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DEVELOPMENT OF TOOTHPASTE FORMULATION MODEL OF MANALAGI APPLE PEEL EXTRACT (Malus Sylvetris) WITH DIFFERENT CONCENTRATIONS AGAINST THE GROWTH OF STREPTOCOCCUS BACTERIA MUTANS SECARA IN VITRO DAN IN VIVO

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ABSTRACT

Background: Caries is a disease caused by damage to the enamel layer that can extend to the nerve part of the tooth. One way to prevent caries is to brush your teeth regularly using antibacterial herbal toothpaste that can reduce the number of colonies of streptococcus mutans bacteria. Apple peel is a useful herbal ingredient as an antibacterial, antioxidant, antifungal and antiproliferative, and polyphenolic compound. Objective: To determine the effect of developing a toothpaste formulation model of manalagi apple peel extract (malus sylvetris) with different concentrations on the growth of mutant Streptococcus bacteria in vitro and in vivo. Method: This study is a true experimental research using pre test and post test approach with control group design. Sampling was carried out using a simple random sampling technique and 15 respondents were obtained. This study was carried out for 3 days, where on the first day measurements were carried out (pretest) by taking saliva samples on respondents before brushing their teeth in the morning, and on the third day (posttest) measurements of respondents' saliva were carried out to test the bacterial colonies contained therein. Statistical analysis of Pre-Test Post-Test difference test using Paired T-Test difference test and One-Way Annova Test and Comparison Analysis with Post Hoc Tukey test. Results: This study showed that the dose of toothpaste in vitro testing was obtained the optimal dose, namely at the concentration of apple peel extract manalagi 25%, then in vivo tests the toothpaste formulation model of Manalagi apple peel extract (Malus Sylvetris) with the optimal dose (25%) was effective in inhibiting the growth of streptococcus mutans bacteria in vivo with an average decrease of -3.30 x 106 CFU / ml, with p value (0.005 < 0.05). the control group + was -0.38 x 106 CFU / ml, and non-herbal toothpaste in the control group - was -0.68 x 106 CFU / ml, but the results of statistical analysis did not show significant differences in the results before and after treatment in the control group + (p value = 0.553 > 0.05) and control - (p value = 0.403 > 0.05). Conclusion: Manalagi apple peel extract toothpaste formulation model (malus sylvetris) with a concentration of 25% effective in inhibiting the growth of mutant Streptococcus bacteria in vitro and in vivo

INTRODUCTION

Dental and oral health is important for general health. Dental and oral health is the client's oral condition that is contained in a continuum starting from optimal health conditions to sick conditions (Harnagea, Lamothe, Couturier, & Emami, 2018). This condition fluctuates over time which is influenced by biological, psychological, spiritual, and developmental factors (Edwards, Dworkin, Sullivan, Turk, & Wasan, 2016). Dental and oral diseases can be a risk factor for other diseases because dental and oral health is an inseparable part of general health, someone who experiences dental and oral health problems will affect health in general (Petersen, 2008). Oral health and general health are conditions that are interconnected and affect each other (Bastos, Celeste, & Paradies, 2018). Dental caries and periodontal disease are still a problem for the health of the teeth and mouth of the general public.

Based on The Global Burden of Disease Study (2019), dental and oral health problems, especially dental caries, are diseases experienced by almost half of the world's population (3.58 billion people) (Reddy, 2019). The prevalence of caries in Indonesia based on Basic Health Research was 25.9% in 2013 and increased to 57.6% in 2018 (Yanis & Agustin, 2020). This shows that the prevalence of dental caries in Indonesia is still high. Streptococcus mutans causes the most dental caries of all oral Streptococcus Dental and oral health conditions are fluctuating and influenced by bioliogical, psychological, spiritual, and developmental factors of oral health and general health. These conditions are interconnected and affect each other. Some problems that occur in the mouth and teeth occur due to lack of maintaining dental and oral hygiene (Coll et al., 2020). This is associated with plaque buildup on the surface of the teeth (Carter, Landini, & Walmsley, 2004). The occurrence of plaque buildup is the beginning of several diseases in the oral cavity including caries and periodontal disease (Prasanna, Karunakar, Sravya, Madhavi, & Manasa, 2018).

Caries is a disease of the hard tissues of the teeth, namely enamel, dentin and cementum, caused by the activity of a tiny body in a carbohydrate that can be leavened (Gullianne, Gultom, & Auerkari, 2023). Unhealthy dental and oral health conditions caused by caries, can result in limited functions so that work activities and decrease. Caries that is already severe will affect health and quality of life which causes pain, difficulty sleeping and eating, discomfort, disharmonious facial profile, decreased body mass index, unable to carry out routines / activities as usual, acute and chronic infections that can cause hospitalization costs and costs incurred for treatment of caries that are already severe will be higher than the initial caries case.

Caries is one of the proofs of the unmaintained condition of the teeth and mouth of the Indonesian people. People generally tend to assume that deciduous teeth do not need to be treated because they will be replaced with permanent teeth. They do not understand that if the first tooth is not maintained properly, it will have cavities. As for efforts to support optimal health, efforts in the field of dental and oral health need to be considered (Lee & Divaris, 2014).

In the oral cavity of a person contains various species of bacteria that are commensal among these bacteria is Streptococcus mutans (S. mutans) which is karyogenic and is the main cause of dental caries (Inchingolo et al., 2022). One of the characteristics of this bacterium is that it has the ability to attach to all surface locations of its habitat in the oral cavity. If left untreated the disease can cause pain, tooth loss and infection (Mathur & Dhillon, 2018).

Streptococcus mutans is a gram-positive bacterium in the oral cavity that usually causes dysbiosis in the ecosystem, this symbiosis is not only responsible for the development of the disease, but is also considered the most relevant bacterium in the transition of non-pathogenic commensal oral microbiota to biofilms that contribute to the process of dental caries (Sureda et al., 2020). Streptococcus mutans has developed various mechanisms to damage tooth surfaces and form bacterial plaque biofilms. The ability of these bacteria to produce organic acids through various

carbohydrate metabolism processes (acidogenicity) and survive in low pH environments (aciduricity) is a major virulence factor in biofilms and causes dental caries.

One way to prevent caries is to brush your teeth regularly using toothpaste. The use of toothpaste as an abrasive serves to clean and smooth the surface of the teeth. Antibacterial ingredients found in toothpaste provide a therapeutic effect so that it can suppress the development of Streptococcus mutans as a cause of caries formation.

The addition of antibacterial ingredients to toothpaste can reduce the number of colonies of bacteria that cause dental caries. Commercial toothpastes containing fluoride play an important role in preventing tooth decay but in the development of teeth when used in concentrations that are not recommended can pose a risk of fluorosis, toxicity, tooth demineralization and can cause enamel discoloration, so a safer alternative antibacterial ingredient option is needed, namely using herbal ingredients. Some studies have concluded that prolonged use of fluoride can cause fluorosis and ingestion causes indigestion.

The use of herbal ingredients in toothpaste is necessary to inhibit the growth of caries-causing bacteria without reducing its quality. In addition, herbal ingredients have several advantages including easy to obtain, cheap, safe and do not harm the surrounding environment. One alternative that can be done is to utilize natural ingredients from chemical compounds. One of the herbal ingredients that has an antibacterial function is apples where not only the flesh on the apple is useful, but the skin of the apple also has a high nutritional content. Apple peel is useful as an antibacterial, antioxidant, antifungal and antiproliferative, even polyphenolic compounds in apple skin value higher than apple danging.

Research by (Deng et al., 2017) on "Antibacterial Power of Manalagi Apple Skin Extract (Malus sylvestris Mill.) Against the Growth of Streptococcus mutans" showed mouthwash preparations with Manalagi apple peel extract at concentrations of 100%, 50%, and 25% had antibacterial power against the growth of S. mutans. The results concluded that a concentration of 25% can still inhibit the growth of S. mutans.

Further testing of manalagi apple peel extract preparations is needed which is converted into Manalagi apple peel toothpaste preparations and used in making toothpaste, which is taken by calculating the Minimum Inhibitory Concentration (KHM) which in previous studies only used bacterial inhibitory power tests and was only tested on humans in the form of plaque removal mouthwashes. Therefore, it is necessary to conduct research to make toothpaste preparations using Manalagi apple peel extract which is done in vitro-in vivo.

Furthermore, human tests were carried out on streptococcus mutans that causes dental caries obtained by taking saliva before and after brushing teeth then tested ALT (Total Plate Number) to count the number of bacterial colonies.

RESEARCH METHODS

This study used a true experimental research method with a pre-test-post test design with control group with a sampling technique using simple random sampling, while previous studies also used true experiments but the technique was a complete randomized design (RAL).

RESULTS AND DISCUSSION

This chapter discusses the results of research that has been done by comparing the results of research that has been described in chapter IV with other theories and research that has been done previously

A. Physical Quality of Manalagi Apple Skin Extract Toothpaste Formulation Preparation (Organoleptis, Homogeneity, pH, Dispersion, High Foam)

1. Organoleptic Test

The results of checking the stability of toothpaste preparations of apple peel extract are carried out against three formulas by looking at the shape, color, aroma, and taste of each preparation. Physical testing of this toothpaste is carried out in order to determine the stability and feasibility of toothpaste. From the results of physical testing of apple skin extract, manalagi is formed in the form of toothpaste preparations Meet the parameters of toothpaste quality tests where from organoleptis tests where the form is half solid, color, aroma, and taste according to the concentration of the extract it contains.

Organoleptic testing aims to see the appearance of pharmaceutical preparations physically including color, texture or shape, taste and aroma, the purpose of organoleptic testing is to determine the level of consumer preference for a product produced.

2. Homogeneity Test

The results of testing the skin extract of the manalgi apple (Malus Sylvetris) on all three formulas produced homogeneous results. Homogeneity testing is carried out to determine when the process of making dental pata is carried out, the active material with other additives mixes with homogeneous. The homogeneous requirements of toothpaste must be met so that the toothpaste is easy to use and evenly distributed on the surface of the teeth. 68 Homogeneity testing aims to analyze the degree or change in homogeneity in toothpaste preparations that may occur due to several factors. For example, storage factors for weeks and human error, such as less fine in sieving grains and lack of stirring. Indicator of homogeneous toothpaste when
There is no coarse grain on the glass of Object 69.

3. pH Test

The pH measurement results of each formula show the pH value obtained from toothpaste in accordance with the oral mucosa so that it is safe for use. pH measurement aims to see the safety of the preparation so as not to irritate the oral mucosa when applied in topical preparations. pH is a measurement of the degree of acidity of a preparation. pH measurement is also intended to determine whether the acidity of the toothpaste is in accordance with the standard pH. The mouth in an acidic state causes bacteria to nest easily, so the pH of toothpaste determines the function of toothpaste as an anti-bacterial power.

pH measurement is an important physicochemical parameter in topical preparations because pH is related to the effectiveness of active substances, stability of active substances and preparations, and comfort in the skin during use. pH values that are too acidic can cause irritation while pH that is too alkaline can cause scaly skin. From the results of pH measurements of apple peel extract preparations, the average pH in the three formulations is 7, this pH value is in accordance with the quality requirements of gel toothpaste in SNI 12-3524-1995, which is 4.5 - 10.5.

4. Dispersion Test

The measurement of the diameter of the spreadability of manalagi apple peel extract toothpaste (Malus Sylvetris) meets the spreadability requirements of 5 – 7 cm. The toothpaste dispersion test is intended to determine the ability to spread toothpaste when applied to toothpaste. Dispersing ability is an important characteristic in formulations because it affects the transfer of active ingredients to the target area at the right dosage, ease of use, pressure required to exit the package and acceptance by respondents.

5. Foam Height Test

The results of observations on the toothpaste base were obtained in all formulas that meet the requirements for the maximum foam height of toothpaste preparations, which is a maximum of 15mm (preparations on the market).

The foam formation test aims to see the amount of foam produced by toothpaste to remove the dirt that cleans the mouth when brushing your teeth. The foam produced in the preparation is generally influenced by the concentration by the detergent used. In this toothpaste base, SLS (Sodium Laury Sulfate) is used as a detergent. SLS is an anionic sulfactant that has a high giving power. Based on the results of the evaluation of foam formation, the three bases of manalagi apple peel extract toothpaste (Malus Sylvetris) can form foam well.

B. Antibacterial Activity of Manalagi Apple Skin Extract Inhibitory Power against Streptococcus mutans Bacteria in vitro

Antibacterial testing of manalagi apple peel extract toothpaste was carried out to determine the inhibitory power of manalagi apple peel extract formulated in toothpaste preparations. The

bacteria used in this study were Streptococcus mutans. Testing is carried out using the agar diffusion method by means of sumur. The welling method is to make holes in solid agar that has been inoculated with bacteria. On the agar plate that has been inoculated with test bacteria, a hole is made which is then filled with test antimicrobial substances. After incubating at a temperature and time (± 24 hours) that matches the test microbes, observations are made by looking at the presence or absence of an inhibitory zone around the hole. This method is the method chosen in activity tests because it has the advantage that the procedure is simple, easy and practical to perform and can be used to see the sensitivity of various types of microbes to antimicrobials at certain concentrations (23).
In this test used positive and negative controls. The positive control used was herbal Pepsodent toothpaste from PT. Unilever on the market. And the negative control used in this study was a formula without extracts.

Based on the results obtained, the concentration of 25% shows the highest diameter of the inhibitory zone, which is 27 mm with an average increase of 22.33 mm, where this result shows the category of inhibitory zones including the classification of very strong inhibitory zones (> 21 mm), and at a concentration of 5% in the third replication shows the lowest inhibitory power of 6 mm, with an average of 7.33 mm, Where this result shows the category of inhibitory zones including the classification of medium inhibitory zones (6-10 mm), then the negative control does not show the occurrence of inhibitory power from the first replication to the last replication. According to the classification of bacterial growth inhibition response (Greenwood) states that antibacterial activity with an inhibitory zone of <5 mm is weak, an inhibitory zone of 5-10 mm is medium, an inhibitory zone of 11-20 mm is strong and an inhibitory zone of >20 mm is very strong.

The post-test difference test shows a significant difference in the inhibitory power at each given concentration, where the value ($p = 0.000 < 0.05$). The average results between the treatment groups had a probability value ($p < 0.05$) which means that each group had differences in growth inhibition of mutant streptococcus bacteria in vitro depending on the concentration given, but the treatment between P2 (10% Concentration) and K + (Herbal Formula Toothpaste) did not show different results from each other in inhibiting streptococcus mutans bacteria with values (p value = 1,000 > 0.05).

The antibacterial activity possessed by apple skin contains several phytochemicals derived from polyphenols including catechins, quercetin, phloridsin and chlorogenic acid. Catechins are a class of secondary metabolites produced by plants and belong to the flavonoid group. Apple peel contains tannins of 42.46 µg/ml in 1 gram of apple peel extracted using methanol solvent. 73
The skin of manalagi apples contains more polyphenolic compounds than the flesh. Other compounds that can inhibit the growth of bacteria on apple skin are flavonoids and tannins. Flavonoids can act directly as antibiotics by interfering with the function of microorganisms such as bacteria or viruses. Apple skin contains many flavonoid compounds that are polar, so it is easier to penetrate the polar peptidoglycan layer on the bacterial cell wall.

Flavonoids will inhibit energy metabolism in bacteria, so that they can inhibit oxygen respiration which then the bacteria will lose the permeability of cell walls, microsomes and lysosomes as an interaction between flavonoids and bacterial DNA. The mechanism of tannin inhibition is by means of bacterial walls that have been lysed due to flavonoid compounds so that tannin compounds can easily enter bacterial cells and coagulate the protoplasm of Streptococcus mutans bacterial cells so that the greater the concentration, the greater the active substance component contained in it so that the inhibitory zone formed will also be different for each concentration.

From these results, it can be concluded that the higher the concentration of apple peel extract in toothpaste preparations, the larger the diameter of the inhibitory zone. This is because the higher the concentration of the test material, which means that the greater the amount of active substance contained in the extract, the greater the ability to inhibit the growth of a bacterium.

C. Effect of Manalagi Apple Skin Extract Toothpaste Formulation with Optimal Dose on Total Plate Number (ALT) Testing in measuring Streptococcus mutans Bacterial Colonies in vivo

Total Plate Number (ALT) is one way to facilitate the testing of microorganisms from a product, and the ALT number indicates the presence of pathogenic or nonpathogenic microorganisms that are observed visually or with a magnifying glass on the planting media studied, then calculated based on the base plate for standard tests on bacteria. The media used is PCA media because it contains sufficient nutrients for the growth of microorganisms. 75

In the total plate number (ALT) test, the average colony of Streptococcus Mutans bacteria in the group before and after treatment changed, where the average decrease in the number of Streptococcus Mutans Bacterial Colonies after being given a toothpaste formulation of manalagi apple peel extract with a concentration of 25% was -3.30 x 106 CFU / ml, with a p value (0.005 < 0.05), which showed an average difference before and after treatment in the intervention group. While the average decrease in the number of Streptococcus Mutans Bacterial Colonies after being given herbal toothpaste formula intervention in the + control group was -0.38 x 106 CFU / ml, and non-herbal toothpaste in the control group - was -0.68 x 106 CFU / ml, but the results of statistical analysis did not show significant differences in the results before and after treatment in the + control group (p value = 0.553 > 0.05) and control - (p value = 0.403 > 0.05).

In the number of bacterial colonies of each group, namely in the intervention group and control group (p = 0.000) and Delta (Δ) Bacterial Colonies of Streptococcus Mutans (p = 0.013) where the p-value (< 0.05). However, the results of the anova are comprehensive, that is, together they have significant differences. To determine whether or not significant differences between groups are carried out with post hoc tests.

The post hoc test showed a significant difference between the intervention group and the positive control group and the negative control group (p = 0.001 < 0.05) with a mean difference (-2.2600*), while between the positive control group and the negative control group did not show a significant difference (p = 0.973 > 0.05) with the mean difference (-0.1000). In the difference between streptococcus mutans colonies, there was a significant difference between the intervention group and the positive control group and the negative control group (p = 0.018 < 0.05) with the mean difference (2.92000*).

The chemical content in apple skin manalagi into anti-bacterial substances is polyphenols, including catechins. Catechins have antibacterial properties due to the presence of pyrigallol groups and galliol groups. Quercetin also has antibacterial active substances by binding to the GyrB subunit of DNA gyrase and inhibiting ATPase activity. Flavonoids can damage bacterial cell walls through differences in polarity between lipids in the preparation of bacterial DNA and alcohol groups of flavonoid compounds. While the content of xylitol which is an anti-bacterial substance is xylitol, the mechanism of action of xylitol is that it can enter the cell nucleus and damage the cell nucleus and can synthesize bacterial proteins so that mutant streptococcus bacteria are disrupted by colony growth in the oral cavity.

D. Effect of Manalagi Apple Skin Extract Toothpaste Formulation on Salivary pH

The pH of saliva is the degree of acidity found in saliva. Under normal conditions salivary pH ranges from 5.6 – 7.0 with an average pH of 6.7. The lower the pH of saliva means the more acidic the content in saliva, conversely if the higher the pH value, the more alkaline the content in saliva.

The average salivary pH in the group before and after treatment changed, where the average increase in salivary pH after being given the toothpaste formulation of apple peel extract manalagi with a concentration of 25% was 1.52, with a p value (0.001 < 0.05), which showed there was a difference in the average before and after treatment in the intervention group, and the average increase in salivary pH after being given the herbal toothpaste formula intervention in the control group (+) was 0. 64, with p value (0.017 < 0.05), while in the control group (-) was 0.54 with p value (0.159 > 0.05), thus showing no significant difference in the results before and after treatment in the control group (-).

Significant differences between the intervention group and the positive control group (p = 0.010 < 0.05) and the negative control group (p = 0.017 < 0.05) with mean differences (0.8600* and 0.7800*), while between the positive control group and the negative control group did not show a significant difference (p = 0.941 > 0.05) with the mean difference (-0.0800). In the difference in salivary pH, there were significant differences between the intervention group and the positive control group (p = 0.047 < 0.05) and the negative control group (p = 0.027 < 0.05) with mean differences (0.08000* and 0.98000*).

The salivary pH measured immediately after using SLS toothpaste > 5% higher than non-SLS toothpaste because the detergent used in SLS toothpaste > 5% is alkaline, causing a significant increase in salivary pH compared to neutral non-SLS toothpaste. However, in long-term use, the use of non-SLS toothpaste is more effective in increasing salivary pH, which in each salivary pH measurement shows a more stable number with an average salivary pH of 7.1. Unlike the case with salivary pH in SLS

toothpaste, it shows results that tend to decrease in every 10 minutes of pH measurement with an average salivary pH of 6.9.

Generally, the increase in salivary pH is caused by the alkaline toothpaste content, the value of the increase in salivary pH using non-SLS toothpaste (herbs) is higher than the the use of SLS toothpaste. The use of excess sodium lauryl sulfate for a long time can cause irritation of the oral cavity, while in the short term it causes allergies in the form of redness. These side effects can be seen if a person has sensitive mucosa. Herbal toothpaste turns out to have an influence on the growth rate of plaque, namely paste

Teeth without sodium lauryl sulfate are more effective in inhibiting plaque in the oral cavity. The interaction of glycolytic enzymes in non-SLS toothpaste serves as an antibacterial capable of killing Streptococcus mutans bacteria that cause dental caries. 78

E. Effect of Debris on the Number of Colonies of Streptococcus mutans Bacteria and Salivary pH

Debris is a major contributing factor to the occurrence of caries periodontal disease. Plaque is a collection of bacteria bound in an organic matrix and tightly attached to the surface of the teeth. Plaque consists of microorganisms that multiply in an intercellular matrix, in the form of stickiness of bacteria and bacterial products. Dental plaque is formed from soft deposits that form a biofilm layer consisting of various species of bacteria in the form of shapeless deposits, which are firmly attached to the tooth surface and are a collection of a number of bacteria attached or embedded in an extracellular polymer matrix.

Debris ($p = 0.004 < 0.05$) has a close relationship with the decrease in streptococcus mutans bacterial colonies in post test measurements, where the strength of the coefficient relationship (correlation) is 0.693 where the relationship between the two variables in these two studies is in the strong category, and the value of R.Square or Coefficient of Determination (KD) obtained is 48%, so it can be interpreted that the debris variable has a contributing influence of 48% to the decrease in the number of streptococcus bacterial colonies mutans.

Streptococcus Mutans is a karyogenic bacterium that is able to metabolize carbohydrates and is able to create an acidic atmosphere in the mouth which is the main cause of caries because its habitat is attached to the crown of the tooth along with plaque. These bacteria have a number of virulence factors such as adhesion, colonization, and forming biofilms on the tooth surface.18 Streptococcus sp has glucosyltransferase and fructosyltransferase enzymes that convert food sucrose into glucans and fructans help other bacteria attach to teeth. 6 The accumulation of bacteria that cause caries causes acid production to increase so that plaque pH drops and caries occurs. Streptococcus sp accumulated in plaque can also be one of the risk factors for caries etiology which can be seen based on the Simplified Oral Hygiene Index (OHI-S) score. The lower the OHI-S score, the higher the risk of caries because the colony of Streptococcus sp bacteria will increase. Dental plaque plays an important role in causing caries by affecting the growth of colonies of streptococcus mutans bacteria.

Saliva is a complex oral fluid consisting of a mixture of secretions from large and small salivary glands present in the oral mucosa. Potential of hydrogen (pH) is a measure that describes the degree of acidity or alkali content of a solution, pH is measured on a scale of 0-14. The degree of acidity or commonly called salivary pH under normal circumstances ranges from 6.8 - 7.2, while the degree of salivary acidity is said to be low if it ranges from 5.2 - 5.5 low salivary pH conditions will facilitate the growth of asedogenic bacteria such as streptococcus mutans bacteria.

In Salivary pH obtained value (p value = $0.131 > 0.05$), so debris has no relationship or contributes to the improvement of Salivary pH value, but the R.Square value or Coefficient of Determination (KD) obtained is 16.6%, so it can be interpreted that the debris variable has a small contribution effect of 16.6% to the pH value of Saliva.

Caries is a focal degeneration of teeth due to the dissolution of minerals that make up tooth structure by exposure to organic acids resulting from carbohydrate fermentation carried out by pathogenic bacteria in the oral cavity, one of which is Streptococcus mutans. The fermented lactic acid will reduce the acidity (pH) of the mouth, where a decrease in oral pH below 5.5 will cause enamel demineralization. 19 The pH of oral saliva is also related to plaque pH which can affect the periodontal state of the oral cavity. Saliva influences the increase in plaque pH and some studies report that plaque pH is higher in regions that receive more salivary flow. Clinically, mouth with periodontal disease

shows a lot of plaque accumulation. Research shows a close relationship between the number of bacteria in plaque and the magnitude of plaque's pathological potential to cause periodontal disease.

F. Research Implications

Manalagi apple peel extract toothpaste (Malus Sylvetris) has the advantage that it is made from natural ingredients of manalagi apple peel extract (Malus Sylvetris) so that it is not toxic, has more affordable economic value, does not cause irritation to the skin, because physical quality tests have been carried out.

G. Research Limitations

In the limitations of the study there are several obstacles faced by researchers, namely:

1. No bacterial staining test was carried out so that in this study the bacteria were not specific.
2. Limited references related to manalagi apple skin and its effect on the growth of Streptococcus Mutans bacteria in vivo.

3. Phytochemical screening tests are not carried out due to limited human resources and inadequate equipment and material facilities.

Physical Quality Test Results of Manalagi Apple Skin Extract Toothpaste Formulation Preparation (Organoleptis, Homogeneity, pH, Dispersion, High Foam)

The three concentrations of manalagi apple peel extract have a fresh, slightly sweet and astringent taste, semi-solid with a distinctive apple aroma, brown in color, but at a concentration (5%) light brown toothpaste, homogeneous with a pH of ± 7, and dispersion and high foam ± 6 which are qualified and suitable for toothpaste.

Test Results of Antibacterial Activity of Manalagi Apple Skin Extract Inhibitory Power against Streptococcus mutans Bacteria in Vitro

a. Bacterial Inhibitory Power Test

A concentration of 25% shows the highest diameter of the inhibitory zone of 27 mm with an average increase of 22.33 mm, where this result shows the category of inhibitory zones including the classification of very strong inhibitory zones (> 21 mm), and at a concentration of 5% in the third replication shows the lowest inhibitory power of 6 mm, with an average of 7.33 mm, where these results show the category of inhibitory zones including the classification of medium inhibitory zones (6-10 mm), Then the negative control does not show the occurrence of inhibition from the first replication to the last replication.

b. Normality Test of Inhibitory Power of Manalagi Apple Skin Extract

The data normality test was carried out using the Shapiro Wilk test because the data used were less than 50 samples. This test aims to determine whether or not the data distribution is normal.

Table 4.3 Normality Test Results of Inhibitory Power of Manalagi Apple Skin Extract

Group	Mean ± SD	*P*
Concentration: 25%	22,33 ± 4.50	0,000
Concentration: 10%	13,33 ± 2.51	0,780
Concentration: 5%	7,33 ± 1.15	0,878
K+	13,33 ± 1.52	0,673

**Shapiro-Wilk*

Based on table 4.3 shows the results of the normality test analysis, the majority of data is normally distributed (p = > 0.05), so the test used is a parametric test (One-Way Anova).

c. Test of Homogeneity of Inhibitory Power of Manalagi Apple Skin Extract

Table 4.4 Test of Homogeneity of Inhibitory Power of Manalagi Apple Skin Extract

Group	Dn	*Df*	*P*
P1 – P2 P1 – P3	4	10	0,109

**Levene Test*

Based on table 4.4 above, it is known that the probability value in the homogeneity test of the Levene Test is 0.109. This shows that the homogeneity test of the Levene Test has a value of (p = > 0.05). The results of the homogeneity test can be concluded that the data has the same variance (homogeneous), then the data can be carried out One-Way Anova test to determine whether there is a difference in each inhibitory power between manalagi apple peel extract and herbal and non-herbal toothpaste.

d. Inhibitory Concentration Difference Test

a. Differences in the inhibitory power of apple peel extract between the intervention group and the control group

Table 4.5 Differences in the Inhibitory Power of Apple Skin Extract between the intervention group and the control group

	Sum Of Square	*Mean Of Square*	*P. Value*
Between Groups	820,267	205,067	
Within Groups	60,667	6,067	0,000
Total	880,933		

**One – Way Anova*

Based on table 4.4 above, it shows that there is a significant difference in the inhibitory power at each given concentration, where the value (p = 0.000 < 0.05).

b. Average Difference in Inhibitory Power of Apple Skin Extract Between Intervention Group and Control Group

Table 4.6 Average Difference in Inhibitory Power of Apple Skin Extract Manalagi between intervention group and control group

Treatment		*Mean Difference*	*P. Value*
P1	P2	9,00000*	0,008
	P3	15,00000*	0,000
	K+	9,00000*	0,008
	K-	22.33333*	0,000
P2	P1	-9,00000*	0,008
	P3	6,00000	0,080
	K+	0,00000	1,000
	K-	13,33333*	0,000
P3	P1	-15,00000*	0,000
	P2	-6,00000	0,080
	K+	-6,00000	0,080
	K-	7,33333*	0,029
K+	P1	-9,00000*	0,008
	P2	0,00000	1,000
	P3	6,00000	0,080
	K-	13,33333*	0,000
K-	P1	-22,33333*	0,000
	P2	-13,33333*	0,000
	P3	-7,33333*	0,029
	K+	-13,33333*	0,000

**Post Hoc Beferoni : Tukey HSD*

Based on table 4.6 above shows that the average results between treatment groups have a probability value (p < 0.05) which means that each group has differences in growth inhibition of mutant streptoccus bacteria in vitro depending on the concentration given, but the treatment between P2 (10% Concentration) and K + (Herbal Formula Toothpaste) does not show different results from each other in inhibiting streptococcus mutans bacteria with values (p value = 1,000 > 0.05).

C. Total Plate Number (ALT) Test Results of Streptococcus mutans Bacterial Colonies

Researchers took samples from 30 postgraduate students, sampling was carried out based on inclusion and exclusion criteria in the population, based on inclusion and exclusion criteria from the population, samples were obtained amounting to 15 people with an age range of 22 years – 25 years, Debris from moderate levels (0.7 – 1.8) – Severe (1.9 – 3.0), Salivary pH (4.5 – 5.5), complete and regular tooth arrangement to light crowding. This study was carried out for 3 days, where on the first day

measurements were carried out (pretest) by taking saliva samples on respondents before brushing their teeth in the morning, and on the third day (posttest) measurements were carried out again by taking respondents' saliva to test the bacterial colonies contained in it. This study aims to determine the effectiveness of brushing teeth with apple peel extract manalagi on the number of streptococcus mutans bacteria in saliva, in calculating the number of streptococcus mutans then in a petri dish one round and clear point is 1 colony of streptococcus mutans (Figure 4.1), then the pH of respondents' saliva was also measured to see how much influence it had on the number of streptococcus mutans bacteria.

CONCLUSION

Based on the research objectives obtained from the results of data analysis and discussion, the researchers' conclusions are as follows:

1. Physical quality tests in the form of organoleptis, homogeneity, pH, foam height, dispersion carried out show that apple peel extract toothpaste has physical stability and is suitable for toothpaste.
2. The inhibitory power of Manalagi apple peel extract (Malus Sylvetris) at each concentration shows differences in inhibitory power from the first replication to the last replication. The higher the concentration, the greater the zone of inhibition that occurs.
3. The dose of Manalagi apple peel extract toothpaste (Malus sylvetris) with the optimal dose is at the dose with the highest concentration of apple peel extract of Manalagi which is 25%, where the diameter of the highest inhibitory zone is 27 mm with an average increase, namely
22.33 mm, where these results show the inhibition zone category including the classification of very strong inhibitory zones (> 21 mm) in inhibiting the growth of Streptococus Mutans bacteria.
4. Manalagi (Malus Sylvetris) apple peel extract toothpaste formulation model with optimal dose (25%) is effective in inhibiting
growth of Streptococcus mutans bacteria in vivo with an average decrease of -3.30 x 106 CFU / ml, with p value (0.005 < 0.05)
5. The formulation model of Manalagi apple peel extract toothpaste (Malus Sylvetris) with optimal dose (25%) was effective in increasing salivary pH values where the average was 1.52 with p values (0.001 < 0.05), which showed that there was an average difference before and after treatment in the intervention group.

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