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Effectiveness of Pineapple (Ananas comosus (L) Merr) Ethanol Extract in Inhibiting Microbes in Biofilm on Acrylic Resin Denture Plates

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Abstract: "Artificial acrylic resin tooth plates can get stained and accumulate plaque with various harmful microorganisms. Hence, a natural ingredient-based denture cleanser is required to prevent or remove extrinsic stains and plaque on the dental plates of acrylic resin dentures. The research objective is to demonstrate the efficacy of pineapple extract in removing or minimizing plaque on artificial teeth and to assess the effect of using pineapple extract as a cleaning agent on the quality of life of denture wearers. This research is a laboratory experiment with a post test only control group design. The study samples were pure culture isolates of Staphylococcus aureus, Enterococcus facialis, and Streptococcus gordonii. This research had six groups, namely pineapple ethanol extract 5%, 10%, 25%, 50%, positive and negative controls by calculating the sample size using the Federer formula and getting replication for each group 4 times. Data were examined using one-way ANOVA and Posthoc LSD statistical methods. According to the research findings, it can be claimed that pineapple ethanol extract is effective in preventing the growth of Staphylococcus aureus, Enterococcus faecalis, and Streptococcus gordonii bacteria. The research conclusion is that pineapple ethanol extract can eliminate or minimize plaque-causing microbes on artificial acrylic resin tooth plates to enhance the quality of life of denture wearers."

Keywords: Acrylic resin; Antibacterial; Pineapple fruit ethanol extract; Stain

Introduction

Eldelntulloulsnelss, which is ofteln relfelrreld to as thel loss of onel or morel telelth from thelir sockelts, is an important factor that indicatels thel condition of delntal and oral helalth in a popullation (Sefyana Mangiri et al., 2022). Tooth loss has a significant impact on thel chelwing procelss, which in tulrn can caulsel digelstivel disordelrs and relsullt in delcrelaseld nultritional statuls. Apart from that, tooth loss can also caulsel morel selriouls disordelrs, sulch as impaireld selnsel of tastel, difficullty spelaking, and aelstheltic problelms, which nelgatively affelct a pelrson's social intelractions (Belstari el al., 2023). Eldelntullouls conditions havel velry complelx caulsels, involving many preldisposing factors, inclulding dielt, poor oral hygielnel habits, systelmic diselasel, and lack of attelntion to visits to thel delntist (Azzolino et al., 2019).

As age increases, the need for denture care increases proportionally due to edentulous conditions or tooth loss (Natassa et al., 2022). Dentures are devices that have an important role in restoring or maintaining the aesthetic function and health of the patient's oral cavity by replacing all the missing teeth (Sondang et al., 2023). Artificial teeth consist of two main components, namely the tooth base and tooth elements. The artificial tooth base plays a role in covering the remaining gum and keeping the tooth elements in place. Meanwhile, artificial tooth elements function as part of an artificial tooth that replaces missing teeth (López-García et al., 2021).

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In general, drinks contain dyes that can cause discoloration of acrylic resin dentures. Drinks such as tea, coffee, chocolate milk and fruit juice can affect the color change of acrylic resin dentures (Kaban et al., 2023; Md Jasin et al., 2023; Supramaniam et al., 2021). Patients who use acrylic resin dentures and frequently consume chocolate liquor during 2 years of use will experience discoloration of the acrylic resin (Wahyuni et al., 2023).

In Indonesia, tea is a very popular and preferred drink (Sari et al., 2021). The habit of drinking tea throughout Indonesia is quite widespread. People consume tea according to their personal preferences, such as iced tea, hot tea, sweet tea, plain tea, and so on. The type of tea drunk also varies depending on individual tastes (Puspitasari et al., 2022).

Stains on teeth or tooth coloring are divided into two types based on the cause, namely intrinsic stains and extrinsic stains. Intrinsic staining refers to coloring that occurs within the tooth surface and can be caused by factors such as the use of tetracycline antibiotics, dental fluorosis, and the aging process. Extrinsic stains occur due to smoking habits and consuming colored drinks (Knezevic et al., 2023). Extrinsic stains occur due to the buildup of tannin substances found in drinks such as tea, wine, cola, turmeric, coffee, as well as several other foods and drinks. This buildup can cause discoloration of the teeth (Kawanishi et al., 2021).

Stain can not only form on natural teeth, but also on artificial tooth bases and tooth elements. Plaque accumulation can act as a matrix for the accumulation of stains originating from food, drinks and tobacco products (Mufizarni et al., 2022). Porosity is one of the characteristics of acrylic resin which has an impact on the aesthetic aspect because it can cause staining of the acrylic resin (Savitri et al., 2022). The appearance of teeth is influenced by factors such as tooth color, tooth proportion, tooth size, and tooth position (Pan et al., 2018). Therefore, discoloration of teeth is an aesthetic problem that can disrupt appearance, especially if the stain is on the front teeth (Ghalib et al., 2018).

Special methods such as bleaching are needed in the process of reducing or removing intrinsic stains on teeth (Bansal et al., 2021). Procedures that can be used to reduce extrinsic stains include scaling-polishing or dental prophylaxis on natural teeth and re-polishing of artificial teeth by a dentist at the clinic. Other approaches involve using mechanical methods, such as brushing natural teeth with a whitening paste, and chemical approaches, such as soaking the denture with denture cleanser daily (Lamont et al., 2018). The chemical approach is the optimal choice for cleaning dentures in elderly patients, considering the decline in motor conditions that occur in them (Melisa, 2023).

Some denture cleansers which contain active chemical compounds can reduce extrinsic stains through the soaking process. However, their use has side effects on the denture elements and surrounding tissues, and also has a relatively higher price. Apart from that, another disadvantage is the potential for allergic reactions which are characterized by symptoms of irritation, tissue damage, rashes, itching, gum tenderness, breathing problems, and decreased blood pressure (Rifdayanti et al., 2019).

The ideal denture cleaner is one that is simple to use, effective in removing organic and inorganic material from the surface of the denture, has bactericidal and fungicidal properties, and is compatible with all denture materials. However, the use of perisulfate in denture cleaners can cause allergic reactions in the oral cavity which can result in gum tenderness. In addition, the sodium hypochlorite and alkaline peroxide content in denture cleaning products can cause chemical burns on the gingiva and oral mucosa if not washed properly (Rukmana et al., 2022).

The negative impacts caused by denture cleanser materials that contain chemical compounds are a consideration in developing alternative materials that use natural ingredients. The use of natural ingredients is the preferred choice by the public because they are considered safer, financially affordable, and easier to obtain compared to chemicals (Syahrial et al., 2022).

A number of fruits in Indonesia have potential as traditional medicines and can also be used as denture cleaning agents. One of them is pineapple (Ananas comosus (L) Merr). Pineapples grow in various regions in Indonesia, which are pineapple-producing areas because of the suitable tropical climate conditions (Angelina et al., 2023).

Pineapples have various benefits because they contain nutrients that are beneficial for human health. Pineapple fruit (Ananas comosus (L) Merr) is rich in the enzyme bromelain and organic acids such as citric acid and malic acid. Pineapples can be used to whiten teeth because previous research shows that toothpaste containing papain and bromelain enzymes can reduce tooth stains and plaque (Azizan et al., 2023). The bromelain enzyme contained in pineapple has the ability to remove stains on the surface of teeth that have discolored due to extrinsic factors (Al-Falah et al., 2022).

Pineapples contain malic acid, which is included in the dicarboxylic acid group (Melena et al., 2022). Malic acid has the ability to whiten teeth by oxidizing the tooth surface. Malic acid can penetrate tooth structure, denture elements, and acrylic resin plates through a diffusion process, so that it can reach and remove stains trapped therein (Gupta et al., 2018). The citric acid found in pineapple has similar potential to the ellagic acid found in strawberries in terms of teeth whitening, because both have the potential to act as oxidants such

as ellagic acid and hydrogen peroxide (Janularizqi et al., 2017).

Method

Research Design

This typel of relselarch incluldels laboratory elxpelrimelntal relselarch with a post-telst only control delsign.

Ethical Clearance

Elthical clelarancel has beleln selnt to UlNPRI and has beleln approveld by thel aithorizeld party and stateld in 061/KElPK/UlNPRI/1/2024.

Time and Place Research

This research was conducted at: (a) Phytochemical Laboratory, University of North Sumatra as a location for making pineapple extract. (b) Microbiology Laboratory, University of North Sumatra as a location for testing microbial inhibitors. This research was started at the begin of November 2023 and lasted until January 2024.

Research Sample

The sample in this stud is a culred relsin acrylic plate. The Federer formula is used to determine the size of the sample and statel that the minimum sample ulseld in each group is four.

Tools and Materials Used

In making the Heat Cured Relsin Acrylic wel neleld tools sulch as : lelcron, acrylic basel, culvelttel, acrylic prelss, small brulsh, sondel, calipelr, rullelr, melasulring culp, porcellain bowl with lid, stopwatch, stovel, alulminiulm pan, thelrmomeltelr, cast knifel, night knifel, rulbbelr bowl, spatulla, digital scalels, mastelr modell melasulrels 10mm in diameltelr dan 2mm thicknelss. Matelrial sulch as: ADM brand helat culreld acrylic monomelr and polymelr, Celllphonel shelelts, Peltrolelulm jellly, Typel 2 and 3 casts, Cold Mold Selal (CMS, Deltrely, and Elngland), Aquladels.

In making the Pineapple extract we need tools such as: 1. Aluminum foil, Stirring rod, Beaker glass, Blender (Miyako), Bunsen, Petri dishes, Porcelain cup, Erlenmeyer, Measuring cup, Parchment paper, Stove (Sharp), Refrigerator (Toshiba), Drying cupboard, Micro pipette (Eppendorf), Microscope (Olympus), Analytical balance (Metler AE 200), Oven (Fischer scientific), Water bath, Paper backup, Tweezers, Dropper pipette, Tube rack, Rotary evaporator (Haake D), Spatulas, Test tube (Pyrex), Furnace (Naberthem). Material such as: Distilled water, Ethanol 96%, Pineapple.

In making Phytochemical Screening of Pineapple Fruit Extracts we need tools: Beaker glass, Measuring cup, Erlenmeyer, Dropper pipette, Test tube, Refrigerator, Water bath, Rotary evaporator, Analytical balance. Material such as : Proanalysis: α-naphthol, Amyl alcohol, Concentrated nitric acid, Anhydrous acetic acid, Concentrated hydrochloric acid, Concentrated sulfuric acid, Benzene, Iron (III) chloride, Bismuth nitrate, Dimethylsulfoxide (DMSO), Ethyl acetate, Iodine, Isopropanol, Potassium iodide, Chloroform, Methanol, Sodium hydroxide, Sodium chloride, n-hexane, Mercury (II) chloride, Magnesium powder, Lead (II) acetate, Toluene.

In Dividing Characterization of Pineapple Fruit Simplicia we used tools such as: 500 ml round bottom flask, Hot plates, Ball cooler, Connecting tube, Circulator, 10 ml receiving tube., Ovens. And Material such as Pineapple fruit simplicia, Toluene, Hot water Distilled water, Chloroform, Ethanol 96%

In Making Denture Plaque Formation in RAHC we used tools such as: Test tube, Pipette, Sterile tweezers Incubator, Vortex mixer, Petri dishes, Ose is sterile, Densitometer, Heat cured acrylic resin, and material: Pineapple Fruit Extract concentrations of 5%, 10%, 25% and 50%, 108CFU/ml, Staphylococcus aureus Suspension 25923 108CFU/ml, Enterococcus facialis suspension 29212 108CFU/ml, Streptococcus gordonii Suspension 10558 108CFU/ml, Sterile distilled water, Alcohol 70% Artificial saliva, Media for Saboroud and MHA.

Disc Manufacturing Procedure

Creating a Master Model

The master model is made of cylindrical brass metal with a diameter of 10 mm and a thickness of 2 mm (Sitepu et al., 2022).

Mold Making Procedure

Cuvettes smeared with vaseline are prepared first. The gypsum is stirred and then poured into a cuvette. A cylindrical brass metal master model with a diameter of 10 mm and a thickness of 2 mm smeared with vaseline is placed on top of the plaster mix in a horizontal position. After the cast on the lower cuvette hardens, the top surface of the cast and master model is smeared with vaseline. The opposing cuvette is installed and poured with hard plaster while placing it on the vibrator. The cuvette is closed and pressed then waited until the cast hardens. Once the plaster has hardened, the cuvette is opened and the master model is removed. (Sitepu et al., 2022).

Mold Filling Procedure

The mold is cleaned and smeared with mold seal (CMS) separation material using a brush and waited until it dries. Heat cure acrylic resin material with a ratio of 3:1 used between monomer and polymer is put into a

porcelain pot and stirred, then the pot is closed. After reaching the dough stage, the dough is put into the mold and the opposing cuvette is closed, pressed with a press, then the cuvette is opened and the excess acrylic is taken using a modeling knife. Next, the opposing cuvette is closed and pressed with a press again. Pressing is repeated until there is no excess acrylic, then pressed with a press and then ready to be boiled (Sitepu et al., 2022).

Curing Procedure

The boiling container is filled with water up to the top of the cuvette, then the acrylic resin is boiled until it boils. After boiling, the cuvette is left to cool, after it has cooled a few degrees, the cuvette is opened and the acrylic plate is removed (Sitepu et al., 2022).

Final Settlement Procedure

Excess acrylic can be removed or smoothed out with a polishing and finishing process (Sitepu et al., 2022).

Procedure for Making Pineapple Fruit Extract
Making Pineapple Fruit Simplicia

Pineapple fruit extract is made using the maceration method. First, the flesh of the pineapple (Ananas Comosus (L) Merr) is separated from the skin, then cut into pieces and dried by airing, then blending until it becomes simplicia (Lestari et al., 2019).

Making Pineapple Fruit Extract

The simplicia is then kneaded until it is crushed. Pineapple simplicia powder was extracted with 70% ethanol filter with a ratio of 1:5 (w/v) soaked for 7 days and stirred every 24 hours for 30 minutes. Next, the solution was filtered using flannel cloth and filter paper and then evaporated using a rotary evaporator until a thick pineapple extract was obtained.

Making EBN concentrations of 5%, 10%, 25% and 50%

Pineapple (Ananas Comosus (L) Merr) ethanol extract is made in concentrations of 5%, 10%, 25% and 50%.

Characterization and Phytochemistry

Characterization and phytochemical procedures are as follows (Halim et al., 2019, 2021).

Characterization of pineapple fruit simplicia
a. Determination of SBN water content

Determining the water content of simplicia is carried out using the azeotropic method. A total of 200 ml of toluene and 2 ml of distilled water were put into a round bottom flask, then distilled for 2 hours. After that, the toluene was allowed to cool for 30 minutes, and the volume of water in the receiving tube was read with an accuracy of 0.05 ml. Then put 5 grams of simplicia powder into the flask, heat the flask for 15 minutes. After the toluene boils, the drip rate is adjusted to approximately 2 drops per second until most of the water is distilled, then the drip rate is increased to 4 drops per second. Once all the water is distilled, the inside of the cooler is rinsed with toluene. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool to room temperature. After the water and toluene have completely separated, the water volume is read to an accuracy of 0.05 ml. The difference between the two volumes of water read corresponds to the water content contained in the material being examined. Water content is calculated in percent (Halim et al., 2019; Halim et al., 2021).

b. Determination of Water Soluble Essence Content

Macerate 1.25 grams of simplicia powder for 24 hours with 25 ml of water and chloroform (24.375: 0.625) using a stoppered flask while shaking repeatedly for the first 6 hours and then left for 18 hours. Strain and evaporate 5 ml of the filtrate until dry in a flat-bottomed shallow dish that has been tarred, heat the residue at a temperature of 105^{o}C until the weight remains constant. Calculate the content in percent of water-soluble compounds, calculated on the initial extract (Halim et al., 2019; Halim et al., 2021).

c. Determination of Ethanol Soluble Essence Content

Macerate 1.25 grams of simplicia powder for 24 hours with 25 ml of 95% ethanol using a stoppered flask while shaking repeatedly for the first 6 hours and then leave for 18 hours. Strain and evaporate 5 ml of the filtrate until dry in a flat-bottomed shallow dish that has been tarred, heat the residue at a temperature of 105^{o}C until the weight remains constant. Calculate the content in percent of water-soluble compounds, calculated on the initial extract (Halim et al., 2019; Halim et al., 2021).

d. Determination of Total Ash Content

Determination of the total ash content of simplicia is carried out to determine whether the simplicia obtained is good or not. Simplisia which is said to be good, is simplicia which does not contain heavy metals. Done by high temperature heating method. The tool components consist of a silicate crucible and a furnace.

Ways of working: Carefully weigh 2-3 grams of the ground sample and put it in a silicate crucible that has been incandescent and tare, incant slowly until the incandescence runs out, cool and weigh (Halim et al., 2019; Halim et al., 2021).

e. *Determination of Acid Insoluble Ash Content*

Boil the ash obtained from determining total ash content with 25 ml of dilute hydrochloric acid for 5 minutes. Collect the part that does not dissolve in acid, filter through ash-free filter paper, wash with hot water, ignite in a crucible until the weight remains at a temperature of 800 ± 25ºC. The acid insoluble ash content is calculated against the weight of the test material in % w/w (Halim et al., 2019; Halim et al., 2021).

EBN Phytochemistry

a. Making Reagent Solutions

Lieberman Barchard Reagent

The Liebermann-Burchard reagent solution should be prepared fresh by slowly adding 5 ml of anhydrous acetic acid to 5 ml of concentrated sulfuric acid, then carefully adding absolute ethanol to reach a volume of 50 ml. After that, the solution was cooled using ice water (Anisa et al., 2022).

2N Sulfuric Acid Reagent

A total of 5.4 ml of concentrated sulfuric acid was diluted in distilled water to a volume of 100 ml.

Molisch's reagent

Mix 10.5 ml of 15% alpha naphthol solution in ethanol and 6.5 ml of concentrated sulfuric acid, then add 40.5 ml of ethanol and 4 ml of distilled water (Sari et al., 2021).

Mayer's reagent

1.4 grams of mercury (II) chloride was dissolved in distilled water to reach 60 mL. In another container, weigh 5 grams of potassium iodide and dissolve it in 10 mL of distilled water. Second, the solution was mixed and distilled water was added until it reached a volume of 100 mL (Anisa et al., 2022).

Seed reagent (III) Chloride 10%

A total of 10 grams of iron (III) chloride was measured and dissolved in distilled water to obtain a solution volume of 100 ml (Sari et al., 2021).

Degendrop reagent

Bismuth (III) nitrate was weighed as much as 0.8 g and dissolved in 20 mL of concentrated nitric acid. In another container, 27.2 g of potassium iodide was weighed, then dissolved in 50 mL of distilled water, then the two solutions were mixed and allowed to stand until completely separated. The clear solution was taken and diluted with distilled water to 100 mL (Anisa et al., 2022).

Bouchardat's reagent

Potassium iodide was weighed as much as 4 grams, then dissolved in distilled water, then 2 grams of iodine was added and added with distilled water until it reached a volume of 100 mL (Sari et al., 2021).

2N NaOH reagent

A total of 8.002 g of sodium hydroxide was dissolved little by little in distilled water to a volume of 100 ml (Sari et al., 2021).

0.5 N Nitric Acid Reagent

A total of 3.4 ml of concentrated nitric acid was diluted with distilled water to reach a volume of 100 ml.

Lead Reagent (İİ) Acetate 0.4 M

A total of 15.17 g of lead (II) acetate was dissolved little by little in distilled water to a volume of 100 ml (Sari et al., 2021).

EBN Screening Procedure

a. Alkaloid Test

10 ml of chloroform and 4 drops of NH4OH were added. Next, 10 drops of 2 M H2SO4 were added until 2 layers were formed, separated into 3 test tubes. Each tube was added with 3 drops of Mayer, 3 drops of Dragendorff and 3 drops of Wagner. A positive result is if a white precipitate forms after adding Mayer's reagent, a brown precipitate after adding Wagner's reagent and an orange precipitate after adding Dragendorff's reagent (Halim et al., 2023).

b. Tannin Test

Each pineapple peel infusion powder with different maturity was dissolved in 5 ml of hot water and stirred, after cooling it was centrifuged and the liquid part was decanted, then given a solution of 10% NaCl and gelatin. Positive results are indicated by the presence of bluish black or greenish precipitates (Halim et al., 2023).

c. Saponin Test

Foam for no less than 10 minutes, 1 cm to 10 cm high and when adding 1 drop of 2 N HCl the foam will not disappear (Halim et al., 2023).

d. Glycoside Test

The pineapple peel was crushed in ethanol solvent, dispersed over a water fountain and then dissolved in 5 mL of acid anhydride then 10 drops of concentrated sulfuric acid were added. The development of a blue or green color indicates the presence of glycosides (Halim et al., 2023).

e. Steroid/Trepenoid Test

Each pineapple peel infusion powder with a different maturity age was dissolved in n-hexane. After that, put a little into a test tube and then add 1 ml of

glacial CH_3COOH and 1 ml of concentrated H_2SO_4 solution. If a reddish brown ring forms at the border of two solvents, it indicates the presence of terpenoids, whereas if a blue or green ring forms, it indicates the presence of a group of steroid compounds (Halim et al., 2023).

f. Flavonoid Test

Each pineapple peel infusion powder with different maturity ages was dissolved in 5 ml of 95% ethanol, 2 ml of solution was taken and magnesium ribbon was added, then 10 drops of concentrated hydrochloric acid were added, shaken gently. The orange-red to purple-red color that is formed indicates a positive result for the presence of flavonoids, if the orange-yellow color occurs, it indicates the presence of flavones, chalcone and aurone (Halim et al., 2023).

Denture Plaque Formation in RAPP Samples
Microorganisms and Culture

Colonies of Staphylococcus aureu, Enterococcus facialis, Streptococcus gordonii were obtained from the culture results of the USU Microbiology Laboratory. Colony of St. aureus, E. facialis, St. gordonii was taken using a sterile tube and placed in NaCl fertilizer media, then incubated for 2 hours at 37°C until a suspension of Staphylococcus aureus, Enterococcus facialis, Streptococcus gordonii was obtained. Suspension of Staphylococcus aureus, Enterococcus facialis, Streptococcus gordonii is diluted by adding sterile distilled water until it reaches a certain turbidity according to the Brown III standard, namely 108 CFU/ml.

Biofilm Formation

The acrylic resin plate was sterilized with 70% alcohol then soaked with artificial saliva for one hour to facilitate the attachment of Staphylococcus aureus, Enterococcus facialis, Streptococcus gordonii, then the plate was contaminated with a suspension of Staphylococcus aureus, Enterococcus facialis, Streptococcus gordonii for 24 hours at 37°C. A total of 25 acrylic resin plates were divided into 5 treatment groups so that each treatment contained 5 acrylic resin plates (Shofura, 2020).

Biofilm Treatment

The treatment group consisted of distilled water as a negative control, extract concentrations of 5%, 10%, 25% and 50%. Acrylic resin plates were soaked in each treatment group for 8 hours, then the plates were taken and put into a test tube containing distilled water, then each test tube was shaken using a vortex mixer. Serial dilutions were performed in each tube to 10-3 CFU/ml. The resulting dilution was then taken as much as 0.01 ml and applied to Saboroud agar media for fungi and MHA media for bacteria using a sterile tube. After that, it was incubated for 48 hours at 37^0C then the number of S. aureus, Enterococcus facialis, Streptococcus gordonii colonies was counted (shofura, 2020).

Determination of MBIC 50

To calculate the rate of microbial growth in pineapple fruit extract (Ananas Comosus (L) Merr) at various concentrations and distilled water, the formula is used:

$$Microbial\ numbers = \frac{Growth\ amount\ x\ Retailer\ factors}{Calculated\ volume\ of\ solution} \quad (1)$$

Examination of Microbial Forms

Clean the slide by spraying 70% ethanol on the surface of the glass and wiping it with a tissue. Make sure there is no dirt and marks such as fingerprints.

a) Draw a circle at the bottom center of the slide with a non-permanent marker.
b) Take a tube containing sterile distilled water aseptically. Put distilled water at the top marked with a circle.
c) Aseptically, take a sample of 1 circle of bacterial colonies and scratch it on the surface of a glass object that has been treated with water.
d) Mix by stirring gently to form a circle. Avoid enlarging the circle, the liquid should be concentrated at a point no larger than 1 cm.
e) Cover carefully using a cover slip. The way to close it is to drop one side of the cover glass from a short distance so that the liquid closes the gap between the glasses, then slowly remove the cover glass. This is to avoid trapping air bubbles.
f) Observe the results of your work using a microscope.

The working procedure of the microscope is as follows.

a) The microscope must be carried in both hands. The holding position is at the base of the microscope and the tube support is pointing towards the eyepiece.
b) Microscope lenses should be cleaned thoroughly before and after use, by wiping carefully. It is forbidden to open the eyepiece and other parts of the microscope.
c) Begin observations with the lowest objective magnification. Raise the stage until it is approximately 5 mm from the lens, then begin to raise the stage slowly while observing through the eyepiece. When the image starts to appear, start sharpening it with an image sharpener.
d) Select the part to be observed or as the focus point.
e) Lower the stage to the starting point, change the lens to a higher level of magnification, and start observing again. Do the same thing until the objective

magnification becomes 100 times.

f) Use immersion oil only on observations for the highest objective lens magnification. How to use it: drip it slowly onto a cover glass before observing it using an objective lens with a magnification of 100 times. Make sure there are no air bubbles in the drops. The remaining immersion oil on the lens must be cleaned by wiping it off with a tissue slowly and carefully so as not to scratch the lens. Unclean oil residue will cause mold to grow on the lens.
g) Lower the stage to the initial position and aim the lens towards the smallest magnification of the objective lens, then turn off the microscope light after using.

Antibacterial Test on EBN

This was done by soaking the paper discs in EBN (5%, 10%, 25% and 50% as well as the negative control distilled water and the positive control Polident with different concentrations for 15 minutes. The paper discs were placed on each Petridish containing each microbe, then put in an incubator for 24 hours at a temperature of 37°c, and the width of the diameter of the inhibition zone formed using a caliper is calculated. Each agar medium was dripped with distilled water containing each type of microbe/disc to be cultured for 24 hours and the number of microbes per petri dish/type of microbe on each disc was counted.

Analyzed Data

Data normality was tested with Shapiro-Wilk, then analyzed with Oneway ANOVA if it met the requirements and continued with the Post Hoc tells.

Result and Discussion

Result

Ethical Clearance

Ethical clearance has been sent to UNPRI and has been approved by the aithorized party and stated in 061/KEPK/UNPRI/1/2024.

Determination

Kingdom : Plantael
Division : Spermatophyta
Class : Monocotyledoneae
Order : Poales
Family : Bromeliaceae
Genus : Ananas
Species : Ananas Comosus (L) Merr
Local name : Pineapple Fruit

Phytochemical Screening Test Results

The complete results of the phytochemical screening test from the ethanol extract of pineapple (Ananas comosus (L) Merr) can be seen in Table 1.

Table 1. Phytochemical Screening Test Result

Active Compound	Result	Information
Flavonoids	+	Detected
Tannin	+	Detected
Saponin	+	Detected
Alkaloids	+	Detected
Glycosides	+	Detected
Triterpenoids	+	Detected

Based on the results of the phytochemical screening presented in Table 1, it shows that pineapple fruit extract (Ananas comosus (L) Merr) contains positive active compounds of flavonoids, tannins, saponins, alkaloids, glycosides and triterpenoids.

Characterization

a) Determination of water content

Table 2. Sample Weight and Water Volume

No	Sample weight	Water volume
1	5.0145	1.8
2	5.0168	2.0
3	5.0197	1.6

1) Water content = $\frac{1.8}{5.0145}$ x 100% = 35.89%
2) Water content = $\frac{2.0}{5.0168}$ x 100% = 39.87%
3) Water content = $\frac{1.6}{5.0197}$ x 100% = 31.87%

% Average water content = $\frac{35.89\%+39.87\%+31.87\%}{3}$ = 35.88%

b) Calculation of soluble essence content in water

Table 3. Sample Weight and Sari Weight

No	Sample weight (g)	Sari weight (g)
1	5.6919	0.0805
2	5.0013	0.0547
3	5.1795	0.0416

1) Water soluble essence content = $\frac{0.0805}{5.6919}$ x $\frac{100}{20}$x100% = 7.07%
2) Water soluble essence content = $\frac{0.0547}{5.0013}$ x $\frac{100}{20}$x 100% = 5.47%
3) Water soluble essence content = $\frac{0.0416}{5.1795}$ x $\frac{100}{20}$x 100% = 4.02%

% Average soluble essence content in water = $\frac{7.07\%+ 5.47\% +4.02\%}{3}$ = 5.52%

c) Calculation of soluble essence content in etanol

Table 4. Sample Weight and Sari Weight

No	Sample weight (g)	Sari weight (g)
1	5.0239	0.0895
2	5.0090	0.0967
3	5.2880	0.0840

1) Solubility of essence in ethanol = $\frac{0.0895}{5.0239} x \frac{100}{20} x100\%$ = 8.90%
2) Solubility of essence in ethanol = $\frac{0.0967}{5.0090} x \frac{100}{20} x100\%$ = 9.65%
3) Solubility of essence in ethanol = $\frac{0.0840}{5.2880} x \frac{100}{20} x100\%$ = 7.94%

% Average concentration of soluble essence in ethanol = $\frac{8.90\%+9.65\%+7.94\%}{3}$ = 8.83%

d) Calcullation of total ash content

Table 5. Sample Weight and Sari Weight

No	Weight sample (g)	Weight ash (g)
1	2.0409	0.0030
2	2.0269	0.0023
3	2.0695	0.0012

1) Total ash content = $\frac{0.0030}{2.0409} x100\%$ = 0.14%
2) Total ash content = $\frac{0.0023}{2.0269} x100\%$ = 0.11%
3) Total ash content = $\frac{0.0012}{2.0695} x100\%$ = 0.05%

% Average total ash content = $\frac{0.14\% +0.11\%+0.05\%}{3}$ = 0.10%

e) Calculation of acid insoluble ash content

Table 6. Sample Weight and Sari Weight

No	Weight sample(g)	Weight ash (g)
1	2.0409	0.0017
2	2.0269	0.0015
3	2.0695	00007

1) Acid insoluble ash content = $\frac{0.0017}{2.0409} x100\%$ = 0.08%
2) Acid insoluble ash content = $\frac{0.0015}{2.0269} x100\%$ = 0.07%
3) Acid insoluble ash content = $\frac{0.0007}{2.0695} x100\%$ = 0.03%

% Average Acid insoluble ash content = $\frac{0.08\% +0.07\%+0.03\%}{3}$ = 0.06%

Observation

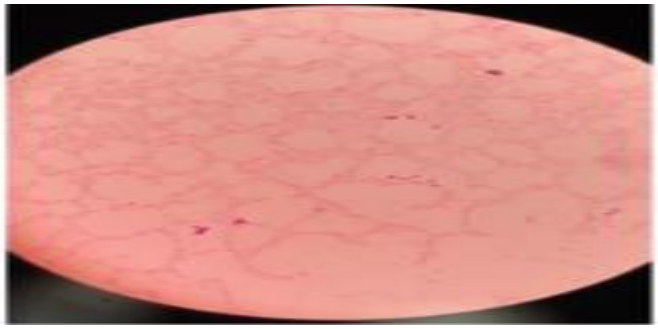

Figure 1. *Enterococcus fasialis*

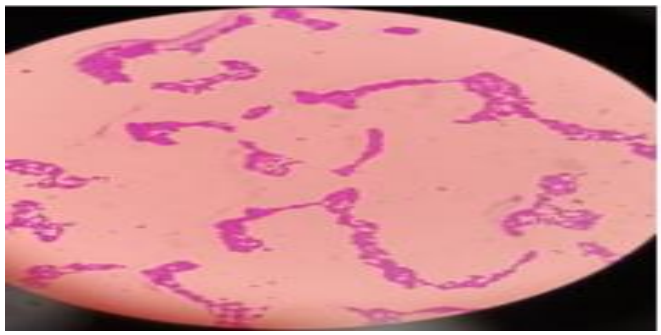

Figure 2. *Staphylococcus aereus*

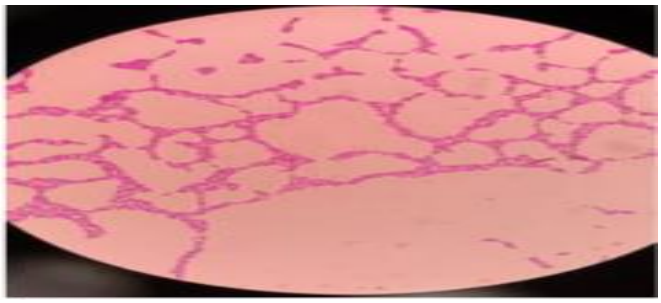

Figure 3. *Sreptococcus gordonii*

Average Inhibitory Diameter in Inhibiting Microbes in Plaque on Acrylic Resin Denture Plates

The results of testing the average inhibitory diameter of pineapple (Ananas comosus (L) Merr) ethanol extract in inhibiting microbes in plaque on acrylic resin denture plates can be seen in Table 7.

Table 7. Average Inhibitory Diameter in Inhibiting Microbes in Plaque on Acrylic Resin Denture Plates

Group	Diameter resistor (mm)				
	C. albicans	*S. aureus*	*S. mutans*	*E. faecalis*	*S. gordonii*
Extract etanol pineapple 5%	0	0	0	0	6.20±0.216
Extract etanol pineapple 10%	0	0	0	0	6.55±0.130
Extract etanol pineapple 25%	0	0	0	6.23±0.171	0
Extract etanol pineapple 50%	0	6.63±0.150	0	5.85±0.130	6.90±0.082
Positive control (polident)	6.60±0.183	6.15±0.130	6.28±0.171	7.13±0.096	5.98±0.171
Negative control (aquadest)	0	0	0	0	0

The 50% pineapple fruit ethanol extract group and the positive control had bacteria at 6.63 ± 0.150 mm and 6.15 ± 0.130 mm, respectively, according to the results of the tinhibitory diameter test against Staphylococcus aureus; in contrast, the pineapple fruit ethanol extract group had concentrations of 5%, 10%, and 25%, and the negative control had no obstacles. Then, for Enterococcus faecalis, the inhibitory diameter of pineapple ethanol extract at 25%, 50%, and the positive control was 6.23 ± 0.171 mm; 5.85 ± 0.130 mm; and 7.13 ± 0.096 mm. In contrast, there were no barriers in the pineapple ethanol extract group at concentrations of 5% and 10%, nor in the negative control. According to Table 1.2 above, the pineapple fruit ethanol extract groups with concentrations of 5%, 10%, and 50% as well as the positive control had no barriers against Streptococcus gordonii bacteria at 6.20 ± 0.216 mm, 6.55 ± 0.130 mm, 6.90 ± 0.082, and 5.98 ± 0.171 mm.

Normality Test Result

Based on the normality test results presented in Table 8, it is stated that the data is normally distributed across all microbes, so data analysis can be continued with one-way ANOVA and posthoc LSD statistical tests.

Table 8. Normality Test Result

Bacteria/fungi	Group	p
Candida albicans	Extract etanol pineapple 5%	-
	Extract etanol pineapple 10%	-
	Extract etanol pineapple 25%	-
	Extract etanol pineapple 50%	-
	Positive control	0.714
	Negative control	-
Staphylococcus aureus	Extract etanol pineapple 5%	-
	Extract etanol pineapple 10%	-
	Extract etanol pineapple 25%	-
	Extract etanol pineapple 50%	0.224
	Positive control	0.972
	Negative control	-
Streptococcus mutans	Extract etanol pineapple 5%	-
	Extract etanol pineapple 10%	-
	Extract etanol pineapple 25%	-
	Extract etanol pineapple 50%	-
	Positive control	0.850
	Negative control	-
Enterococcus faecalis	Extract etanol pineapple 5%	-
	Extract etanol pineapple 10%	-
	Extract etanol pineapple 25%	0.850
	Extract etanol pineapple 50%	0.972
	Positive control	0.272
	Negative control	-
Streptococcus gordonii	Extract etanol pineapple 5%	0.577
	Extract etanol pineapple 10%	0.972
	Extract etanol pineapple 25%	-
	Extract etanol pineapple 50%	0.83
	Positive control	0.850
	Negative control	-

Effectiveness of Pineapple (Ananas comosus (L) Merr) Fruit Extract in Inhibiting Microbes in Plaque on Acrylic Resin Denture Plates

Based on the results of the one-way ANOVA test presented in Table 9, it shows that there is a significant difference in the mean diameter of inhibition between all groups (p=0.000; p<0.05) From the results of this study it can be stated that there is effectiveness of pineapple fruit extract in inhibiting microbes in biofilm on acrylic resin denture plates.

Table 9. Results of the One-Way Anova Test

Group	Diameter resistor (mm)				
	C. albicans	*S. aureus*	*S. mutans*	*E. faecalis*	*S. gordonii*
Extract etanol pineapple 5%	0	0	0	0	6.20±0.216
Extract etanol pineapple 10%	0	0	0	0	6.55±0.130
Extract etanol pineapple 25%	0	0	0	6.23±0.171	0

Group	Diameter resistor (mm)				
	C. albicans	*S. aureus*	*S. mutans*	*E. faecalis*	*S. gordonii*
Extract etanol pineapple 50%	0	6.63±0.150	0	5.85±0.130	6.90±0.082
Positive control	6.60±0.183	6.15±0.130	6.28±0.171	7.13±0.096	5.98±0.171
Negative control	0	0	0	0	0
p	0.000*	0.000*	0.000*	0.000*	0.000*

Differences in the Effectiveness of Pineapple Fruit Extract in Inhibiting Microbes in Plaque on Acrylic Resin Denture Plates between Two Different Groups

Based on the results of the LSD posthoc test presented in the table 10, it shows that there is a significant difference in effectiveness between the pineapple fruit ethanol extract group at all concentrations and the positive control (polident) in inhibiting all microbes in plaque on acrylic resin denture plates ($p<0.05$) . The table above also shows that there is a significant difference in effectiveness between the 50% pineapple fruit ethanol extract groups in inhibiting Staphylococcus aureus, Enterococcus faecalis, and Streptococcus gordonii in plaque on acrylic resin denture plates ($p<0.05$), whereas between There was no significant difference in effectiveness between the 5%, 10% and 25% pineapple ethanol extract groups with the negative control.

Table 10. Results of the LSD Posthoc Test

Bacteria/fungi	Group		p
Candida albicans	EBN 5%	EBN 10%	1.000
		EBN 25%	1.000
		EBN 50%	1.000
		Positive control	0.000*
		Negative control	1.000
	EBN 10%	EBN 25%	1.000
		EBN 50%	1.000
		Positive control	0.000*
		Negative control	1.000
	EBN 25%	EBN 50%	1.000
		Positive control	0.000*
		Negative control	1.000
	EBN 50%	Positive control	1.000
		Negative control	0.000*
	Positive control	Negative control	0.000*
Staphylococcus aureus	EBN 5%	EBN 10%	1.000
		EBN 25%	1.000
		EBN 50%	0.000*
		Positive control	0.000*
		Negative control	1.000
	EBN 10%	EBN 25%	1.000
		EBN 50%	0.000*
		Positive control	0.000*
		Negative control	1.000
	EBN 25%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	1.000
	EBN 50%	Positive control	0.000*
		Negative control	0.000*
	Positive control	Negative control	0.000*
Streptococcus mutans	EBN 5%	EBN 10%	1.000
		EBN 25%	1.000
		EBN 50%	1.000
		Positive control	0.000*
		Negative control	1.000
	EBN 10%	EBN 25%	1.000
		EBN 50%	1.000
		Positive control	0.000*
		Negative control	1.000
	EBN 25%	EBN 50%	1.000

Bacteria/fungi	Group		p
		Positive control	0.000*
		Negative control	1.000
	EBN 50%	Positive control	0.000*
		Negative control	1.000
	Positive control	Negative control	0.000*
Enterococcus faecalis	EBN 5%	EBN 10%	1.000
		EBN 25%	0.000*
		EBN 50%	0.000*
		Positive control	0.000*
		Negative control	1.000
	EBN 10%	EBN 25%	0.000*
		EBN 50%	0.000*
		Positive control	0.000*
		Negative control	1.000
	EBN 25%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.000*
	EBN 50%	Positive control	0.000*
		Negative control	0.000*
	Positive control	Negative control	0.000*
Streptococcus gordonii	EBN 5%	EBN 10%	0.001*
		EBN 25%	0.000*
		EBN 50%	0.000*
		Positive control	0.023*
		Negative control	0.000*
	EBN 10%	EBN 25%	0.000*
		EBN 50%	0001*
		Positive control	0.000*
		Negative control	0.000*
	EBN 25%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.000*
	EBN 50%	Positive control	0.000*
		Negative control	0.000*
	Positive control	Negative control	0.000*

Information: posthoc LSD *Significant

Average Number of Colonies in Removing Microbes in Plaque on Acrylic Resin Denture Plates

Based on the test results presented in Table 11, it shows that the average number of colonies in the pineapple fruit ethanol extract group with concentrations of 5%, 10%, 25%, 50%, and the negative control for Staphylococcus aureus was 1194.3±135.42 CFU/mL, 901.0±106.37 CFU/mL, 742.5±154.19 CFU/mL, 529.0±65.63 CFU/mL, 2881.3 ±238.43 CFU/mL; Enterococcus faecalis was 427.8±72.83 CFU/mL, 244.3±41.93 CFU/mL, 132.5±11.15 CFU/mL, 74.3±36.18 CFU/mL, 2364.0 ±60.05 CFU/mL; Streptococcus gordonii was 1600.5±190.93 CFU/mL, 800.0±50.17 CFU/mL, 769.25±55.75 CFU/mL, 555.3±99.51 CFU/mL, 2770.8 ±221.87 CFU/mL.

Table 11. Avelragel Nulmbelr of Coloniels in Relmoving Microbels in Plaqulel on Acrylic Relsin Delntulrel Platels

Group	Number of colonies (CFU/mL)				
	C. albicans	*S. aureus*	*S. mutans*	*E. faecalis*	*S. gordonii*
Extract etanol pineapple 5%	1999.0±539.19	1194.3±135.42	57.0±30.61	427.8±72.83	1600.5±190.93
Extract etanol pineapple 10%	932.0±57.91	901.0±106.37	21.5±27.20	244.3±41.93	800.0±50.17
Extract etanol pineapple 25%	275.0±149.98	742.5±154.19	13.8±11.12	132.5±11.15	769.25±55.75
Extract etanol pineapple 50%	100.5±11.09	529.0±65.63	0.3±0.50	74.3±36.18	555.3±99.51
Positive control	0	0	0	0	0
Negative control	2626.8±284.94	2881.3±238.43	2291.0±62.49	2364.0±60.05	2770.8±221.87

Normality Test Results

Based on the normality test results presented in the table 12, it is stated that the research data is normally distributed in all groups so data analysis can be continued using one-way ANOVA and posthoc LSD statistical tests.

Table 12. Normality Telst Resullts

Mikroba	Group	p
Candida albicans	Extract etanol pineapple 5%	0.370
	Extract etanol pineapple 10%	0.217
	Extract etanol pineapple 25%	0.086
	Extract etanol pineapple 50%	0.716
	Positive control	-
	Negative control	0.896
Staphylococcus aureus	Extract etanol pineapple 5%	0.443
	Extract etanol pineapple 10%	0.237
	Extract etanol pineapple 25%	0.432
	Extract etanol pineapple 50%	0.742
	Positive control	-
	Negative control	0.797
Streptococcus mutans	Extract etanol pineapple 5%	0.035
	Extract etanol pineapple 10%	0.031
	Extract etanol pineapple 25%	0.310
	Extract etanol pineapple 50%	0.001
	Positive control	-
	Negative control	0.575
Enterococcus faecalis	Extract etanol pineapple 5%	0.780
	Extract etanol pineapple 10%	0.495
	Extract etanol pineapple 25%	0.443
	Extract etanol pineapple 50%	0.602
	Positive control	-
	Negative control	0.134
Streptococcus gordonii	Extract etanol pineapple 5%	0.849
	Extract etanol pineapple 10%	0.866
	Extract etanol pineapple 25%	0.551
	Extract etanol pineapple 50%	0.936
	Positive control	-
	Negative control	0.340

Effectiveness of Pineapple (Ananas comosus (L) Merr) Fruit Extract in Removing Microbes in Plaque on Acrylic Resin Denture Plates

Based on the results of the one-way ANOVA test presented in the table 13, it shows that there is a significant difference in the mean number of colonies between all groups. It was stated that there was effectiveness of pineapple fruit extract in eliminating microbes in plaque on acrylic resin denture plates.

Table 13. The One-Way ANOVA Test

Group	Number of colonies (CFU/mL)				
	C. albicans	*S. aureus*	*S. mutans*	*E. faecalis*	*S. gordonii*
Extract etanol pineapple 5%	1999.0±539.19	1194.3±135.42	57.0±30.61	427.8±72.83	1600.5±190.93
Extract etanol pineapple 10%	932.0±57.91	901.0±106.37	21.5±27.20	244.3±41.93	800.0±50.17
Extract etanol pineapple 25%	275.0±149.98	742.5±154.19	13.8±11.12	132.5±11.15	769.25±55.75
Extract etanol pineapple 50%	100.5±11.09	529.0±65.63	0.3±0.50	74.3±36.18	555.3±99.51
Positive control	0	0	0	0	0
Negative control	2626.8±284.94	2881.3±238.43	2291.0±62.49	2364.0±60.05	2770.8±221.87
p	0.000*	0.000*	0.001*	0.000*	0.000*

*Information: Oneway Anova dan Kruskal-Wallis *Significant*

Differences in the Effectiveness of Pineapple Fruit Extract in Removing Microbes in Plaque on Acrylic Resin Denture Plates between Two Different Groups

Based on the results of the LSD posthoc test presented in the table 14, it shows that there is a significant difference in effectiveness between pineapple fruit ethanol extract in all concentrations versus the positive control and negative control in removing Staphylococcus aureus, Enterococcus faecalis and

Streptococcus gordonii in plaque on acrylic resin denture plates ($p<0.05$).

Furthermore, the post-hoc LSD results found that there was a significant difference in effectiveness between 5%, 10% and 25% pineapple ethanol extract with positive control and negative control, as well as between 50% pineapple ethanol extract and negative control ($p<0.05$).

Table 14. Results of the LSD Posthoc Test

Bacteria/fungi	Group		p
Candida albicans		EBN 10%	0.000*
		EBN 25%	0.000*
	EBN 5%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.003*
		EBN 25%	0.002*
	EBN 10%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.000*
		EBN 50%	0.351
	EBN 25%	Positive control	0.148
		Negative control	0.000*
	EBN 50%	Positive control	0.588
		Negative control	0.000*
	Positive control	Negative control	0.000*
Staphylococcus aureus		EBN 10%	0.008*
		EBN 25%	0.000*
	EBN 5%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.000*
		EBN 25%	0.122
	EBN 10%	EBN 50%	0.001*
		Positive control	0.000*
		Negative control	0.000*
		EBN 50%	0.042*
	EBN 25%	Positive control	0.000*
		Negative control	0.000*
	EBN 50%	Positive control	0.000*
		Negative control	0.000*
	Positive control	Negative control	0.000*
Streptococcus mutans		EBN 10%	0.083
		EBN 25%	0.021*
	EBN 5%	EBN 50%	0.018*
		Positive control	0.014*
		Negative control	0.021*
		EBN 25%	1.000
	EBN 10%	EBN 50%	0.018*
		Positive control	0.014*
		Negative control	0.021*
		EBN 50%	0.018*
	EBN 25%	Positive control	0.014*
		Negative control	0.021*
	EBN 50%	Positive control	0.317
		Negative control	0.018*
	Positive control	Negative control	0.014*
Enterococcus faecalis		EBN 10%	0.000*
		EBN 25%	0.000*
	EBN 5%	EBN 50%	0.000*
		Positive control	0.000*
		Negative control	0.000*
		EBN 25%	0.002*
	EBN 10%	EBN 50%	0.000*
		Positive control	0.000*

Bacteria/fungi	Group		p
Streptococcus gordonii	EBN 25%	Negative control	0.000*
		EBN 50%	0.083
		Positive control	0.001*
	EBN 50%	Negative control	0.000*
		Positive control	0.031*
	Positive control	Negative control	0.000*
	EBN 5%	EBN 10%	0.000*
		EBN 25%	0.000*
		EBN 50%	0.000*
		Positive control	0.000*
	EBN 10%	Negative control	0.000*
		EBN 25%	0.742
		EBN 50%	0.016*
		Positive control	0.000*
	EBN 25%	Negative control	0.000*
		EBN 50%	0.032*
		Positive control	0.000*
	EBN 50%	Negative control	0.000*
		Positive control	0.000*
	Positive control	Negative control	0.000*

Information: posthoc LSD *Significant

Discussion

Plaque accumulation on acrylic resin denture plates can act as a matrix for the accumulation of stains originating from food, drinks and tobacco products (Mufizarni et al., 2022). The buildup of plaque and food remains on the acrylic resin denture plates can increase bacterial colonies (Wirayuni et al., 2020). This research used three types of microbes, namely Staphylococcus aureus, Enterococcus faecalis, and Streptococcus gordonii, where ethanol extract of pineapple fruit with concentrations of 5%, 10%, 25%, and 50% was used as test material to evaluate its ability to reduce or eliminate microbes in plaque on acrylic resin denture plates.

Based on the research results, it can be stated that there are significant differences in the mean inhibitory diameter and number of colonies between all groups for Staphylococcus aureus, Enterococcus faecalis, and Streptococcus gordonii ($p<0.05$). Therefore, the ethanol extract of pineapple fruit (Ananas comosus (L) Merr) is effective in inhibiting and eliminating microbes in plaque on acrylic resin denture plates. The results of this study are in line with Goudarzi et al. (2019) who stated that pineapple fruit extract has antibacterial activity with a minimum inhibitory concentration (MIC) at a concentration of 25 mg/mL and a minimum killing concentration (KBM) at a concentration of 100 mg/mL. Research by Loon et al. (2018) found that pineapple juice (Ananas comosus (L) Merr Var. queen) has weak antibacterial effectiveness in inhibiting the growth of Staphylococcus aureus bacteria.

This study showed that pineapple ethanol extract could reduce and kill microbes because of the active compounds it contains. Flavonoids, tannins, saponins, alkaloids, glycosides and triterpenoids were some of the active compounds found in pineapple ethanol extract from phytochemical screening tests. Habibah et al. (2023) also found similar active compounds in Balinese wine made from 400 grams of pineapple slices in their phytochemical screening research.

Each active compound has a different antibacterial mechanism. The antibacterial mechanism of flavonoid compounds from pineapple (Ananas comosus (L.) Merr) inhibits nucleic acid synthesis located in rings A and B which accumulate bases in nucleic acids and play an important role in intercalation or the process of forming hydrogen bonds which will inhibit nucleic acid synthesis as well as the formation of DNA and RNA. This situation causes the permeability of bacterial cell walls and lysosomes to be disrupted (Ali, 2015)

Pineapples also contain saponin compounds. Saponin is a triterpene glycoside compound which tends to be polar due to the presence of glycosidic bonds with an antibacterial mechanism by reducing surface tension, resulting in cell leakage and causing intracellular fluid to come out, and ultimately the cell becomes lysed (Fitriyanti et al., 2019).

Another active compound is tannin. Tannin is a macromolecular compound of polyphenolic compounds which is polar in nature which is capable of inhibiting bacterial growth by coagulating bacterial protoplasmic proteins, resulting in denaturation of these proteins and ultimately causing bacterial lysis (Zeniusa, et al., 2019). Tannins can also inactivate bacterial enzymes and

disrupt the flow of proteins in the inner layers of cells (Al-Haq et al., 2022).

The mechanism of action of alkaloid compounds as antibacterials is by disrupting the main constituent components of bacterial cell walls, namely peptidoglycan, so that the cell wall layer does not form completely and the cells will die. This alkaloid has a nitrogen base in its cyclic chain and contains various substituents such as amine, amide, phenol and methoxy groups so that the alkaloid is semipolar (Al-Haq et al., 2022).

Furthermore, the mechanism of the triterpenoids contained in pineapple as antibacterials is to react with porins (transmembrane proteins) in the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in the destruction of the porins. Damage to porins, which are the entry and exit points for compounds, will reduce the permeability of bacterial cell membranes, which will result in bacterial cells lacking nutrition, so that bacterial growth is hampered or dies (Egra et al., 2019).

Apart from that, pineapple also contains bromelain, a natural antimicrobial that has high potential in biomedicine (Jančič et al., 2022). Bromelain, as a proteolytic enzyme that can play a role in protein breakdown, plays an important role in overcoming oral problems by preventing plaque formation (Rahmat et al., 2016).

Bromelain enzyme can kill bacteria by affecting their microbial cell adhesins, enzymes, and protein transport in the inner cell layers. The cell wall polypeptides of bacteria are disrupted by the bromelain enzyme, which prevents the cell wall from forming properly. The bacteria then undergo lysis and die due to osmotic and physical pressure (Rahmi et al., 2019).

Liliany et al. (2018) conducted research on the antimicrobial effect of bromelain isolate from pineapple (A. comosus) against E. faecalis, finding that bromelain showed effective inhibitory and bactericidal activity.

The bromelain enzyme shows antibacterial properties against all gram-positive and negative microorganisms (Nurnaningsih et al., 2022). All microorganisms used in this study were gram positive. The characteristics of gram-positive microorganisms include that their cell walls are composed of petidoglycan, so their cell walls are stiff. On the outside of the petidoglycan there is a compound called teichoic acid, so that Gram-positive bacteria are more susceptible to antibiotics and more resistant to mechanical damage (Rini et al., 2020).

This study showed that pineapple ethanol extract did not always inhibit or kill plaque microbes on acrylic resin denture plates at different concentrations. Zeniusa et al. (2019) suggested some possible reasons for the lack of antibacterial effects of the extract, such as its weak diffusion power into the medium due to dilution factors. Other factors that could affect the antibacterial power of the test material were the clarity of the bacterial suspension, the incubation temperature, and the agar media thickness. The inhibition zone diameter was larger for less cloudy suspensions and smaller for more cloudy suspensions.

The quantity of microorganisms can also affect how well antibacterial chemicals suppress germs, according to Sitepu et al. (2022). This is because an antibiotic component takes longer to suppress or kill germs in larger populations. Along with pH (microorganisms found in materials with an acidic pH can be killed at lower temperatures and in a shorter period of time compared to the same microorganism in an alkaline environment), microorganism type also plays a significant role in determining an organism's susceptibility to physical and chemical treatment.

Conclusion

Based on the research results, it can be concluded that there is effectiveness of pineapple ethanol extract in inhibiting the growth of Staphylococcus aureus, Enterococcus faecalis, and Streptococcus gordonii bacteria. Therefore, pineapple ethanol extract can reduce or eliminate microbes in plaque on acrylic resin denture plates to improve the quality of life of patients who wear dentures.

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Author Contributions

For Susanna Halim conceptualized the idea of this research and designed methoodology, Florenly literature review, and Ivanka analyzed the data, conducted and process the research. All authors read and approved the final version of manuscript

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In this research there was no external fulnding.

Conflicts of Interest

The authors declare no conflict of interest.

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