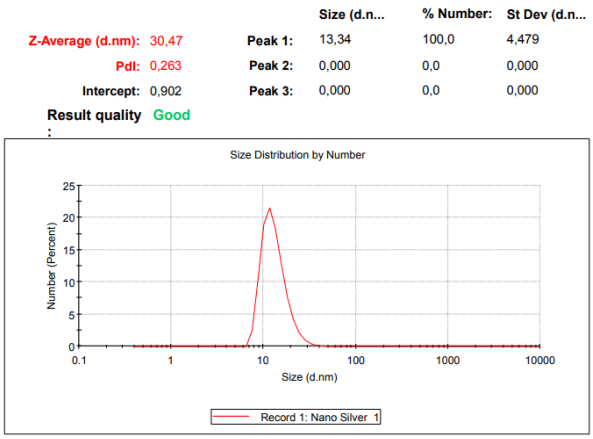

Figure 1. PSA Nanosilver test results

The synthesized composite does not meet the requirements of a nanoparticle, but the synthesized nanosilver has the characteristics of nanoparticles where the nanoparticles have a size of <100 nm [24]. It can be caused by chemical composition [25] and the influence of environmental factors such as long storage time [26].

FTIR Test Result

To find out the functional groups contained in the HAp-silver-clove oil sample, characterization was carried out using the FTIR instrument. The results of characterization with FTIR can be seen in Figure 1.

Figure 2 shows the HAp-nanosilver-clove oil spectra. In the FTIR spectra, the groups that appear are OH, CO_3^{2-}, PO_4^{3-}, C-H, C-O, and C=O groups. The OH^- functional group is shown at 3373.55 cm^{-1}. The C-H absorption spectrum is indicated by wave number 2923.76 cm^{-1}, which indicates the presence of

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conjugated aromatic compounds due to the stretching vibrations C-H sp_3 at wave number 1464.21 cm^{-1}. Meanwhile, the C=O group is shown at a wavelength of 1637.29 cm^{-1} and 1514.64 cm^{-1}. The CO_3^{2-} functional group is shown at wave numbers 1349.88 cm^{-1} and 1273.60 cm^{-1}. The C-O group in the above spectra is shown at wave number 1080.89 cm^{-1}. The PO_4^{3-} group is shown at wave numbers 944.59 cm^{-1} and 591.45 cm^{-1}.

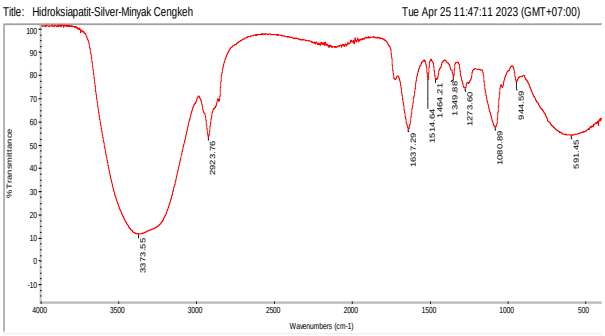

Figure 2. FTIR Spectra of HAp-Nanosilver-Clove Oil

pH, Spreadability, Adhesion, and Homogeneity Test

The synthesized toothpaste was then tested for pH, spreadability, adhesion, and homogeneity so that it was obtained, as shown in Table 2.

Table 2. Test results characterization of pH, spreadability, adhesion, and homogeneity

Formula	pH	Spreadability (mm)	Adhesion (s)	Homogeneity
F_1	9,0	5.47	12.85	Homogen
F_2	8,9	5.63	9.56	Homogen
F_3	8,8	5.85	7.59	Homogen
F_4	8,8	6.10	7.32	Homogen

Based on Table 2 above, the pH of the toothpaste produced meets the pH requirements specified by SNI. Namely, it must have a pH between 4.5-10.5. In addition, the spreadability of the resulting toothpaste has a range of 5-6 cm, which meets the requirements for the spreadability of the paste, which is 5-7cm [27].

The adhesion test aims to see the strength of the toothpaste to stick to the brush and tooth surface. There are no definite parameters for adhesion value; however, toothpaste should ideally have 1 – 6 seconds of adhesion. High adhesion shows that the consistency of the preparation is denser, elastic, and easily attached to the toothbrush but has poor distribution. Conversely, pastes with a thinner consistency usually have low adhesion and are not so attached to a toothbrush but can spread the active ingredients well [22]. The decrease in adhesion from adding 1% to 4% clove oil indicates that clove oil affects reducing the viscosity in toothpaste preparations.

Hydroxyapatite-nanosilver-clove oil toothpaste meets homogeneous requirements with no bubbles or clumping particles. The texture of the toothpaste depends on how much clove oil is added. The more clove oil used, the smoother the texture and the more liquid the consistency

Antibacterial Test

At this stage, the synthesized toothpaste was then tested for its bioactivity against S. mutans bacteria. The antibacterial activity test was carried out using the disc diffusion method. Observations were made after passing the incubation period for $1x_{24}$ hours with the focus of observation on the clear zone, which indicates the sensitivity of the bacteria to the sample, which is an antibacterial material. The inhibition zone formed was then measured using a caliper [28].

HAp-Nanosilver-Clove oil toothpaste samples with variations on the addition of clove oil, namely 1%, 2%, 3%, and 4%, with negative control using distilled water and positive control using sensitive toothpaste made from HAp. The antibacterial test results for the negative control did not show a clear zone, while the positive control showed the formation of a clear zone. The area of inhibition of the formation of the clear zone is shown in Figure 3.

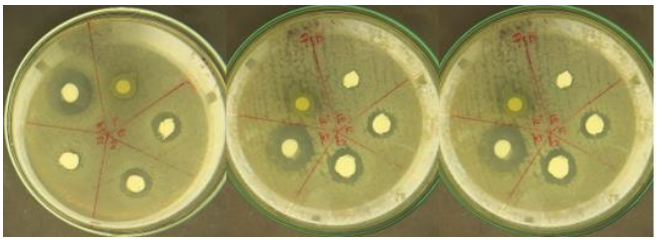

Figure 3. The clear zone formed from the results of the antibacterial test

At this stage, the clear zone formed indicates the antibacterial activity of the toothpaste with the active ingredient HAp-Nanosilver-Clove oil. Below is presented data on the antibacterial test results in Table 3.

Table 3. Antibacterial test results of hydroxyapatite-nanosilver-clove oil toothpaste

Formulation	Clear Zona (mm) Repetition			Average
	I	II	III	
F1	12.96	12.94	12.67	12.85
F2	13.55	13.38	14.62	13.85
F3	14.62	13.38	13.65	13.88
F4	15.79	14.98	14.22	14.99
Positive Control	21.40	21.92	20.92	21.41
Negative Control	0	0	0	0

The results of measuring the inhibition of Streptococcus mutans bacteria were tested using statistics. In the normality test, it obtained a p-value>

0.05 (Table 4), so it can be said that the data is normally distributed.

Table 4. Shapiro-Wilk Normality Test

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Sample	Statistic	df	Sig.	Statistic	df	Sig.	
Activity	Clove oil 1%	3	.363	.801	3	.118	
	Clove oil 2%	3	.339	.851	3	.242	
	Clove oil 3%	3	.306	.904	3	.398	
	Clove oil 4%	3	.177	1.000	3	.965	

a. Lilliefors Significance Correction

Furthermore, testing the data statistically with the homogeneity test resulted in p > 0.05 (Table 5), which is considered homogeneous.

Table 5. Homogeneity Statistical Test

		Levene Statistic	df_1	df_2	Sig.
Activity	Based on Mean	1.411	3	8	.309
	Based on Median	.502	3	8	.691
	Based on the Median and with adjusted df	.502	3	6.099	.694
	Based on trimmed mean	1.330	3	8	.331

Finally, a one-way ANOVA test with a significance level of 5% was used to determine significant differences between materials and try statistically. The results of the ANOVA test yielded a significant probability of p <0.05 (Table 6).

Table 6. One Way ANOVA Test Results on Clear Zone Data.

Activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.882	3	2.294	6.038	.019
Within Groups	3.040	8	.380		
Total	9.921	11			

The One Way ANOVA test results show a significance level of 0.019. If the significance value <0.05 indicates that, on average, the four variations of clove oil addition have a significantly different effect on each other.

Table 3 above shows that the greatest antibacterial activity is the area where the clear zone is formed by 14.99 mm at a concentration of 4% clove oil addition. Antibacterial activity appeared starting from adding 1% clove oil concentration and continued to increase until 4% clove oil was added. It shows that toothpaste with the addition of 4% clove oil has good stability resulting in the largest antibacterial clear zone because the greater the concentration, the faster the diffusion process so that the diameter of the formed clear zone is wider [19].

The inhibition zone can be categorized based on the diameter of the clear zone formed, namely the range <5 mm is categorized as low, the range 5-10 is categorized as medium, the range 10-20mm is categorized as strong, and the inhibition zone > 20mm is categorized as very strong [29].

From the inhibition zones obtained, the hydroxyapatite-nanosilver-clove oil formulation proved capable of suppressing Streptococcus mutans' growth. Nanosilver can penetrate the surface of bacteria and cause changes in the structure of the cell wall and cell membrane. Silver, too, is a weak acid, which tends to react with sulfur and phosphorus in DNA which is weak language. The nanoparticles will react with the weak base and inhibit bacterial DNA replication, causing bacterial cell death [15].

CONCLUSION

Based on this study's results, the FTIR characterization of hydroxyapatite-nanosilver-clove oil shows the functional groups OH, C-H, C-O, C=O, PO_4^{3-}, and CO_3^{2-}. In PSA testing, the smallest particle size is shown in the 5th repetition, 2350 nm. At the same time, the results of the nanosilver PSA test have the smallest value of 30.47nm. Toothpaste characterization with pH, spreadability, adhesion, and homogeneity followed existing standards. Activity test measurement antibacterial activity showed that the hydroxyapatite-nanosilver-clove oil formulation inhibited the growth of Streptococcus mutans bacteria with the largest diameter of inhibition on the addition of 4% clove oil of 14.99mm.

REFERENCES

[1] Hafizah, H. (2021). Sistem Pakar Untuk Pendiagnosaan Karies Gigi Menggunakan Teorema Bayes. *Jurnal Teknologi Sistem Informasi dan Sistem Komputer TGD*, *4*(1), 103-111.

[2] Jain, N., Dutt, U., Radenkov, I., & Jain, S. (2023). WHO's global oral health status report 2022: Actions, discussion and implementation. *Oral Diseases*.

[3] Suhadi, R. S. S., & Mauludin, A. (2023, January). Scoping Review: Rokok Sebagai Faktor Risiko terhadap Kejadian Karies Gigi. In Bandung

Conference Series: Medical Science (Vol. 3, No. 1, pp. 77-83).

[4] Tampoliu, M. K. K., Ratu, A. P., & Rustiyaningsih, R. (2021). Formula dan Aktivitas Antibakteri Obat Kumur Ekstrak Batang Serai Wangi (Cymbopogon nardus L.) Terhadap Bakteri Streptococcus mutans. *JPP (Jurnal Kesehatan Poltekkes Palembang)*, *16*(1), 29-39.

[5] Moelyaningrum, A. D. (2017). Timah hitam (Pb) dan karies gigi. Stomatognatic-Jurnal Kedokteran Gigi, 13(1), 28-31.

[6] Lely, M. A. (2017). Pengaruh (pH) saliva terhadap terjadinya karies gigi pada anak usia prasekolah. *Indonesian Bulletin of Health Research*, *45*(4), 241-248.

[7] Zulsantritus, Z., Edrizal, E., & Busman, B. (2016). Potensi Remineralisasi Pada Pasta Gigi Berflorida dan Tidak Berflorida. *B-Dent: Jurnal Kedokteran Gigi Universitas Baiturrahmah*, *3*(2), 139-144.

[8] Indrawati, R., Arundina, I., & Trisnadyantika, A. (2014). Efektivitas pasta gigi yang mengandung herbal terhadap Streptococcus mutans. *Oral Biology Dental Journal*, *6*(1), 56-60.

[9] Souza, B. M., Comar, L. P., Vertuan, M., Fernandes Neto, C., Buzalaf, M. A. R., & Magalhães, A. C. (2015). Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralization and remineralization in situ. *Caries Research*, *49*(5), 499-507.

[10] Anggresani, L., Perawati, S., & Rahayu, I. J. (2019). Limbah tulang ikan tenggiri (Scomberomorus guttatus) sebagai sumber kalsium pada pembuatan hidroksiapatit. *Jurnal Katalisator*, *4*(2), 133-140.

[11] Kattimani, V. S., Chakravarthi, P. S., Kanumuru, N. R., Subbarao, V. V., Sidharthan, A., Kumar, T. S., & Prasad, L. K. (2014). Eggshell derived hydroxyapatite as bone graft substitute in the healing of maxillary cystic bone defects: a preliminary report. *Journal of international oral health: JIOH*, *6*(3), 15.

[12] Lawarti, R. A., & Cahyaningrum, S. E. (2022). Potential Antibacterial Activity of Hydroxyapatite Composite And Green Betel Leaf Extract (Piper betel L.) Against Streptococcus mutans Bacteria. *Indonesian Journal of Chemical Science*, *11*(1), 41-49.

[13] Afifah, F., & Cahyaningrum, S. E. (2020). Sintesis Dan Karakterisasi Hidroksiapatit Dari Tulang Sapi (Bos Taurus) Menggunakan Teknik Kalsinasi Synthesis And Characterization Of Hydroxyapatite From Cow Bones (Bos Taurus) Using Calcination Techniques. *UNESA Journal of Chemistry*, *9*(3), 189-196.

[14] Azarsina, M., Kasraei, S., Yousefi-Mashouf, R., Dehghani, N., & Shirinzad, M. (2013). The antibacterial properties of composite resin containing nanosilver against Streptococcus mutans and Lactobacillus. *The journal of contemporary dental practice*, *14*(6), 1014.

[15] Lestari, N. R. D., & Cahyaningrum, S. E. (2022). Synthesis and characterization of hydroxyapatite-nanosilver as anti-bacteria that cause dental caries. *Indonesian Journal of Chemical Science*, *11*(1), 33-40.

[16] Ristian, I., Wahyuni, S., & Supardi, K. I. (2014). Kajian Pengaruh Konsentrasi Perak Nitrat Terhadap Ukuran Partikel Pada Sintesis Nanopartikel Perak. *Indonesian Journal of Chemical Science*, *3*(1).

[17] Putra, F. S., & Mintjelungan, C. N. (2017). Efektivitas pasta gigi herbal dan non-herbal terhadap penurunan plak gigi anak usia 12-14 tahun. *e-GiGi*, *5*(2).

[18] Hafizah, H. (2021). Sistem Pakar Untuk Pendiagnosaan Karies Gigi Menggunakan Teorema Bayes. *Jurnal Teknologi Sistem Informasi dan Sistem Komputer TGD*, *4*(1), 103-111.

[19] A Akbar, A. F., & Cahyaningrum, S. E. (2022). Characterization and Antibacterial Activity Testing of the Nano Hydroxyapatite-Clove (Eugenia Caryophyllus) Against Streptococcus Mutans Bacteria. *Indonesian Journal of Chemical Science*, *11*(1), 1-8.

[20] Nandasyah, I., & Cahyaningrum, S. E. Synthesis and Characterization of Hydroxyapatite Gel-Nanosilver-Clove Flower Extract (Syzygium Aromaticum L.) as a Toothpaste Forming Gel.

[21] A. Mahdalin, E. Widarsih, and K. Harismah, "Pengujian Sifat Fisika dan Sifat Kimia Formulasi Pasta Gigi Gambir dengan Pemanis Alami Daun Stevia," *6th Univ. Res. Colloq. 2017*, pp. 135–138, 2017.

[22] Gratia, B., Yamlean, P. V., & Mansauda, K. L. (2021). Formulasi pasta gigi ekstrak etanol buah pala (Myristica fragrans Houtt.). *Pharmacon*, *10*(3), 968-974.

[23] Nurhayati, L. S., Yahdiyani, N., & Hidayatulloh, A. (2020). Perbandingan pengujian aktivitas antibakteri starter yogurt dengan metode difusi sumuran dan metode difusi cakram. *Jurnal Teknologi Hasil Peternakan*, *1*(2), 41-46.

[24] Kantharia, N., Naik, S., Apte, S., Kheur, M., Kheur, S., & Kale, B. (2014). Nano-hydroxyapatite and its contemporary applications. *Bone*, *34*(15.2), 1-71.

[25] Xu, T., Gao, C., Feng, X., Huang, M., Yang, Y., Shen, X., & Tang, X. (2019). Cinnamon and clove essential oils to improve physical, thermal, and antimicrobial properties of chitosan-gum arabic polyelectrolyte complexed films. *Carbohydrate polymers*, *217*, 116-125.

[26] Xu, Y., Chu, Y., Feng, X., Gao, C., Wu, D., Cheng, W., ... & Tang, X. (2020). Effects of zein stabilized clove essential oil Pickering emulsion on the structure and properties of chitosan-based edible films. *International journal of biological*

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macromolecules, *156*, 111-119.

[27] Garg, A., Aggarwal, D., Garg, S., & Singla, A. K. (2002). Spreading of semisolid formulations: an update. *Pharmaceutical Technology North America*, *26*(9), 84-84.

[28] Palilling, A., Posangi, J., & Anindita, P. S. (2016). Uji daya hambat ekstrak bunga cengkeh (Syzygium aromaticum) terhadap bakteri Porphyromonas gingivalis. *e-GiGi*, *4*(2).

[29] Lomboan, E. R., Yamlean, P. V., & Suoth, E. J. (2021). Uji Aktivitas Antibakteri Sediaan Sabun Cair Ekstrak Etanol aun Cengkeh (Syzygium aromaticum) Terhadap Bakteri Staphylococcus aureus. *PHARMACON*, *10*(1), 767-773.