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PIENNO-MOASH: Formulation of Nanoparticle Mouthwash from Pineapple Peel Extract (Ananas comosus L.) as an Antibacterial Agent Against Streptococcus mutans via In Silico and In Vitro Studies

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KEYWORDS
Antibacterial; Nanoparticle mouthwash; Pineapple peel extract; Streptococcus mutans

ABSTRACT
Dental caries cases are an increasing problem in adult and pediatric patients, one of the causes of which is the Streptococcus mutans bacteria. Pineapple peel (Ananas comosus L.) is a plant part that has been known to have antibacterial activity due to the content of secondary metabolites epicatechin and quercetin. However, the exploration of pineapple peel as a nanoparticle mouthwash has not been widely done. This study aims to extract pineapple peel, analyze the phytochemical profile of the extract, conduct molecular docking tests on dental caries protein targets, test the antibacterial activity of pineapple peel extract on Streptococcus mutans cultures, optimize and evaluate the formulation of "PIENNO-MOASH" nanoparticle mouthwash preparations. The extraction method was carried out by maceration of 70% ethanol solvent then the extract results were qualitatively tested for phytochemical screening. In silico antibacterial activity testing was carried out by molecular docking on Ag I/II and GbpC proteins. While testing antibacterial activity in vitro using disc diffusion method with pineapple peel extract concentration. The results of maceration obtained yield of pineapple peel extract 6.88%. Qualitatively positive extracts contain alkaloids, saponins, flavonoids, quinones, polyphenols, and triterpenoids. In silico antibacterial testing results showed epicatechin as the best Ag I/II protein inhibitor and quercetin as the best GbpC protein inhibitor. The results of antibacterial testing in vitro could not be analyzed due to the appearance of bacterial contamination. The results of optimization and evaluation showed that formulation F2 had particle size, specific gravity, pH, and viscosity that met the nanoparticle preparation. So it can be concluded that pineapple peel extract contains secondary metabolites that can affect the growth of Streptoccocus mutans bacteria and formula F2 has a composition that meets the requirements of nanoparticle mouthwash preparations.

Introduction

Health is one aspect that plays an important role in life, so it must be maintained and treated regularly. Especially oral health, which if ignored will cause a variety of diseases, one of which is dental caries. According to the WHO Global Oral Health Status Report (2024) an estimated 2 billion people suffer from permanent dental caries, and 514 million children suffer from primary dental caries. This is also based on data from WHO, dental caries in European, American, and Asian countries, including Indonesia; the prevalence reaches 80 - 90% of children under the age of 18 years, namely 6-12 years old, are affected by dental caries. It is estimated that 90% of school-age children worldwide have suffered from caries (Adam & Ratuela, 2021). Caries is damage to the tooth coating caused by the acidic environment produced by fermentative microorganisms as a product of carbohydrate metabolism. The fermentation of carbohydrates will produce organic acids, such as lactic acid, acetic acid, and propionate which reduce the pH to a critical number (5.2 - 5.7). This supports the colonization of microorganisms that are both tolerant and acid-producing. Several studies have discussed the microbiota of infecting dental caries, of which one is Streptococcus mutans (Se, 2014). Medically, Streptococcus mutans infection can be inhibited through the administration of antibiotics. However, sourced from research on the test of Streptococcus mutans bacteria against antibiotics for dental caries patients, the results were obtained in the form of antibiotic-resistant properties (Borty et al., 2015). Therefore, alternatives to resistance are needed through other antibiotic treatments, including herbal antibiotics derived from plants.

Indonesia is one of the mega biodiversity countries; this is evidenced by as many as 15,000 Indonesian plants that have the potential to become efficacious drugs (Setiawan, 2022). The utilization of plants as herbal medicines comes from the skin of fruits that often become organic waste, such as pineapple skin. There are about 596,000 tons per year of pineapple peel waste that is just thrown away (Kholifah et al., 2022). Pineapple fruit (Ananas comosus L.) contains vitamins A and C, calcium, phosphorus, magnesium, sodium, potassium and the enzyme bromelain (Damogalad et al., 2013). Simplisia of pineapple fruit peel, which is an ethanol extract and water juice of pineapple fruit peel, contains antibiotic compounds of alkaloids, flavonoids, tannins, saponins, steroids / triterpenoids and glycosides (Nadia et al., 2023). Based on research, pineapple peel extract containing the enzyme bromelain and secondary metabolites can act as antibiotics in inhibiting the growth of Streptococcus mutans bacteria. Pineapple peel extract (Ananas comosus L.) made in concentrations of 100%, 50%, 25%, 12,5%, 6,25%, 3.125%, 1.56%, 0.78%, and 0.39% were tested against Streptococcus mutans. The study showed the results of the Minimum Inhibitory Level (KHM) of Streptococcus mutans at a concentration of 6.25% (Anggraeni, 2021).

Based on this, researchers want to maximize the principles of Green Chemistry in dealing with pineapple peel waste through the use of antioxidant compounds so that it can be used as an innovative alternative to herbal-based antibiotics in overcoming dental caries. Especially the utilization of Subang pineapple peel (Ananas comosus L. Cayenne), which is based on the determination of the total protein of the bromelain enzyme extract, which produces 44.43%, a value that is relatively high because the bromelain enzyme is composed of quite a lot of proteins (Nuraeni

et al., 2021). Therefore, researchers tried to make a nanoparticle mouthwash preparation from pineapple peel extract of Subang varieties into PIENNO-MOASH as an antibiotic against Streptococcus mutans bacteria. Innovation in the form of nanoparticles is chosen based on the structure in the size of 1-100 nm so as to facilitate the absorption process of the extract. In addition, the use of Mouthwash can make it easier for extracts to reach hard-to-reach areas so that the particle distribution process becomes more evenly distributed throughout the tooth substance. Nanoparticle mouthwash is antimicrobial and, as a consequence, can prevent the formation of dental caries (Vasiliu et al., 2021). In addition, practical use, giving a mint sensation and leaving a fragrant aroma after use are the advantages of these preparations.

The purpose of this research is to analyze several things based on the formulation of the problems that have been described. This study aims to analyze the extraction results and phytochemical profile of pineapple peel extract with 70% ethanol solvent and to analyze the effectiveness of pineapple peel extract on the inhibition of Streptococcus mutans bacteria both in silico and in vitro. In addition, this study aims to analyze the optimization and evaluation of PIENNO-MOASH formulations based on pineapple peel extract.

Based on the focus of the author's study, this research is expected to provide benefits for the author, medical personnel, and the government. The benefits of this research include providing information about innovations in the utilization of herbal plants as local natural resources and house plants, which can be used as ingredients for making mouthwash to treat dental caries and help solve existing problems in the world of health. In addition, this research is expected to contribute in the form of products and knowledge for medical practitioners in the treatment of Streptococcus mutans bacterial infections through the utilization of the content of pineapple peel extract (Ananas comosus L.) in the form of mouthwash nanoparticle preparations.

Materials and Methods

The data collection methods used in this research are literature study, observation, laboratory experiments, and documentation. The literature study was conducted by reviewing national and international literature, books, and research journals that are relevant to the issues discussed in this study. The observation method is carried out using observation, direct sampling and recording the overall symptoms that occur in the object of research, as well as observing the results of laboratory test examinations. The laboratory experimental method was carried out by the process of making, calculating, and testing phytochemicals, analyzing the particle size of the formula, and measuring the specific gravity, pH, and viscosity of the formula. The documentation method was carried out by taking pictures personally and collecting data from various sources of documents originating from the internet, which was carried out from the beginning of the research until the research was completed.

This research was conducted by the author in the Laboratory of SMA Negeri 1 Lhokseumawe, Chemistry Laboratory FKIP Syiah Kuala University, Material and Energy Physics Laboratory FMIPA Syiah Kuala University, Environmental Quality Testing Engineering

Laboratory FT Syiah Kuala University, and TDMRC Laboratory Syiah Kuala University in April to August 2024.

The research variables in this study are categorized into independent, control, and bound variables. The independent variable is the pineapple peel extract concentration. The control variables include PEG-400, sorbitol, Tween-80, virgin coconut oil (VCO), distilled water, and sodium benzoate. The bound variables are the percentage yield of the extract, organoleptic properties, specific gravity, particle size, pH acidity, and viscosity.

The tools used in this research are oven, blender, glass jar, digital balance, dropper pipette, filter paper, autoclave, bunsen, petri dish, sterile cotten swab, vernier, thermometer, electric stove, vessel, glass funnel, stirring rod, baker's glass, measuring cup, sterile tweezers, test tube, sonicator, water bath, magnetic stirrer, spectrophometer, PSA (Particle Size Analyzer), brookfield viscometer, and pycnometer. The material used is pineapple peel. The chemicals used were 70% ethanol, dragendroff reagent, mayer reagent, wagner reagent, hot water, distilled water, propanol, HCl, NaOH, 10% ammonia, chlorophome, HCl, H2SO4, gelatin, FeCl3, liebermen burchaardat, MHA (Mueller Histon Agar), physiological NaCl, paper disk, Tween 80, VCO, PEG-400, sodium benzoate, sorbitol, papermint oil, and amoxaxylin. Using SPSS 16.0 software, AuttoDockTool version 1.5.6, and Discovery Studio 2021.

Data Processing and Analysis

1) Yield Calculation

   Performed to determine the percentage of thick extract obtained after evaporation. formula used is:

$$\%\text{Rendemen} = \frac{\text{mass of extract obtained (gram)}}{\text{mass of initial extract (gram)}} \times 100\%$$

2) Specific gravity analysis

   Performed to calculate the specific gravity of the nanoparticle mouthwash formula. The formula used is:

$$\text{Bobot Jenis} = \frac{\text{Sampel pycnometer} - \text{Empty pycnometer}}{\text{Water pycnometer} - \text{Empty pycnometer}} \times \text{Specific gravity of water}$$

3) Viscosity Analysis

   Performed to determine the viscosity level of the nanoparticle mouthwash formula. Formula used are:

$$\text{Viskositas} = \frac{\text{Sample specific gravity} - \text{Sample Time}}{\text{Water specific gravity} - \text{Water time}} \times \text{Viscosity of water}$$

4) Statistical Analysis with One-Way ANOVA

   Based on the data from the test results of the antibacterial activity of extracts against the growth of Streptococcus mutans bacteria analyzed using SPSS 16.0. The number of samples used is <30, so the analysis with the On-Way ANOVA test. This analytical test aims to analyze two variables, namely the dependent variable and the independent variable, to determine whether

there is an effect of giving the sample on inhibiting the growth of Streptococcus mutans bacteria.

Results and Discussion

Extraction and phytochemical profiling of pineapple peel extracts

1) Pineapple Peel Extraction

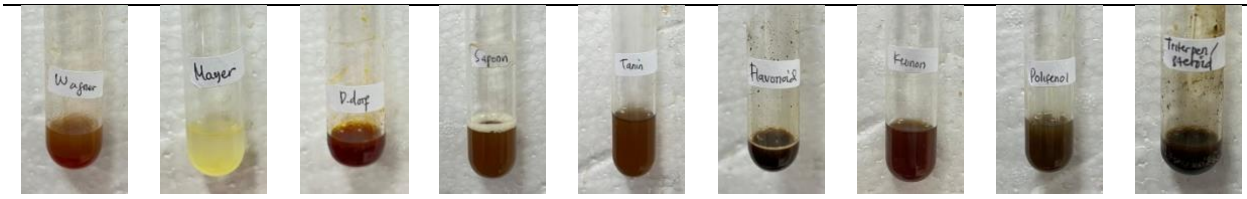
The maceration method extraction was carried out by dissolving pineapple skin simplisia using 70% ethanol solvent in a ratio of 1 10. A total of 500 grams of pineapple skin simplisia was dissolved in 2,500 ml of ethanol solvent and allowed to stand for 3 × 24 hours. The maceration results were then filtered using Gater F-802 paper to obtain 2,000 ml of liquid extract. Furthermore, the maceration process was carried out by dissolving 200 g of the same simplisia with 1000 ml of 70% ethanol solvent. Then, it was allowed to stand for 3 × 24 hours and filtered again so that 840 ml of remacerated liquid extract was obtained. The liquid extract from maceration and maceration was then evaporated using the water bath method. Thus, a thick extract of 34.4 grams was obtained with a percentage yield of 6.88% which can be seen in (Figure 1).

(A) (B)

Figure 1. (A) Pineapple Peel, and (B) Pineapple Peel Extract

2) Identification of Phytochemical Profile

Alkaloid identification was carried out using mayer reagent, dragendorff reagent and wagner reagent. Mayer reagent showed positive results with the formation of a cloudy white solution. The dragendorff reagent showed positive results with the formation of an orange brown precipitate solution, as well as the wagner reagent which showed positive results with the formation of a reddish colored solution. Saponin identification showed positive results with the formation of bubbles or foam on the surface of the sample. The results obtained from the identification of tannins showed negative results with no cloudy white solution formed. Identification of flavonoids showed positive results with the formation of a yellow solution. Identification of quinones showed positive results with the formation of a red solution. Polyphenol identification shows positive results with the formation of a blue solution. Identification of steroids and triterpenoids on lieberman burchard reagent formed a red solution that produces positive triterpenoids and shows negative results of steroids by not forming a green color solution. So that the results of phytochemical identification of pineapple peel extract qualitatively positive pineapple peel extract contains alkaloid compounds, saponins, flavonoids, quinones, polyphenols, and triterpenoids. However, pineapple peel extract negatively contains tannins and steroid compounds. The following are the results of phytochemical identification in (Table 1) and (Figure 2).



(A) (B) (C) (D) (E) (F) (G) (H) (I)

Figure 2. Phytochemical Identification (A) Alkaloid Wagner's Reagent (B) Alkaloid Mayer's Reagent C) Alkaloid Dragendorff's Reagent D) Saponin (E) Tannin (F) Flavonoids (G) Quinones (H) Polyphenols (I) Steroid & Triterpenoids

Table 1. Phytochemical Identification Results

Testing	Reagent Name	Results	Description
	Dragendroff	+	Formed Orange Brown Precipitate
Alkaloids	Mayer	+	Formed cloudy white
	Wagner	+	Formed Reddish Color
Saponins	HCl	+	Bubbles Form
Tannins	Gelatin	-	No White Turbid Solution Formed
Flavonoids	HCl + Propanol	+	Yellow Solution Forms
Quinone	NaOH	+	Red Solution Forms
Polyphenols	$FeCl_3$	+	Blue Solution Formed
Steroids	Liebermen Burchard	-	No Green Color Formed
Triterpenoids	Liebermen Burchard	+	Red Color Formed

The results of phytochemical identification of pineapple peel extract obtained are even better than research conducted by previous researchers. Based on the results of qualitative phytochemical screening research, the ethanol extract of pineapple peel positively contains secondary metabolite compounds of flavonoids, alkaloids, tannins, and saponins, and negative results contain secondary phenolic compounds, steroids, and triterpenoids (Reiza et al., 2019). However, identification obtained negative results for tannin and steroid compounds. This is because the maceration method is influenced by temperature, time, and the type of solvent used; the use of temperature and time that is too long causes low levels of tannin to be produced (Fakhruzy et al., 2020). In addition, steroid identification also obtained negative results due to the use of 70% ethanol solvent, which is semipolar. Steroid compounds are nonpolar compounds so they cannot be extracted perfectly in these solvents (Ergina et al., 2014). The phytochemical identification obtained positive results for polyphenols, in line with previous research. The LC/MS-MS phytochemical test results of pineapple peel ethanol extract quantitatively contained six polyphenolic compounds consisting of four phenolic acids (gallic acid, catechin, epicatechin, and ferulic acid) and two flavonoids (quercetin and kaempferol) (Jatav et al., 2022).

1) Validation Stage

Validation was performed on Ag I/II (Antigen I/II) protein with ID: 3IPK and GbpC (Glucan Binding Protein C) with ID: 6CAM. Ligand selection is based on the results of the protein preparation so that the phenylmethanol sulfonic acid (PMS) ligand of Ag I/II and beta-D-glucopyranose (BGC) ligand of GbpC are obtained. Ligands and receptors from both proteins were validated by setting the grid box dimensions (X: 40, Y: 40, Z: 40) and grid spacing (0.375 A) at the coordinates of Ag I/II (X: 6.787, Y: 40.372, Z: 22.711) and GbpC (X: 241.316, Y: -26.092, Z: 7.265). So, from the validation, the binding affinity result of the Ag I/II ligand-receptor is -4.96 kcal/mol with RMSD of 1.042 Å. While GbpC obtained a binding affinity value of -5.87 kcal/mol with an RMSD of 0.953 Å. The validation results of both proteins obtained RMSD ≤ 2 Å, which meets the validation requirements. Settings and validation results of the molecular docking method are in (Table 2).

Table 2. Molecular Docking Validation Results with AgI/II and GbpC Proteins

Parameters	Ag I/II	GbpC
GDP ID	3IPK	6CAM
Ligand Co-crystal ID	PMS	BGC
Ligand	Phenylmethanol sulfonic acid	beta-D-glucopyranose
Grid Box Size	40 x 40 x 40	40 x 40 x 40
	6.787	241.316
Grid Box Coordinate (x, y, z)	40.372	-26.092
	22.711	7.265
Grid Spacing	0.375	0.375
RMSD (Å)	1,042	0,953
Binding affinity (kcal/mol)	-4,96	-5,87

2) Molecular Docking Stage

Docking is done to find compounds that have the potential to become candidate protein inhibitors through the analysis of binding affinity values and inhibitory constants as well as amino acid interaction similarities from native ligands. The selected compounds were obtained from the phytochemical test results of pineapple peel ethanol extract. The LC/MS-MS phytochemical test results of pineapple peel ethanol extract quantitatively contained six polyphenolic compounds consisting of four phenolic acids (gallic acid, catechin, epicatechin, and ferulic acid) and two flavonoids (quercetin and kaempferol) (Jatav et al., 2022). Furthermore, the compound was docked on Ag I/II and GbpC proteins with the same settings as the validation results, so that the molecular docking results obtained are listed in Table 3.

Table 3. Molecular Docking Results of Pineapple Peel Ethanol Extract Compounds against Ag I/II and GbpC Proteins

Compound	Ag I/II (ID: 3IPK)		GbpC (ID: 6CAM)	
	G(kcal/mol)	Ki(µM)	G(kcal/mol)	Ki(µM)
Catechin	-6.41	19.91	-8.65	0.458
Quercetin	-6.26	25.96	-8.71	0.411
Epicatechin	-7.61	2.66	-8.12	1.12
Ferulic Acid	-4.52	487.94	-5.95	43.63
Gallic Acid	-3.82	1590	-4.76	321.81
Kaempferol	**-6**.54	16.12	-8.46	0.630
Amoxicillin	-7.59	2.72	-7.73	2.15
Native Ligand	-4.96	230.16	-5.87	49.49

From the docking results, epicatechin, kaempferol, catechin, and quercetin were obtained as compounds that have binding affinity values to Ag I/II protein receptors that are smaller than the native ligand. The four compounds have binding affinity values of -7.61 kcal/mol, -6.54 kcal/mol, -6.41 kcal/mol, and -6.26 kcal/mol, respectively. While the inhibitory constant values were 2.66 µM, 16.12 µM, 19.91 µM, and 25.96 µM, respectively. , the docking results on GbpC protein showed quercetin, catechin, kaempferol, and epicatechin had smaller binding affinity values than the native ligand. The four compounds have binding affinity values of -8.71 kcal/mol, -8.65 kcal/mol, -8.46 , and -8.12 kcal/mol and inhibitory constant values of 0.411 µM, 0.458 µM, 0.630 µM, and 1.12 µM, respectively.

Through the docking analysis results, epicatechin has a lower binding affinity value than the native ligand which is -4.96 kcal/mol. Even lower than Amoxicillin's binding affinity value of -7.59 kcal/mol. Meanwhile, the docking result of GbpC protein showed that quercetin had a lower binding affinity value than the native ligand which was -5.87 kcal/mol. Even lower than the binding affinity value of amoxicillin worth -7.73 kcal/mol. In this case, the score is a parameter of the strength of the binding affinity of the test ligand to the receptor. The more stable the ligand-receptor interaction is reflected by the lower the score. The stability of this interaction is proportional to the potential of the compound in providing the same effect as the reference ligand virtually (Adelina, 2014). So, from the docking results, epicatechin has the potential to be a candidate Ag I/II protein inhibitor and quercetin also has the potential to be a candidate GbpC protein inhibitor due to the high level of stability of ligand-receptor interactions compared to other compounds.

3) Visualization of Amino Acid Interactions

Through visualization results, the similarity of binding sites between quercetin, ferulic acid, and kaempferol with native ligand to the receptor of Ag I/II protein is shown in (Table 4). Quercetin has two similarity binding sites with native ligand on amino acid Serine (Ser697) through hydrogen bond and on amino acid Tryptophan (Trp816) through hydrophobic bond (van der walls). Meanwhile, ferulic acid also has two binding site similarities with native ligands on the amino acid Arginine (Arg824) and on the amino acid Tryptophan (Trp816) through

hydrophobic bonds. While kaempferol has two binding site similarities with native ligand at the amino acid Arginine (Arg824), and Serine (Ser697) through hydrogen bonds. The visualization results of the GbpC protein have three compounds that have the same binding site to the native ligand, namely quarcetin, gallic acid, and kaempferol as shown in (Table 5). Quercetin and kaempferol have three binding sites in common with the native ligand on the amino acids Serine (Ser347), Serine (Ser346), and Alanine (Ala453) through hydrogen bonds. Meanwhile, gallic acid has three binding sites in common with the native ligand on amino acids Tryptophan (Trp451), Phenylalanine (Phe452), and Serine (Ser346) through hydrogen bonds.

Table 4. Visualization of Amino Acid Interaction of Pineapple Peel Ethanol Extract Compounds with Ag I/II Protein

No.	Identified compound	Hydrogen bonds (H-bonds)	Hydrophobic interaction
1	Catechin	ASP760, THR586, TRP816, GLU706, VAL587	**TRP816**
2	Quercetin	TRP816, ASP760, **SER697**, SER762, VAL586	**TRP816**
3	Epicatechin	THR586, TRP816, **SER697**, ASP760	ASP760
4	Ferulic Acid	THR586, **ARG824**, ASP760	**TRP816**, VAL587
5	Gallic Acid	ASN820, **ARG824**, GLU706, SER818	ALA696
6	Kaempferol	ASP760, **ARG824**, **SER697**, TRP816	ASP760
7	Amoxicilin antibiotic	TRP816, THR586, ASP760, **ARG824**	**TRP816**
8	Native Ligand	**ARG824**, **SER697**	**TRP816**, **ARG824**

Table 5. Visualization of Amino Acid Interaction of Pineapple Peel Ethanol Extract Compounds with GbpC Protein

No.	Identified compound	Hydrogen bonds (H-bonds)	Hydrophobic interaction
1	Catechin	GLY414, ALA457, SER344, ASN455, **ALA453**, **SER346**	TRP451, PRO416, SER346
2	Quercetin	ILE190, ASP413, THR300, **SER347**, **SER346**, **ALA453**, ASN455	ALA457, ALA453
3	Epicatechin	**TRP451**, GLY414, ASP413, **ALA453**	SER347, ALA453, GLY414
4	Ferulic Acid	ASP408, **ALA453**, **TRP451**	PHE452, ALA453
5	Gallic Acid	GLY414, ASN455, **TRP451**, **PHE452**, **SER346**	ALA453
6	Kaempferol	**SER346**, **ALA453**, ASN455, **SER347**,	VAL410, ALA453
7	Amoxicilin antibiotic	GLY414, **ALA453**, ASP408	TRP451, ALA453

8	Native Ligand	**SER346**, **PHE452**, **SER347**, **TRP451**, **ALA453**	-

From the visualization of amino acid bonds in Ag I/II proteins, kaempferol is the compound that has the most similarity in the number of amino acids through hydrogen bonds to native ligands. Meanwhile, the amino acid visualization of GbpC protein shows that quercetin has the most amino acid interactions through hydrogen bonds to the ligand-receptor. In this case, the visualization result of amino acid interaction on GbpC protein is in line with its docking result that shows quercetin as the compound that has the most stable bond. However, the amino acid visualization results of Ag I/II protein showed different compounds compared to the docking results. The level of stability of amino acid interaction is not only judged by the number of the same amino acids but the number of bonds formed as a result of interaction is also part of the parameter. The more hydrogen bonds formed with amino acid residues, the stronger and more stable the bond (Tallei et al., 2020). Epicatechin has six interaction bonds with amino acids through hydrogen bonds and two hydrophobic bonds. The bond is more stable as quercetin has five hydrogen bonds and one hydrophobic bond. Kaempferol has four hydrogen bonds and two hydrophobic bonds while ferulic acid has three hydrogen bonds and two hydrophobic. So through the visualization results, epicatechin is a compound that has the strongest amino acid interaction with Ag I/II protein, while quercetin is a compound that has the strongest amino acid interaction with GbpC protein.

Effectiveness of Pineapple Peel Extract on Antibacterial Activity of Streptoccocus mutans In Vitro

The antibacterial activity test phase of Streptococcus mutans was carried out by disc diffusion method based on Kirby & Bouer test. The observation results obtained are the appearance of a clear area formed around the disc paper which shows the inhibition zone on growth. These results will be analyzed using the One-Way ANOVA statistical test to see whether or not there is an effect between different concentrations of pineapple peel extract on the growth of Streptococcus mutans. However, the results of antibacterial testing in this study cannot be analyzed due to the appearance of contamination. Contamination can be seen by the formation of bacterial colonies that are different from the colonies of Streptoccocus mutans. Microorganisms that cause contamination can sometimes be found in large numbers in microbiology laboratories. Microorganisms that cause contamination can come from the air, work surfaces, floors, human activity, or equipment used. Sterilization of instruments and equipment is necessary to prevent contamination (MicrobeHolic, 2024). So the presence of this contamination causes the secondary metabolite compounds in pineapple peel extract to be unable to inhibit Streptoccocus mutans. This is indicated by the absence of a clear area as a zone of inhibition of bacterial growth which can be observed in (Figure 3).

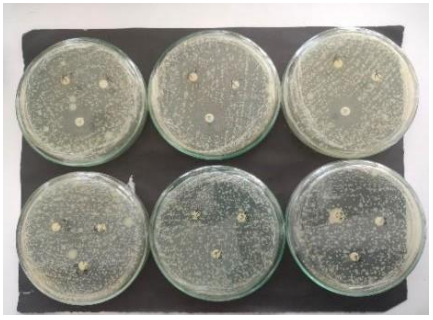


Figure 3. Antibacterial Test Result of Pineapple Peel Extract

However, secondary metabolite compounds in pineapple peel extract have antibacterial properties. Flavonoid compounds act to bind to proteins in the bacterial cell membrane and form complex compounds so that the plasma membrane in bacteria becomes weak and causes plasma membrane leakage (Redha, 2013). Terpenoids act as antibacterial by reacting with the outer membrane of the bacterial cell wall, thus forming a strong polymer bond to reduce the permeability of the bacterial cell wall (Retnowati et al., 2011). Alkaloids have the ability as antibacterial because they can interfere with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed perfectly and causes cell death (Darsana et al., 2012). Meanwhile, saponin and tannin compounds are known to have strong toxic activity that causes leakage of proteins and enzymes from bacterial cells. In addition, saponins can also diffuse through the outer membrane and vulnerable cell walls then bind to the cytoplasm, causing damage to the plasma membrane and lysis of bacterial cells (Tampongangoy et al., 2019).

Composition and Evaluation of Nanoparticle Mouthwash Formula of Pineapple Peel Extract

1) Preformulation and Formulation Results

Preformulation is done to get the optimum ratio of each concentration so that it can be used as an ideal composition for mouthwash. The best nanoparticle mouthwash formula can be made with a ratio of Tween-80 (surfactant) and PEG-400 (cosurfactant) of 1:2 (Widyaningrum, 2015). In addition, the ratio between VCO (carier oil) with Tween 80 and PEG-400 (surfactant-cosurfactant) was made based on research Anindhita and Oktaviani, (2016) with a ratio of 1:6 which is included in the stable composition category. However, the ratio between VCO and pineapple peel extract was made with a ratio of 3:2 in 3% extract, 6:2 in 6% extract, and 9:2 in 9% extract. For mouthwash preparations, benzoic acid concentration was used in the range of 0.01%-0.5%, with an average concentration of 0.15% (Storehagen et al., 2003). Meanwhile, the concentration of sorbitol (Humectant) was made at 10% and the addition of peppermint oil, as much as 3 drops, as a sweetener and aroma enhancer (Rahmadhani et al., 2019). So, by referring to previous research, the formulation composition of nanoparticle material concentration and pineapple peel extract is made as in (Table 6) and the formulation results are shown in (Figure 4).

Table 6. Nanoparticle Mouthwash Preformulation Composition

Material	F1	F2	F3
Pineapple Peel Extract	3%	6%	9%
VCO	2%	2%	2%
Tween 80	8%	8%	8%
PEG-400	4%	4%	4%
Sodium Benzoate	0,5%	0,5%	0,5%
Sorbitol	10%	10%	10%
Peppermint Oil	3 drops	3 drops	3 drops
Aquades	Add 100 ml	Add 100 ml	Add 100 ml

Figure 4. Nanoparticle Mouthwash Formulation Results

2) Organoleptical Test

Organoleptical testing was carried out by testing the color, taste, smell, and texture of the mouthwash. The results of the organoleptical test of the three formulas showed that pineapple peel extract nanoparticle mouthwash preparation had a brown color, smelled typical of pineapple accompanied by mint, had a sweet taste typical of pineapple accompanied by mint, and had a liquid texture and was not thick. Organoleptical testing shows that pineapple peel extract nanoparticle mouthwash preparation has a liquid form with a brownish orange color, smells typical of mint, and tastes sweet followed by a mint sensation (Rahmadhani et al., 2019). Thus, the organoleptical results of the three nanoparticle mouthwash formulas are not much different from the research conducted by previous researchers. The results of the organoleptical test can be seen in (Table 7).

Table 7. Organoleptical Test Results of Nanoparticle Mouthwash

Parameters	F1	F2	F3
Color	Chocolate	Chocolate	Chocolate
Smell	Pineapple flavor with mint	Pineapple flavor with mint	Pineapple flavor with mint
Taste	Pineapple sweetness with mint	Pineapple sweetness with mint	Pineapple sweetness with mint
Texture	Liquid and not viscous	Liquid and not viscous	Liquid and not viscous

3) Specific gravity test

Specific gravity testing to determine the purity of substances through the comparison between substances in the air to the weight of water with the same volume and temperature using a pycnometer. So, from the test results, the specific gravity of F1, F2, and F3 was obtained as 1.060 g, 1.070 g, and 1.076 respectively which are listed in (Table 8) and (Figure 5). If the specific gravity is close to the predetermined value, it can be said that the preparation has high purity (Lismayani et al., 2023). In this study, the specific gravity of the formula was determined by comparing the specific gravity of water 1 g. So that these results show the specific gravity of the formula, which is not much different from the specific gravity of water, even though the three formulas have a specific gravity that is slightly greater than the specific gravity of water. This is because in the sample other substances are dissolved so that they affect the specific gravity of the preparation (Rahmadhani et al., 2019). So from these results, the three formulas meet the provisions of the optimum specific gravity for mouthwash.

Table 8. Specific gravity test results of Nanoparticle Mouthwash

Formula	Specific gravity (gr)	Specific gravity >1 Meets (Yes/No)
F1	1,060	Yes
F2	1,070	Yes
F3	1,076	Yes

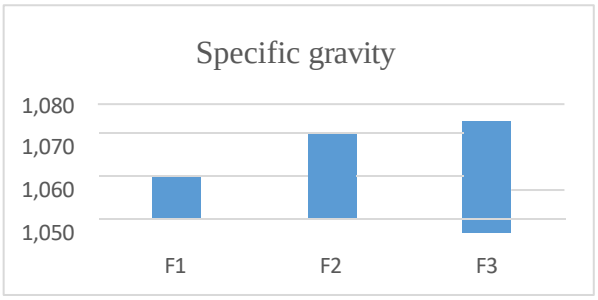


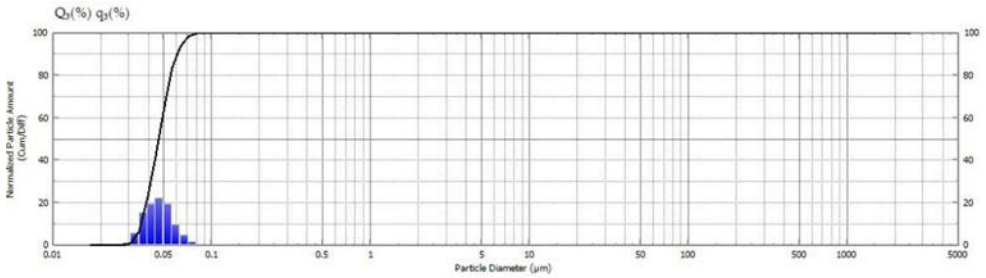
Figure 5. Specific gravity test results of Nanoparticle Mouthwash

4) Nanoparticle Test

The nanoparticle test on the nanoparticle mouthwash formula obtained the results of formula F1 and formula F2, which met the nanoparticle requirements, while formula F3 did not meet the nanoparticle requirements. Nanoparticle is a particle with a nanometer size, which is about 1-100 nm (Masykuroh & Puspasari, 2020). Formula F1 obtained test results with an average particle size of 46, and Formula F2 obtained test results with an average particle size of 43 nM. While Formula F3 obtained test results with an average particle size of 762 nM. The nanoparticle test results obtained are listed in (Table 9) and (Figure 6).

Table 9. Nanoparticle Test Results

Formula	Particle Size (nM)	Meets 1-100 nM (Yes/No)
F1	46	Yes
F2	43	Yes
F3	762	No



(A)

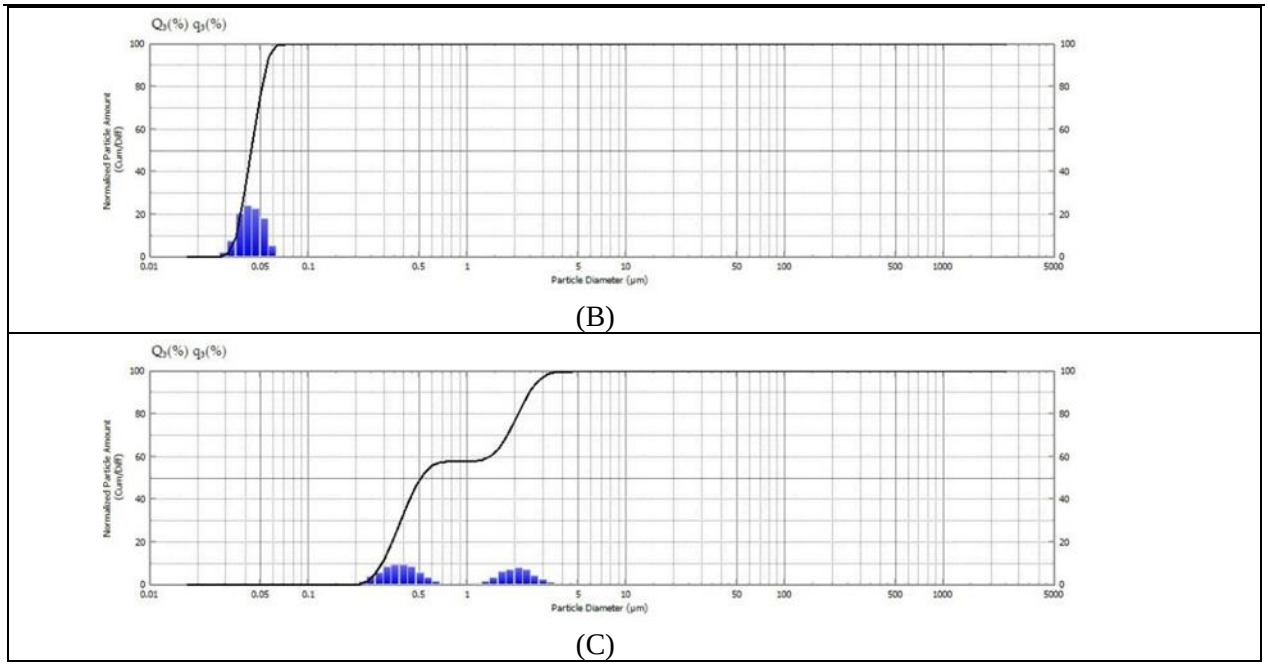


Figure 6. Graph of Nanoparticle Test Results, (A) F1, (B) F2, and (C) F3

Through the nanoparticle test results, the resulting droplet size depends on several factors including the type of homogenizer used, manufacturing temperature, energy intensity and time, as well as sample conditions in the form of oil type, oil concentration, type of emulsifier/surfactant used and physicochemical properties of the sample (interfacial tension and viscosity) (Lee & McClements, 2010). In addition, the concentration ratio of extract, oil, surfactant, and cosurfactant also affects the size and stability of the nanoparticle preparation. From the test results, formula F2 is the preparation with the smallest particle size and tends to be more stable with a homogeneous size as in (Figure 4 (B)). Although formula F1 has a size that meets nanoparticle preparations, the particle size is less homogeneous than formula F2 so that the properties of the preparation become less stable as in (Figure 4 (A)). Plus formula F3 which does not meet the nanoparticle preparation at all so that the preparation properties become inhomogeneous and unstable as in (Figure 4 (C)). In this case, formula F2 has a composition that is not much different from previous studies. The best manis jangan nanoparticle formula can be made with a composition of VCO and manis jangan oil 1:3, Tween 80 and PEG-400 in a ratio of 2:1 in 100 grams of formula (Widyaningrum, 2015).

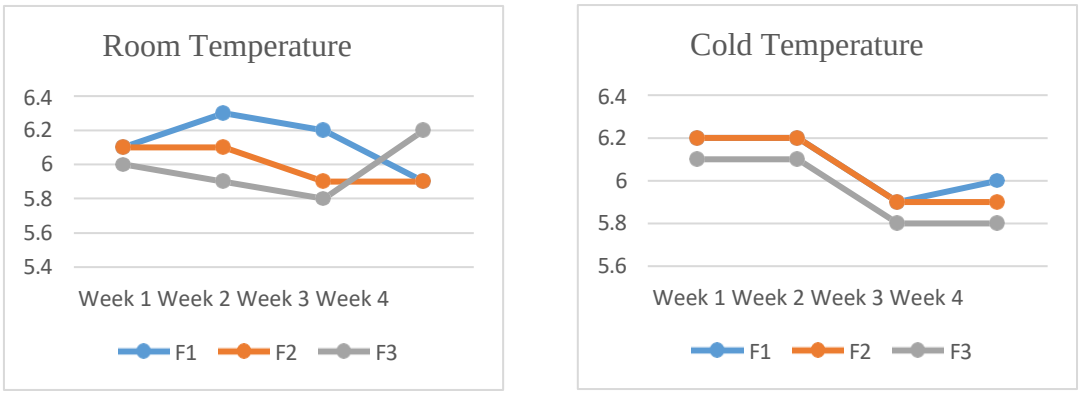
5) Stability test (pH and viscosity)

The pH test was conducted to evaluate the level of acidity of nanoparticle mouthwash that is safe for the mouth. The quality standard for herbal mouthwash is a pH between 5-7 (Hidayanto et al., 2017). The pH test was conducted using a pH meter to obtain more accurate test results. Thus, the pH test results of each formula were obtained in (Table 11). The three nanoparticle mouthwash formulas stored at room temperature and cold temperature with observation for four weeks, still meet the safe pH requirements for herbal mouthwash. Although there is a small change in pH every week. The highest pH result was obtained in formula F1 at room temperature observation week 2, while the lowest pH was obtained in formula 3 at room temperature observation week 3 and cold temperature week 3 and week 4. The decrease in pH that occurs is due to the decomposition of phenol groups in polyphenol compounds contained in pineapple peel extract. This decomposition causes an increase in H^+ so that the pH of the mouthwash decreases (Lismayani

et al., 2023).

Table 10. pH Stability Test Results of Nanoparticle Mouthwash Formula

Observation		pH test (Criteria: pH 5-7)				Average
		Week 1	Week 2	Week 3	Week 4	
Room Temperature (20-25)℃	F1	6,1	6,3	6,2	5,9	6,1
	F2	6,1	6,1	5,9	5,9	6,0
	F3	6,0	5,9	5,8	6,2	6,0
Cold Temperature (0-5)℃	F1	6,2	6,2	5,9	6,0	6,0
	F2	6,2	6,2	5,9	5,9	6,0
	F3	6,1	6,1	5,8	5,8	5,9



(A) (B)

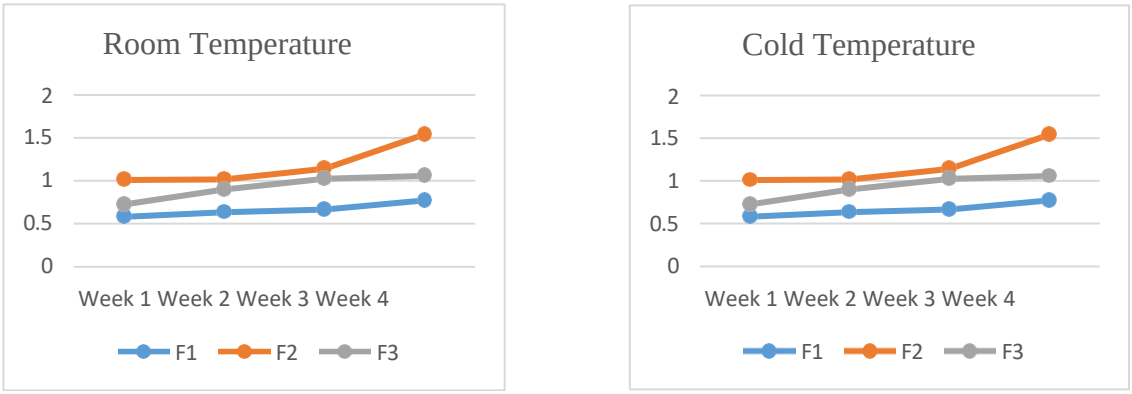
Figure 6. Graph of pH Stability Test Results of Nanoparticle Mouthwash Formula (A)Room Temperature, and (B)Cold Temperature

Viscosity testing is carried out to evaluate the viscosity level of nanoparticle mouthwash preparations that are safe for the mouth. The viscosity level of pure water is 1 Cp, while the standard viscosity of mouthwash on the market is 7.25 (Rowe et al., 2009). Thus, the viscosity test results of each formula are obtained in (Table 12). Through these results, it was found that formula F2 at room temperature and cold temperature observations for four weeks had a viscosity value higher than the viscosity of water. This shows that the preparation has a very good viscosity. addition, the closer the viscosity level of a formulated product is to the viscosity of water, the easier and more comfortable the product is used for gargling.

Table 11. Viscosity Test Results of Nanoparticle Mouthwash Formula

Observation		**Viscosity Test (Criteria: > Cp_{Water}= 1)**				**Average**
		Week 1	**Week 2**	**Week 3**	**Week 4**	
Room Temperatur	F1	0,582	0,636	0,668	0,774	0,665
	F2	1,011	1,021	1,145	1,543	1,180
	F3	0,726	0,903	1,027	1,058	0,929

e (20-25)℃						
Cold Temperatur e (0-5)℃	F1	0,655	0,657	0,708	0,708	0,682
	F2	1,050	1,016	1,058	1,145	1,067
	F3	0,993	0,955	0,976	1,013	0,994



(A) (B)

Figure 7: Viscosity Stability Test Results Graph of Nanoparticle Mouthwash Formula (A)Room Temperature, and (B)Cold Temperature

Conclusion

The pineapple peel extract, when dissolved in 70% ethanol solvent, contains secondary metabolites such as alkaloids, saponins, flavonoids, quinones, polyphenols, and triterpenoids, although it is negative for tannins and steroids. In molecular docking studies, six polyphenolic compounds four phenolic acids (gallic acid, catechin, epicatechin, ferulic acid) and two flavonoids (quercetin and kaempferol) were tested as inhibitors of Streptococcus mutans bacteria in silico. The Ag I/II protein showed that epicatechin acted as an inhibitor with a binding affinity of -7.61 kcal/mol, while the GbpC protein showed quercetin as an inhibitor with a binding affinity of -8.71 kcal/mol. In vitro antibacterial testing of the pineapple peel extract against Streptococcus mutans growth could not be analyzed due to contamination, but it is evident that the extract contains secondary metabolites that could affect bacterial growth. Furthermore, optimization and evaluation of nanoparticle mouthwash formulas revealed that Formula F1, with a particle size of 46 nm, and Formula F2, with a particle size of 43 nm, both met the particle size requirements for nanoparticle preparations. Organoleptic evaluation showed that both formulas had a brown color, a typical pineapple smell, a sweet taste, and a liquid texture. Specific gravity testing showed that all three formulas met weight standards. Stability tests for pH and viscosity indicated that Formula F2 maintained stable properties, with a pH of 6.0 at both room and cold temperatures and an average viscosity of 1.180 cP at room temperature, decreasing slightly to 1.067 cP at cold temperatures.

Further research is expected on the phytochemical identification of pineapple peel extract quantitatively using LC/MS test. There is further testing of the antibacterial activity of pineapple peel extract against the growth of streptoccocus mutans and other bacteria and further research is

needed with the in vivo test method. There is further research on the composition of pineapple peel extract nanoparticle mouthwash formula with different types of surfactants and cosurfactants. There is further research on zeta potential testing, SEM test of nanoparticle mouthwash formula.

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