Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 8, No. 3, July 2023

Research Paper

Hydroxyapatite-ZnO Biomimetic Toothpaste Formulation from Rice Snail Shell Waste

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Abstract
Toothpaste is a preparation for dental treatment to clean, beautify, and replace minerals that decay from the surface of the teeth. Prevention of damage to the mineral layer of the teeth, the addition of remineralizing agents to toothpaste preparations in the form of hydroxyapatite-ZnO composites is carried out. This study aims to synthesize and characterize hydroxyapatite-ZnO, as well as to formulate hydroxyapatite-ZnO toothpaste from rice field conch shell waste. Hydroxyapatite-ZnO composites have been successfully synthesized using the sol-gel method and characterized using X-Ray Diffraction (XRD), Fourier Transform Infra-Red (FTIR), and Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX). The XRD result obtained a crystal size of 59.90 nm with a crystallinity percentage of 68.85%. The absorption band at a wave number of 452 cm^{-1} is an indication that the ZnO compound has been successfully combined with hydroxyapatite. SEM-EDX analysis showed that the morphology of the compound was granular with a rough surface, uneven size, and shape. The results of the hydroxyapatite-ZnO toothpaste formulation in the 45% formula had good physical stability compared to other formulas. The antibacterial properties of hydroxyapatite-ZnO toothpaste preparations showed a very strong inhibitory effect on Streptococcus mutans bacteria. The results of the hydroxyapatite-ZnO toothpaste formulation in the 45% formula had good physical stability compared to other formulas. The antibacterial properties of hydroxyapatite-ZnO toothpaste preparations showed a very strong inhibitory effect on Streptococcus mutans bacteria. The results of the hydroxyapatite-ZnO toothpaste formulation in the 45% formula had good physical stability compared to other formulas. The antibacterial properties of hydroxyapatite-ZnO toothpaste preparations showed a very strong inhibitory effect on Streptococcus mutans bacteria.

Keywords
Antibacterial, Hydroxyapatite-ZnO, Rice Snail Shell, Toothpaste

Received: 17 April 2023, Accepted: 26 June 2023
https://doi.org/10.26554/sti.2023.8.3.486-493

1. INTRODUCTION

Consumption of food and drink that humans do every day can cause tooth enamel damage. Tooth enamel damage that often occurs is dental caries. Dental caries can cause pain even though it is a non-communicable chronic disease (Moynihan, 2016). Although it can be prevented, dental caries is one of the most common diseases and requires medical care. As many as 80% of humans experience dental caries, of which 20-25% of sufferers are children, where 10% of all dental caries sufferers cannot be treated, and they come from low socioeconomic communities (Amaechi et al., 2019). As many as 90.9% of the community takes preventive action by cleaning their teeth using toothpaste, and the rest utilize the services of dentistry (Ghasemianpour et al., 2019).

For more than 50 years the most commonly used fluoride-containing toothpaste has been recommended. Although it is an effective strategy to reduce the percentage of dental caries sufferers, it should be noted that toothpaste containing fluoride can increase the risk of developing dental fluorosis(Bossù et al., 2019). Dental fluorosis is a disorder that occurs during the formation of tooth enamel thereby inhibiting the development of tooth enamel. This can occur due to excess fluoride exposure as tooth enamel forms. The enamel will be porous, have larger patches, or turn yellow to light brown in the tooth enamel area. Acute toxicity can also occur when the accumulation of fluoride in the body increases, especially in the organs in the stomach. The first organ affected by fluoride toxicity will cause initial symptoms of abdominal pain, nausea, and diarrhea until some cases show serious symptoms (Kanduti et al., 2016). Efforts to overcome fluoride toxicity by exploring fluoride replacement toothpaste.

The development of fluoride replacement toothpaste is attempted to maintain the ability to inhibit caries formation by maintaining the availability of calcium and phosphate ions. Hydroxyapatite (HAp) has been proposed as a substitute for fluoride as a remineralizing agent which has anti cariogenic characteristics (Amaechi et al., 2021). Hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2.14H_2O$) is a constituent of tooth enamel with sizes ranging from 20 to 40 nm. As these particles mature, hydroxyapatite will harden, limiting its ability to undergo biological remodeling during demineralization. Hydroxyapatite has been shown to have a remineralizing effect on artificial carious lesions and form new enamel layers (Anil et al., 2022). Hydroxyapatite in nanoparticle size can be synthesized from natural precursors, namely clam shells, egg shells, conch shells, and several other natural precursors. The high content of calcium carbonate from rice snails can also produce hydroxyapatite with a fairly high purity (Charlena, 2019).

Nanohydroxyapatite has been used extensively in biomedical applications because it closely resembles human hard tissue with excellent biocompatibility and osteogenic capacity, especially for use in toothpaste (Ebadifar et al., 2017). Nanohydroxyapatite can reduce pain due to dentin hypersensitivity because it has nanoparticle size that can enter the dentinal tubules. The nanoparticles settle within the tubules and consequently block neural interactions with external stimuli (Coelho et al., 2019). Hydroxyapatite-ZnO nanoparticles in toothpaste formulations is expected to show the deposition of calcium and phosphorus ions on the tooth enamel surface. Therefore, tooth remineralization will increase (Toledano et al., 2019). Nanohydroxyapatite added with Zn showed a decrease in plaque score, Full Mouth Plaque Score (FMPS), and reduced bleeding, Full Mouth Bleeding Score (FMBS). This indicates an anti-inflamm atory and antibacterial role by adding Zn to the nanohydroxyapatite toothpaste formulation (Monterubbianesi et al., 2020).

Previous studies used abrasive materials in the form of $CaCO_3$ Syurgana (2017) while hydroxyapatite was not widely used, one of which was Anggresani et al. (2021), namely the use of hydroxyapatite from Tenggiri Fish Bones. It also has not done the substitution of zinc oxide on hydroxyapatite. The importance of this research is to obtain toothpaste containing hydroxyapatite-ZnO nanoparticles as a remineralizing agent and has antibacterial activity.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this study were rice snail shells from Menduran Village, Grobogan Regency, Central Java, CMC Na, methyl paraben, glycerin, Na saccharin, titanium dioxide, $(NH_4)_2HPO_4$, ZnO, Mueller Hinton Agar from Merck, Na lauryl sulfate. (Techno Pharmachem), oleum menthae (Fuyang Best), Streptococcus mutans and aquadest.

2.2 Methods

HAp synthesis in this study was carried out using the wet reaction method (sol-gel). The five stages of the study included preparation of $CaCO_3$, synthesis of hydroxyapatite-ZnO, characterization of HAp using XRD, FTIR, and SEM, preparation of gel toothpaste, and physical evaluation of gel and antibacterial toothpaste preparations.

2.3 Calcination of Rice Snail Shells (Charlena, 2015)

The rice field snail shells were cleaned of dirt and then dried in the oven for 1 hour. The rice field snail shell was mashed and then sieved with a size of 100 mesh. Powdered snail shells were calcined in a furnace at 1000°C for 2 hours to calacify into CaO. The CaO powder was left in contact with air at room temperature for one week to turn into $Ca(OH)_2$.

2.4 Hydroxyapatite Synthesis Using the Sol-Gel Method (Moldovan et al., 2015)

The synthesis of hydroxyapatite-ZnO was carried out by adding a solution of $(NH_4)_2HPO_4$ and ZnO to $Ca(OH)_2$ powder resulting from calcination. $(NH_4)_2HPO_4$ solution with a concentration of 0.3 M was added dropwise to 0.5 M $Ca(OH)_2$ suspension. The suspension was then decanted for 120 hours. ZnO solution with a concentration of 0.01 M was added to the hydroxyapatite gel and heated again at 80-90°C. The resulting suspension was stirred for 1 hour. After 12 hours, the suspension was filtered using a Buchkner funnel and washed with distilled water 2-3 times. The filtered results were transferred to a porcelain cup and dried in an oven at 120°C for 4 hours. The powder obtained was then put into the furnace and heated at 1000°C for 2 hours.

2.5 Toothpaste Preparation

The preparation of gel toothpaste preparations is carried out by weighing all the ingredients according to the calculation (Table 1). Then developed Na CMC using warm water as much as 20 times the amount of Na CMC weighed, let stand for 30 minutes, and homogeneously crushed. In a different container, glycerin and TiO_2 were mixed, then methyl paraben was added and stirred until homogeneous. Na CMC which had expanded was added with sodium saccharin which had previously been dissolved with the rest of the water, then ground until homogeneous. Next, hydroxyapatite-ZnO from rice snail shells was added, Na lauryl sulfate, and a few drops of oleum menthae. At each addition of the material, crushed until homogeneous. So that the final result is obtained, all the ingredients are mixed homogeneously and a paste mass is formed.

2.6 Evaluation of Gel Toothpaste Preparations

Evaluation of gel toothpaste preparations was carried out by testing the shape (consistency), color, and aroma of the preparations objectively using the five senses. In addition, pH, homogeneity, foam formation, and antibacterial activity tests were carried out using Streptococcus mutans with the Kirby-Bauer modified agar diffusion method.

3. RESULTS AND DISCUSSION

3.1 Calcium Hydroxide from Rice Field Snail Shells

Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is a bioceramic material that is widely used as a substitute for bones and teeth. One of the reasons for using hydroxyapatite is that its chemical composition is similar to the mineral phase of human bones and teeth. Hydroxyapatite material is a bioactive ceramic that has

Table 1. Formulation of Toothpaste

Material	The Concentration of Preparation Ingredients (%)			Function
	F1	F2	F3	
Hydroxyapatite-ZnO	40	45	50	Abrasive Agent
Na CMC	3.5	3.5	3.5	Gelling/Binder Agent
Glycerin	35	35	35	Humectants
Methyl Paraben	0.1	0.1	0.1	Preservative
Na Saccharin	0.2	0.2	0.2	Sweetener
Na Lauryl Sulfate	1	1	1	Surfactant
TiO_2	0.1	0.1	0.1	Bleach
Oleum Menthae	qs	qs	qs	Taste
Aquades	ad 100	ad 100	ad 100	Solvent

Note: qs = quantum sufficient (to taste)

Table 2. Absorption Range (cm^{-1}) and Hydroxyapatite-ZnO Functional Groups

Functional Groups	Absorption Range (cm^{-1})	
	This Research	Charlena et al. (2019)
OH^-	726, 1655, 1956, 3461	630, 1600, 1900, 3440
$PO_4{}^{3-}$	552, 603, 972, 1024	471, 567, 961, 1050
$CO_3{}^{2-}$	1513	1500
ZnO	452	416

biocompatibility capable of accelerating bone regeneration, and osseointegration properties that can blend with bone. Hydroxyapatite can be synthesized from inexpensive natural sources such as beef bones, fish bones, clam shells, egg shells, and rice field snail shells (Gunawan, 2011). The shell of the paddy snail (Bellamnya javanica) taken from the shallow waters of Menduran Village, Grobogan Regency, is rich in various minerals including calcium. Waste of rice field snail shells is used as a source of calcium because of its abundant availability and low price. The rice field snail shell waste was cleaned of dirt, then dried and ground into 100 mesh size powder.

The source of calcium obtained from rice field snail shells is calcium carbonate ($CaCO_3$). Calcium carbonate is calcined at 1000°C for 2 hours to obtain calcium oxide. Calcination of calcium carbonate produces calcium oxide (CaO) and carbon dioxide gas (CO_2) according to the following reaction:

$$CaCO_3(s) \rightarrow CaO(s) + CO_2(g) \qquad (1)$$

Calcium oxide is converted to $Ca(OH)_2$ through the hydration process. Hydration was carried out by allowing CaO to come into contact with open air at room temperature for one week. Hydration occurs spontaneously between CaO and water vapor. The reactions that occur are as follows:

$$2CaO(s) + 2H_2O(g) \rightarrow 2Ca(OH)_2(s) \qquad (2)$$

The results of the calcination and hydration phase were characterized using XRD, where the expected phase was $Ca(OH)_2$ (Figure 1).

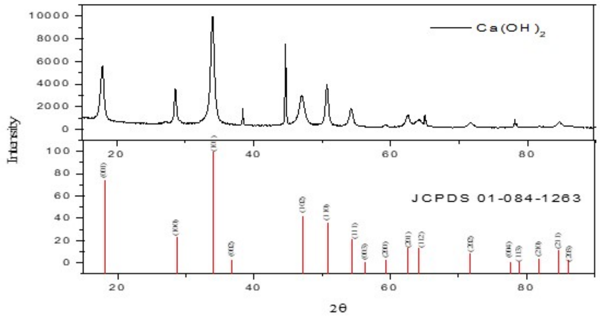

Figure 1. X-ray Diffractogram of Rice Field Snail Shell After Hydration

The presence of $Ca(OH)_2$ is indicated by diffraction peaks with the highest intensity at 2θ: 17.89°, 28.56°, 33.94°, 46.88°, 50.66°, 54.18°, 62.43 °, 63.90°, 71.54°, 78.74° and 84.50°. The results of the XRD analysis correspond to the standard diffraction pattern of calcium hydroxide according to JCPDS 01-084-1263 where the highest intensity peak pattern is at 2θ rated 28.60°, 34.10°, 47.10°, and 50.80°. There are also peaks at 2θ: 38.34° and 44.62° which are impurities from the tools used. Based on the XRD results obtained, it is possible to

Table 3. Results of Physical Evaluation of Toothpaste Preparations

Evaluation	Formulas F1	F2	F3
Organoleptic			
Color	White-gray	White-gray	White-gray
Aroma	Mint	Mint	Mint
Consistency	Less Viscous	Thick	Thick
Homogeneity	Enough Homogeneous	Enough Homogeneous	Not Enough Homogeneous
pH	8.12	8.09	8.24
Formation of Foam(cm)	7.7	7.8	8.1

Table 4. Organoleptic Test Results for F1 Toothpaste Base for 4 Weeks of Storage

Organoleptic			Week-		
	0	1	2	3	4
Color	Gray White	Gray White	Gray White	Gray White	Gray White
Aroma	Mint	Mint	Mint	Mint	Mint
Consistency	Less Viscous	Less Viscous	Less Viscous	Less Viscous	Less Viscous
Homogeneity	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous
pH	8.12	8.05	7.98	7.74	7.79
Formation of Foam(cm)	7.7	7.5	7.6	7.5	7.6

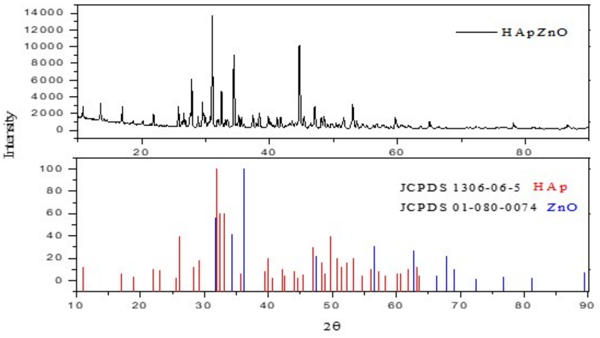

Figure 2. Hydroxyapatite-ZnO X-ray Diffractogram

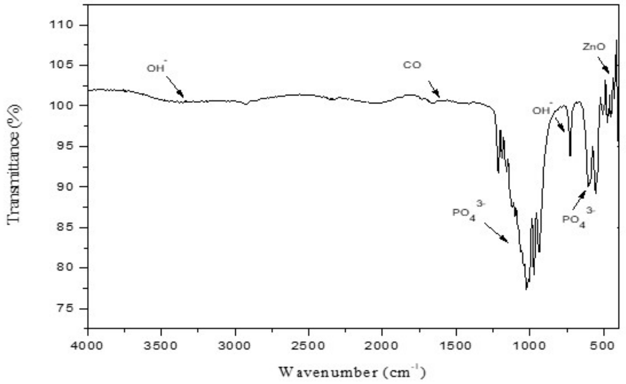

Figure 3. Hydroxyapatite-ZnO Infra-red Spectrum

find the crystalline particle size of $Ca(OH)_2$ using the Debye Scherrer equation. The average crystal size based on this equation is 13.80 nm. The experiment results show that the smaller crystal size, the wider diffraction pattern obtained, this is due to the fact that crystals with small sizes have a more limited number of diffraction fields (Gunawan, 2011).

3.2 Hydroxyapatite-ZnO

The material used as a toothpaste formulation is expected to have antibacterial activity. One method to achieve this is by compositing the material with zinc oxide. The HAp-ZnO composite material was characterized using XRD (Figure 2).

Further heating at 1000°C for 2 hours caused the peaks to overlap with other phases such as tricalcium phosphate and octacalcium phosphate. The appearance of the last phase is probably a consequence of the attachment of the ZnO composite crystal structure to hydroxyapatite. The average size of hydroxyapatite-ZnO crystals based on the Debye Scherrer equation was obtained at 59.90 nm. The crystal size of the synthesized results is smaller than that of Zelaya et al. 2019 has an average diameter of 77 nm. The crystallinity of the synthesized HAp-ZnO was 68.65% smaller than that of the synthesized HAp according to Galindo et al. (2016) 75.44%. This indicates a decrease in crystallinity due to the substitution of Zn (radius 0.6 nm) with calcium ions (radius 0.1 nm) in the HAp structure.

ZnO-substituted hydroxyapatite was then characterized by FTIR. The functional groups $–CO_3^{2-}$, $-OH^-$, and $–PO_4^{3-}$ are functional groups that indicate the presence of the hydroxyap-

Table 5. Organoleptic Test Results for F2 Toothpaste Base for 4 Weeks of Storage

Organoleptic	Week- 0	1	2	3	4
Color	Gray White	Gray White	Gray White	Gray White	Gray White
Aroma	Mint	Mint	Mint	Mint	Mint
Consistency	Thick	Thick	Thick	Thick	Thick
Homogeneity	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous	Pretty homogeneous
pH	8.09	7.83	7.75	7.74	7.71
Formation of Foam(cm)	7.8	8.0	8.1	7.7	7.6

Table 6. Organoleptic Test Results for F3 Toothpaste Base for 4 Weeks of Storage

Organoleptic	Week- 0	1	2	3	4
Color	Gray White	Gray White	Gray White	Gray White	Gray White
Aroma	Mint	Mint	Mint	Mint	Mint
Consistency	Thick	Thick	Thick	Thick	Thick
Homogeneity	Less homogeneous	Less homogeneous	Less homogeneous	Less homogeneous	Less homogeneous
pH	8.24	8.20	7.96	7.85	7.88
Formation of Foam(cm)	8.1	8.3	8.1	8.0	8.0

atite phase. The FTIR spectrum of hydroxyapatite-ZnO can be observed with absorption bands in the range of 4000-400 cm^{-1} as shown in Figure 3. The absorption ranges obtained are comparable to those reported (Table 2). The presence of the functional group $–CO_3{}^{2-}$ is shown at wavenumber 1400-1500 cm^{-1}. The absorption bands at wavenumbers 726, 1655, 1956, and 3461 cm^{-1} indicate the presence of the $–OH^-$ functional group. The functional group $–PO_4{}^{3-}$ is indicated by absorption peaks at wavenumbers 552, 603 937, 972, 1024, and 1213 cm^{-1}. ZnO compounds show vibrations at wavenumber 400-600 cm^{-1} (Azam et al., 2012).

The results of the HAp-ZnO composite spectrum show that there is an absorption band at wavenumber 452cm^{-1}. This absorption band appears as an indication that the ZnO compound has been successfully composited with hydroxyapatite. The presence of ZnO affects the hydroxyapatite spectrum due to steric strain leading to a reduction in the vibrational energy of the HAp molecule. The low intensity of the OH peak when substituted for ZnO indicates that new bonds are formed, where ZnO interacts strongly with HAp around the OH bonds or directly with the OH groups (Moldovan et al., 2015).

Hydroxyapatite-ZnO morphological analysis can be identified using Scanning Electron Microscopy (SEM). SEM analysis functions to determine the shape or surface of a material, besides that the results of SEM analysis can show the pore size of a sample. The porous structure of hydroxyapatite will trigger new cell growth. Pores are indispensable for media infiltration of osteoblast cells thereby accelerating the mineralization of bones and teeth. Characterization using SEM was carried out at 5000x magnification (Figure 4a) and 20,000x (Figure 4b). The EDX result of the Ca/P ratio is 1.91 which means more than 1.67. This is due to the interaction of hydroxyapatite with Zn^{2+} through a different scheme, namely O from $(PO_4{}^{3-})$; O from OH; Ca from $Ca(OH)_2$, and P from $(PO_4{}^{3-})$. Based on research by Athawi (2013) the morphology of hydroxyapatite shows the formation of pores with a size of 0.09-0.04 μm. The resulting particle shape is granular with a rough surface, uneven size, and shape. Sizes that appear not uniform occur due to agglomeration. Agglomeration occurs due to the adhesion of particles due to temperature treatment. As the temperature increases, the movement between particles becomes more reactive, so the crystallization rate increases rapidly. Agglomeration can result in a larger readable particle size. The non-uniformity affects the mechanical properties of the hydroxyapatite-ZnO composite.

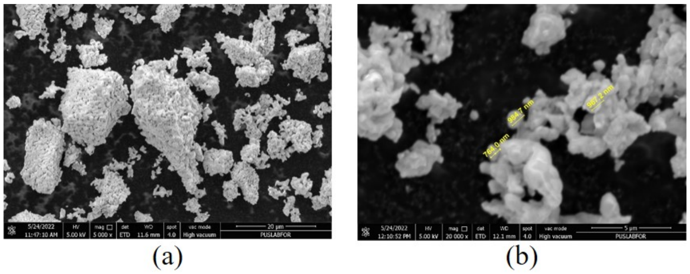

Figure 4. Results of HAp-ZnO SEM Analysis at 5000x Magnification (a) and 20,000x Magnification (b)

3.3 Hydroxyapatite-ZnO Gel Toothpaste Formulation

The formulation of hydroxyapatite-ZnO toothpaste can be seen in Table 1. Optimization of the three formulas is expected to obtain the best basic formula as a hydroxyapatite-ZnO toothpaste preparation. Optimization was carried out by making three variations of the formula for the concentration of hydroxyapatite-ZnO used, namely, 40%, 45%, and 50%. The formulation consists of various ingredients, including Na-CMC which acts as a thickening agent, and glycerin as a humectant.

Table 7. Diameter of the Bacterial Inhibition Zone

Sample	Inhibition Zone Diameter(mm) First Repeat	Inhibition Zone Diameter(mm) Second Repetition	Mean ± SD (mm)	Classification
Formula 40%	20	19	19.5 ± 0.707	Strong
Formula 45%	22	21	21.5 ± 0.707	Very Strong
Formula 50%	23	22	22.5 ± 0.707	Very Strong
Positive Control	25	26	25.5 ± 0.707	Very Strong
Negative Control	0	0	0	Not Inhibit

Both of these ingredients affect the consistency of toothpaste preparations. The results of the evaluation of the physical parameters of toothpaste preparations are following Table 3.

Based on the results obtained, the three formulas for organo leptic evaluation were not much different, namely having a white-gray color with a minty aroma coming from oleum menthae, and the consistency slightly increased with increasing concentration. Homogeneity is an important factor as a benchmark for the distribution of each material. If the homogeneity is good, the distribution of each ingredient is evenly distributed, so that the ingredients are dispersed and mixed optimally due to the effectiveness of the toothpaste preparation when it is applied.

The homogeneity requirement is based on SNI 12-3524-1995 which is characterized by no observed air bubbles and clumps of particles that separate from the toothpaste preparation. Based on the evaluation results, it was stated that the hydroxyapatite-ZnO toothpaste preparation at a concentration of 50% was less homogeneous because there were few air bubbles and particle clumps. This is caused by the amount of concentration of Na-CMC used, so it is necessary to carry out further research on the impact of different concentrations of Na-CMC on hydroxyapatite-ZnO toothpaste preparations. The pH test was carried out to determine the level of acidity or alkalinity of the hydroxyapatite-ZnO gel toothpaste preparation. The suitability of the pH to the standard is expected so that irritation of the oral and dental mucosa does not occur. The pH requirements based on SNI 12-3524-1995 for toothpaste are 4.5-10.5.

The foam formation test was carried out to determine the amount of foam produced by the preparation formula. The foam acts to remove dirt and clean the mouth when it is applied. The foam formation test does not have a standard reference for foam height in toothpaste, but this is related to the aesthetic value preferred by consumers. Foam in toothpaste preparations is produced due to the addition of surfactants in the form of sodium lauryl sulfate (SLS). SLS is an anionic surfactant that is contained in soap, shampoo, toothpaste, and other cleaning products. In addition, SLS is known as a surfactant with good foam-forming power and high cleaning power. Based on the evaluation results of hydroxyapatite-ZnO toothpaste preparations, the three formulas showed a good ability to form foam. Based on the results of the overall evaluation it is known that the best basis for the formula is F2 where the physical stability parameters are following the standard for each parameter. The evaluation results for each parameter of the physical characteristics of the base and hydroxyapatite-ZnO toothpaste preparations during a 4-week storage time at room temperature are presented in Tables 4, 5, and 6.

3.4 Antibacterial Activity

The antibacterial activity test was carried out to compare the antibacterial abilities of the three formulas with positive control, namely commercial toothpaste with the antibacterial activity that has been traded to the public. Streptococcus mutans bacteria is a gram-positive bacteria that cause dental caries. The results of the antibacterial test showed an increase in antibacterial activity in the material which was composited with ZnO. The increase in antibacterial ability is evidenced by the increase in the width of the clear zone (inhibition zone). The results of the antibacterial test obtained the diameter of the inhibition zone in the F1 formulation (40%) of 20 and 19 mm. The results of the antibacterial test obtained the diameter of the inhibition zone in the F2 formulation (45%) of 22 and 21 mm, while the results of the antibacterial test obtained the diameter of the inhibition zone in the F3 formulation (50%) of 23 and 22 mm (Table 7).

Based on the results of the study, it was shown that hydro xyapatite-ZnO toothpaste preparations produced strong and very strong inhibition zone diameters for Steptococcus mutans bacteria. The test results were compared with the antibacterial activity of hydroxyapatite-ZnO against Escherichia coli (Gram-negative bacteria) and Staphylococcus aureus (gram-positive bacteria) according to (Charlena, 2019) of 16 and 12 nm, respectively. The difference in inhibition is due to differences in the characteristics of the cell wall between groups of bacteria. Gram-positive bacteria can protect the inside of cells from penetration of antibacterial compounds due to their thick peptidoglycan layer (Bhowmick et al., 2015). The increase in the inhibition zone against Streptococcus mutans was due to the addition of the formulation composition, namely oleum menthae which also has antimicrobial activity (Kapp et al., 2020).

The comparison gel toothpaste obtained inhibition zone diameters of 25 and 26 mm. If the diameter of the inhibition zone is 5 mm or less, then the inhibitory activity is categorized as weak. The diameter of the inhibition zone is 5-10 mm, it is categorized as moderate. The inhibition zone diameter of 10-20 mm is categorized as strong, and if the inhibition zone diameter is 21 mm or more, then the activity is categorized as very strong (David and Stout, 1971). The greater the concentration of hydroxyapatite-ZnO in the toothpaste formulation, the greater the inhibition obtained. The results of the antibacterial activity test of the 40%, 45%, and 50% formulas were not significantly different whereas the three formulas had antibacterial activity based on the ANOVA test where the probability value was <0.05.

4. CONCLUSION

The hydroxyapatite-ZnO composite has been successfully synthesized and the results of characterization using XRD, FTIR, and SEM are following the literature. The addition of ZnO compounds causes a decrease in the degree of crystallinity. The hydroxyapatite-ZnO composite has been successfully formulated into toothpaste preparations. The formulation of hydroxyapatite-ZnO toothpaste has stable color, odor, and consistency, fairly homogeneous preparation, acceptable pH value, and good foam formation. The results of the formulation of hydroxyapatite-ZnO toothpaste in the second formula (45% hydroxyapatite-ZnO) have good physical properties compared to other formulas. Toothpaste preparations have a good effect on inhibiting the growth of bacteria, especially Streptococcus mutans bacteria. The three formulas showed strong and very strong inhibition against these bacteria.

5. ACKNOWLEDGMENT

We want to thank the lecturers and the manager of the Laboratory of Anorganic Chemistry, IPB University, for supporting the establishment of this research.

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