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Potential Inhibition of Ethanol Extract of Red Betel Leaves (*Piper crocatum* Ruiz & Pav) Against Microorganisms in Biofilms in *Hot Curing Acrylic Denture Plates*

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Abstract: Tooth loss reduces quality of life by affecting chewing and speech function and can interfere with social activities. *Hot curing acrylic* dentures have the disadvantage of high porosity and surface roughness, so they are susceptible to food residues and microorganisms that can lead to the formation of biofilm and denture stomatitis. Red series leaves *(Piper crocatum Ruiz & Pav*) have chemical content such as flavonoids, alkaloids, and essential oils that have the potential to be antimicrobials. This study aims to determine the potential of red betel leaf ethanol extract in inhibiting microorganisms in biofilms in denture plates made of *hot curing acrylic*. This study used the liquid dilution method to test the effectiveness of red betel leaf ethanol extract with concentrations of 10%, 25%, 50% and 75% on the growth of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* contaminated on round denture plates made of *hot curing acrylic*. The results showed that the ethanol extract of red betel leaf significantly reduced the number of bacterial and fungal colonies, with a concentration of 75% showing the highest effectiveness, especially against the bacterium *Lactobacillus acidophilus* with an average colony count of 45.67 ± 4.04. Red betel leaf ethanol extract contains chemical compounds that are effective as antimicrobials in biofilms in denture plates made of *hot curing acrylic*. This extract has the potential to be an alternative natural ingredient for denture cleaning.

Keywords: Antimicrobial; Biofilm; *Candida albicans;* Denture plates; Ethanol extract of red betel leaf; Hot curing acrylic; *Lactobacillus acidophilus; Streptococcus mutans*

Introduction

Tooth loss or edentulous is the loss of some or all of the teeth in the jaw arch (Khoman et al., 2024). Tooth loss decreases quality of life by affecting chewing and speech function, lowering self-confidence and interfering with social activities (Ratnasari et al., 2019). Therefore, to restore full oral function without interruption, the missing teeth need to be replaced with dentures. Dentures are prostheses designed to replace missing natural teeth and prevent the negative impact they cause (Ayu & Pintadi, 2020)

Acrylic resin, which is often used as a base material for dentures, is a *hot curing* acrylic resin because it is non-toxic, does not cause irritation, is insoluble in oral fluids, is cheap, easy to manipulate, and can be repaired in the

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event of damage (Kusmawati & Putri, 2019). One of the disadvantages of *hot curing* acrylic resin is that it has a very high porosity and surface roughness, so that the base surface of unpolished dentures such as the area facing the mucosa is susceptible to food residue and if not cleaned it can become a place for food residues and microorganisms to accumulate. Acrylic resin tends to absorb water when it comes into contact with saliva, so it can lead to the formation of plaque where microorganisms accumulate (Ayu & Pintadi, 2020)

Plaque in dentures is not the same as dental plaque, although the microbial composition of plaque in dentures is affected to some extent by dental plaque because the microbiota on the surface of dentures and the surface of teeth come from the same oral cavity (Angela et al., 2024). Some of the microorganisms that reside in denture plates are *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*, which are difficult to clean mechanically and chemically, thus interfering with the cleanliness and health of denture plates. If these bacteria and fungi interact with food debris, it can cause plaque buildup, bad breath, damage to tooth structures, and when the immune system is weakened, they cause infections in the oral cavity, resulting in the appearance of pathogens that result in inflammation of the mucosa. As a result of plaque formation, these microorganisms attach to the oral mucosa and react so that it can cause *denture stomatitis* (Ayu & Pintadi, 2020).

According to research (Ayu & Pintadi, 2020), people who use dentures who experience denture stomatitis on their mucous membranes have *Candida albicans* 66.7%, *Staphylococcus aureus* and *Streptococcus mutans* as much as 49.5%. Red betel leaves, or in Latin referred to as *Piper crocatum* Ruiz & Pav, are known to treat a wide variety of diseases. However, there is still little use of red betel leaves for evidence-based treatment or commonly known as *Evidence Based Medicine* (EBM). The chemical content found in red betel leaves includes alkaloids, saponins, flavonoids, tannins, essential oils, polyphenols, quinones, steroids, isoprenoids, nonprotein amino acids, cyanogenic, and isoprenoids (Jayadi et al., 2023). The content of essential oils in red betel leaves can function as antibacterial and antifungal. This content can inhibit the growth of *Streptococcus mutans* bacteria (Saka Abeiasa et al., 2022) and *Candida albicans* fungi (Sulastri et al., 2024)

From the above problems, this study was conducted to determine the potential of red betel leaf ethanol extract against the inhibition of microorganisms in biofilms, namely *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans* in dentures made of hot curing acrylic. The objective of this research is to determine the most effective concentration of red betel leaf ethanol extract, ranging from 10%, 25%, 50%, and 75%, against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* biofilms on hot-curing acrylic denture plates. The benefit of this research is that it can be used as a basis or consideration for further research to develop the potential of red betel leaf extract as a natural alternative cleaning agent for hot-curing acrylic dentures.

Method

Ethical Clearance

Ethical clearance has been submitted and approved, as stated in 005/KEPK/UNPRI/X/2024.

Research Design

This study uses *the post-test only control group design*, which is observation of the treatment group and compares it with the control group.

Location and Time of Research

The research locations include the Medanese Herbarium Laboratory of FMIPA USU, the Phytochemistry Laboratory of the Faculty of Pharmacy USU, the Microbiology Laboratory of the Faculty of Medicine USU and the Laboratory of Pharmaceutical Microbiology USU. This research was carried out from October to November 2024.

Tools and Materials

The tools used in this study are in the form of *rubber bowls,* spatula, cuvettes, vibrators, *hydraulic bench presses,* acrylic pots, cement spatels, lecrons, pots, stoves, *straight handpieces* and *carbide* burs, *abrasive papers grit* 280, 360, 400, knives, blenders, mesh sieves no. 60, extraction jars, digital scales, *rotary evaporators*, oven, ash-free filter paper, test tube, filter paper, measuring cup, ose, *spreader*, test tube, autoclave, incubator, spectrophotometer, sterile tweezers, *microplates,* vortex, *colony counter*.

The materials used in this study were nightshade, type II cast, water, vaseline, CMS separator, *hot curing acrylic resin*, cellophane plastic, red betel leaf, 70% ethanol, DMSO, filtrate, 25 mL dilute hydrochloric acid, 100 mL ethanol, 100 mL chloroform, concentrated HCl, magnesium metal powder, amyl alcohol, Mayer reagent, Dragendroff reagent, FeCl3 solution, aquaades, ether, concentrated H2SO4 solution, artificial saliva, *suspension of S. mutans*, *L. acidophilus*, *C. albicans*, NA media, *acrylic hot curing plates*, polydense, PBS pH 7.0 solution, BHIB media, PCA media.

Research Population and Sample

The population in this study was the bacteria *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*. The sample in this study is a denture

plate made of hot curing acrylic. The determination of the sample size used in this study was calculated using Federer's formula and a result of 3 was obtained. So the number of samples used in this study is 54 acrylic hot curing plates with three experimental replications for each group.

Making Simplisia

The red betel leaves collected are then cleaned and washed with running water. The next step is the display of simplicia materials to make the drying and grinding process easier. Drying is carried out by drying without sunlight or aeration, then making simplicia powder by smoothing dry simplicia using a blender and sifting with mesh no.60 (Farchati et al., 2023)

Making Red Betel Leaf Ethanol Extract

The extraction method by maceration uses ethanol solvents. Simplisia as much as 200 g is soaked with 1.5 L of ethanol for 5 days and stirred occasionally, then filtered. The pulp is soaked with 1.5 L of ethanol for 2 days with occasional stirring then filtered. Maserat is combined and then concentrated with a rotary evaporator until the extract is obtained (Halim et al., 2021). After that, dilution was carried out using DMSO so that red betel leaf extract was obtained with concentrations of 10%, 25%, 50%, and 75%.

Characterization Testing of Red Betel Leaf Simplicia

Testing the characteristics of red betel leaf simplicia includes the determination of water content to provide a minimum limit or range regarding the amount of water content in simplisia, the determination of the solubility of fruits aims to determine the water solubility (polar) of the simplisia, the determination of the solubility of ethanol to determine the dissolved compounds in ethanol both polar and non-polar, the determination of the total ash content to provide an overview of the internal mineral content and external from the initial process to the formation of the extract, and the determination of the total ash content to provide an overview of the internal and external mineral content from the initial process to the formation of the extract, and the determination of the insoluble ash content in acids to assess the simplicia against contamination of materials containing silica, heavy metals such as lead/lead (Pb) (Halim et al., 2019)

Phytochemical Testing of Red Betel Leaf Ethanol Extract
Flavonoid Test

Each red betel leaf infusion powder with a different maturity age is dissolved in 5 ml of 70% ethanol, 2 ml of solution is taken and magnesium tape is added, then 10 drops of concentrated hydrochloric acid are added, beaten slowly. The orange-red to purple-red color that is formed shows a positive result of the presence of flavonoid compounds, if an orange-yellow color occurs, it indicates the presence of flavonoid compounds, kalkon and auron (Halim et al., 2023).

Alkaloid Test

Added 10 ml of chloroform and 4 drops of NH4OH. Next, 10 drops of H2SO4 2 M are added until 2 layers are formed, separated into 3 test tubes. Each tube is added 3 Mayer drops, 3 Dragendorff drops, and 3 Wagner drops. Positive results if a white precipitate is formed after the addition of Mayer's reagent, a brown precipitate is formed after the addition of Wagner's reagent, and an orange precipitate is formed after the addition of Dragendorff reagent (Halim et al., 2023).

Tannin Test

Each simplicia of different maturity is dissolved in 5 ml of hot water and stirred, after cooling is centrifuged and the liquid part is poured, then given a 10% NaCl solution and gelatin. Positive results are indicated by the presence of bluish-black or greenish deposits (Halim et al., 2023).

Saponin Test

Saponins are active compounds that are easily detected by forming foam. The appearance of foam and bubbles indicates the presence of glycosides that have the ability to form bubbles in water and the addition of HCl can make the foam more stable. The foam that arises is caused by saponin compounds containing compounds that are partially soluble in water and compounds that are soluble in nonpolar solvents as surfactants that can reduce surface tension (Najmudin *et al*., 2023). Foam for at least 10 minutes, 1 cm to 10 cm high and when added 1 drop of HCI 2 N the foam does not disappear (Halim et al., 2023).

Glycoside Test

Red betel leaves are crushed in ethanol solvent, spread over a fountain, then dissolved in 5 mL of acid anhydride and then added 10 drops of concentrated sulfuric acid. The appearance of blue or green indicates the presence of glycosides (Halim et al., 2024)

Triterpenoid/Steroid Test

Each pineapple peel infusion powder with different maturation gels is dissolved in n-hexane. After that, put a small amount into the test tube and then add 1 ml of glacial CH3COOH and 1 ml of concentrated H2SO4 solution. If a reddish-brown ring forms at the border of the two solvents, it indicates the presence of terpenoids, and if it is as if a blue or green ring is formed, it indicates the presence of a group of steroid compounds (Halim et al., 2024).

Acrylic Hot Curing Resin Sample Making

Steps for making acrylic resin samples are (Amanda et al., 2024)

a. Making plates from cylindrical red night with a diameter of 10 mm and a thickness of 2 mm a total of 54 plates.
b. The Plaster of Paris dough is stirred until homogeneous in a rubber bowl with a spatula. Then inserted into the lower cuvette, the prepared night plate is planted in the dough in a horizontal position after which it is vibrated on top of the vibrator.
c. The surface of the cast on the lower cuvette is smeared with vaseline and the upper cuvette is filled with a cast and waited for it to harden. The cuvette is opened so that a mold is obtained.
d. The mold is applied with CMS material using a brush and waited for it to dry. Acrylic resin dough in a ratio of 3:1 (according to the manufacturer's instructions) then stir in an acrylic pot with spatel cement until the dough stage process.
e. The acrylic resin dough is inserted into the moldspace then covered with cellophane plastic, then the antagonist cuvette is installed and pressed. The cuvette and cellophane are opened and then cleaned of excess resin. The cuvette is closed and then repressed.
f. Cuvettes that have been filled with acrylic molds are cured with a waterbath for 90 minutes at a temperature of 100 °C. The cuvette is waited until it cools completely then the acrylic resin plate is removed from the cuvette.
g. Finally, continue by tidying up the excess acrylic using a straight handpiece and carbide bur.

Plaque Formation On Hot Curing Acrylic Resin Denture Plates

A 5 mL solution of BHI-B was put into a test tube and then 1 ose of Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans was added after which it was incubated for 24 hours at a temperature of 37°C. Bacterial and fungal suspensions are added with aqueducts to achieve a standard Mc. Farland turbidity of 0.5 (1 x 106 CFU/ml) (Amanda et al., 2024).

Then the acrylic plates are soaked in sterile aqueducts for 48 hours, then sterilized by autoclave at 121°C for 15 minutes. Soak the acrylic plate in artificial saliva for 1 hour. Rinse the acrylic plate with PBS 2 times every 15 seconds. Acrylic plates were taken using sterile tweezers and then immersed into each test tube on BHI-B media containing 10 ml of suspension *of Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans* and incubated at 37°C for 24 hours (Amanda et al., 2024)

Observation of Morphology of Colonies and Microbial Cells

Sterilize the round ose needle, then touch it on the surface of the bacterial colony and then inoculate it on the surface of the NA medium by the scratching method. This is done several times so that a colony is obtained that is pure from microbes. Furthermore, it was incubated at 37°C for 48 hours (Junaedi, 2023).

From the colonies obtained, the morphological properties are observed, namely the shape of the edges, elevation, and color. Meanwhile, the observation of bacterial cell characterization includes cell shape, gram staining, spore staining, and physiological and biochemical tests (Junaedi, 2023).

Antimicrobial Testing

This study used a liquid dilution method by means of *acrylic hot curing* plates that had been contaminated *with Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* bacteria were put into *microplates* divided into six groups that would be soaked for 30 minutes (Amanda et al., 2024). The six groups included negative control (aquadest), positive control (*alkaline peroxide*: polydent), red betel leaf ethanol extract with a concentration of 10%, 25%, 50%, and 75%.

After soaking, the acrylic plate is rinsed with PBS 2 times every 15 seconds. The acrylic plate was inserted into 10 ml of BHIB media and then vibrated using a vortex for 30 seconds to remove the bacteria *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* fungus attached to the acrylic plate.

Calculation of the Number of Colonies

Each tube was diluted in a series by *the pour plate method* until it reached a suspension of 10-2 CFU/ml for *Streptococcus mutans* and *Lactobacillus acidophilus* bacteria and *Candida albicans* fungus. The suspension obtained after shaking the tube was then taken as much as 0.1 ml and then bacteria and fungi were cultured by the *plate spread* method on Plate Count Agar (PCA) medium and incubated for 24 hours at a temperature of 37°C. Furthermore, the number of cells (CFU/ml) was calculated using a colony counter and data analysis was carried out (Amanda *et al*., 2024).

Data Analysis Methods

The data obtained was then analyzed using the normality test, namely the Kolmogorov-Smirnov test to determine the normality of the distribution of research data. Furthermore, a homogeneity test was carried out using the Levene test to determine the homogeneity of the research data. However, the normality test shows that the research data is not distributed normally, so it can be continued with *the Kruskal-Wallis* and *Mann U Whitney test*.

Results and Discussion

Result

Results of Red Betel Leaf Determination

The determination was carried out at the Medanese Herbarium Laboratory, University of North Sumatra, from the results of the determination will be obtained data on the results of the plant taxonomy used as follows:

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotylendoneae
Order : Piperales
Family : Piperaceae
Genus : Piper
Species : *Piper crocatum* Ruiz & Pav.
Local Name : Red Betel

Results of Characterization of Red Betel Leaf Simplicia

The results of the characterization test of red siren leaf simplicia were obtained as shown in table 1. next.

Table 1. Results of the red betel simplicia characterization test

Parameters	Yield(%)
Determination of moisture content	7.99
Determination of water soluble juice rate	17.45
Determination of soluble sari content in ethanol	2.43
Determination of total ash content	13.88
Determination of acid insoluble ash content	1.44

Results of Phytochemical Screening of Ethanol Extract of Red Betel Leaves

The results of phytochemical screening tests on red betel leaf ethanol extract show that red betel leaf ethanol extract contains flavonoids, alkaloids, triterpenoids, glycosides, saponins and tannins which can be seen in full in table 1. next.

Table 2. Results of Phytochemical Screening of Ethanol Extract of Red Betel Leaves

Active Compounds		Result	Information
Flavonoids	$MgHCl+H_2SO_4$	+	Detected
	Meyer	+	Detected
Alkaloids	Dragendof	+	Detected
	Bouchardat	+	Detected
Triterpenoids/ Steroids	Lieberman-Bouchardat	+	Detected
Glycosides	$Molish+H_2SO_4$	+	Detected
Saponins	Aquades	+	Detected
Tannins	$FeCl_3$	+	Detected

Description:
Bouchardat : KI + Aquadest +Iodine
Meyer : HgCl + Aquadest + KI
Lieberman-Bourchat : $H_2SO_4(p)+CH_3COOH$

Morphological Observation Results of Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans

The isolates obtained were rejuvenated in NA media to see colonies from *Streptococcus* mutans and *Lactobacillus acidophilus bacteria*, as well as SDA media to see colonies from *Candida albicans*. The morphology results of bacteria and fungi obtained by gram staining.

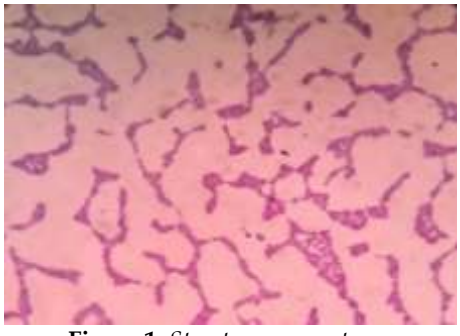


Figure 1. *Streptococcus mutans*

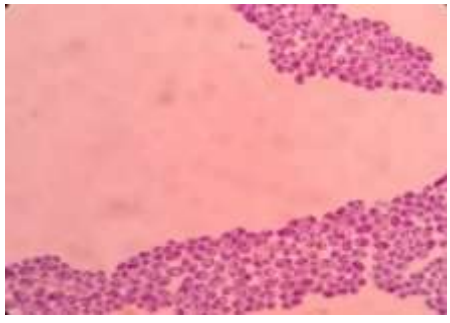


Figure 2. *Lactobacillus acidophilus*

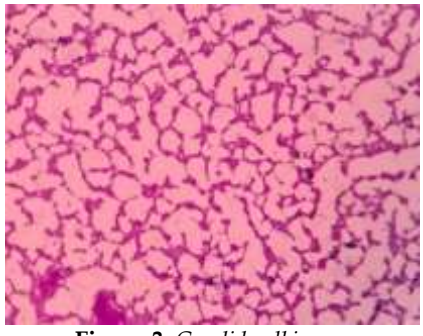
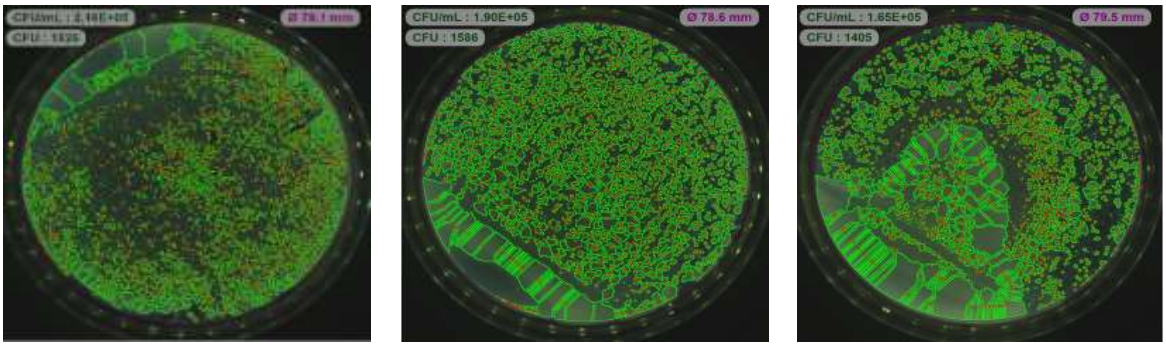


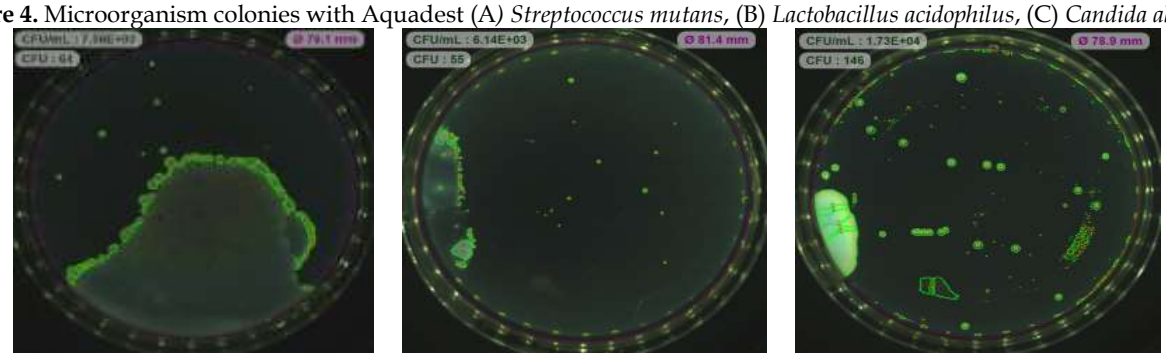
Figure 3. *Candida albicans*

Results of Testing the Number of Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans Colonies on Hot Curing Acrylic Denture Plates



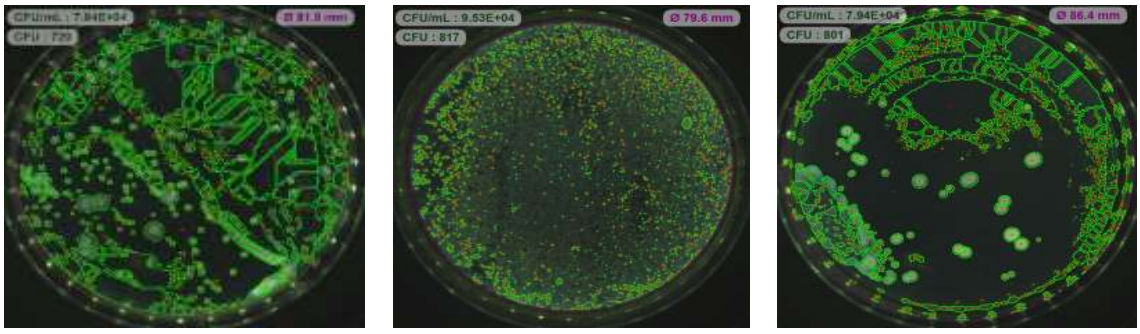
(A) (B) (C)

Figure 4. Microorganism colonies with Aquadest (A*) Streptococcus mutans*, (B) *Lactobacillus acidophilus*, (C) *Candida albicans*



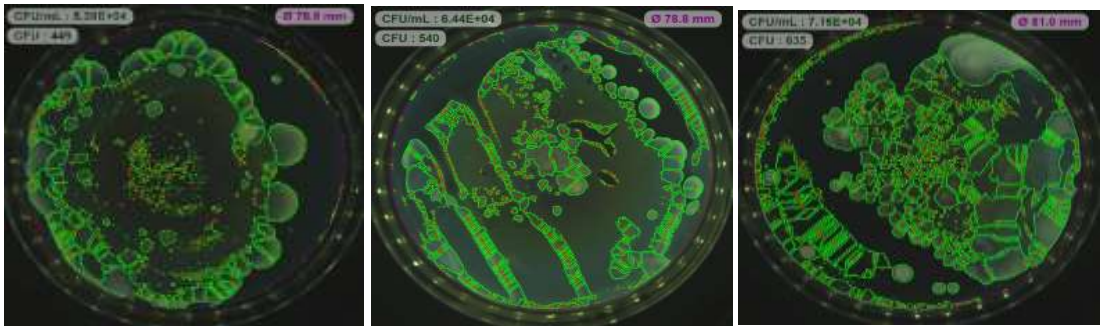
(A) (B) (C)

Figure 5. Colonies of microorganisms with Polydent (polyden) (A) *Streptococcus mutans,* (B) *Lactobacillus acidophilus,* (C) *Candida albicans*



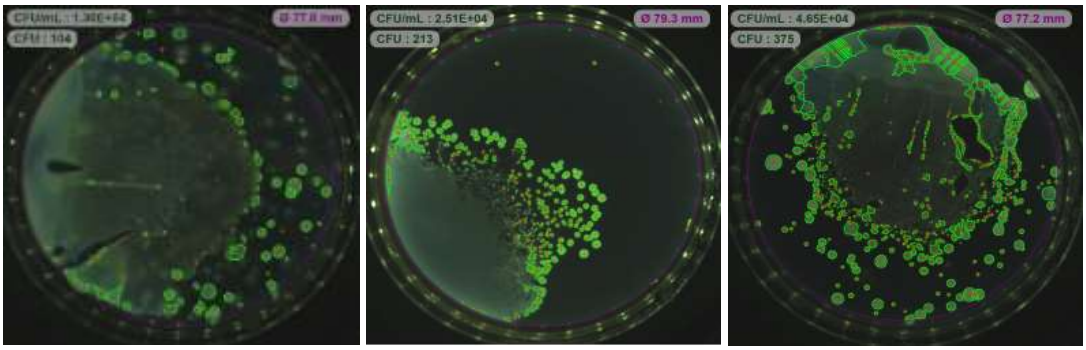
(A) (B) (C)

Figure 6. Microorganism colonies with ethanol extract of red betel leaf concentration 10% (A) *Streptococcus mutans*, (B) *Lactobacillus acidophilus*, (C) *Candida albicans*



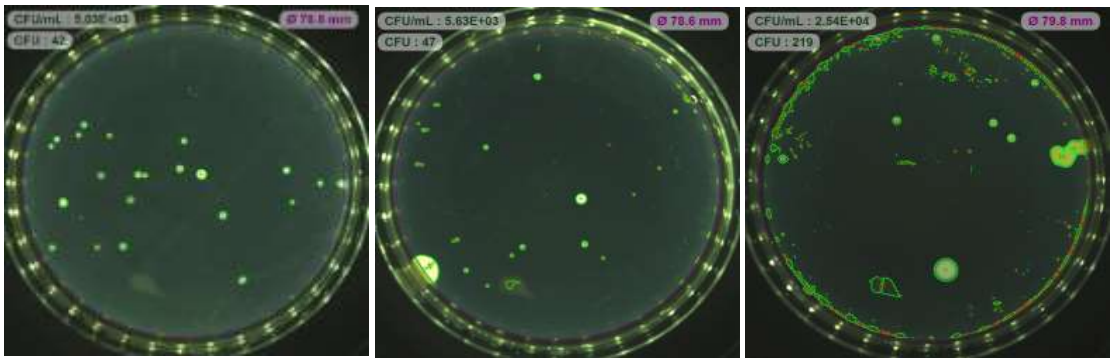
(A) (B) (C)

Figure 7. Microorganism colonies with ethanol extract of red betel leaf concentration 25% (A) *Streptococcus mutans*, (B) *Lactobacillus acidophilus*, (C) *Candida albicans*



(A) (B) (C)

Figure 8. Microorganism colonies with ethanol extract of red betel leaf concentration 50%
(A) *Streptococcus mutans*, (B) *Lactobacillus acidophilus*, (C) *Candida albicans*



(A) (B) (C)

Figure 9. Microorganism colonies with ethanol extract of red betel leaf concentration 75%
(A) *Streptococcus mutans*, (B) *Lactobacillus acidophilus*, (C) *Candida albicans*

Statistical Test Results

Based on the results of the normality test, it was stated that the research data was not normally distributed in all groups, namely for *Streptococcus mutans, Lactobacillus acidophilus,* and *Candida albicans* , namely with a p >value of 0.05, so the data analysis continued with *Kruskal-Wallis* and *Mann-Whitney*.

Table 3. *Kruskal-Wallis Test*

Microorganism	*Group*	*P*
Streptococcus mutans	EEDSM 10%	0.005*
	EEDSM 25%	
	EEDSM 50%	
	EEDSM 75%	
	Polydent	
	Aquadest	
Lactobacillus acidophilus	EEDSM 10%	0.006*
	EEDSM 25%	
	EEDSM 50%	
	EEDSM 75%	
	Polydent	
	Aquadest	
Candida albicans	EEDSM 10%	0.005*
	EEDSM 25%	
	EEDSM 50%	
	EEDSM 75%	
	Polydent	
	Aquadest	

Description:
EEDSM = Red Betel Leaf Ethanol Extract
*The result of this test is significant

The Kruskal-Wallis test was carried out to find out whether there was a difference in the number of colonies *of Streptococcus mutans*, *Lactobacillus acidophilus*, and Candida *albicans* fungi after administration of red betel leaf ethanol extract with various different concentrations. From the results *of the Kruskal-Wallis* test that has been carried out, it was found that *the value of p= 0.005* was obtained in Streptococcus mutans *bacteria, in the bacterium Lactobacillus acidophilus* p=0.006, and in the fungus *Candida albicans* the value of p=0.005 as seen in table 3. Therefore, p<0.05, it can be concluded that there is a significant difference in the number of colonies for *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* in each treatment group.

Table 4. *Mann-Whitney Test*

Microorganism		Group	*P*
Streptococcus mutans	EEDSM 10%	EEDSM 25%	0.050
		EEDSM 50%	0.050
		EEDSM 75%	0.050
		Polydent	0.050
		Aquadest	0.050
		EEDSM 50%	0.050

Microorganism		Group	*P*
	EEDSM	EEDSM 75%	0.050
	25%	Polydent	0.050
		Aquadest	0.050
	EEDSM	EEDSM 75%	0.050
	50%	Polydent	0.050
		Aquadest	0.050
	EEDSM	Polydent	0.050
	75%	Aquadest	0.050
	Polydent	Aquadest	0.050
Lactobacillus acidophilus	EEDSM	EEDSM 25%	0.050
	10%	EEDSM 50%	0.050
		EEDSM 75%	0.050
		Polydent	0.050
		Aquadest	0.050
	EEDSM	EEDSM 50%	0.050
	25%	EEDSM 75%	0.050
		Polydent	0.050
		Aquadest	0.050
	EEDSM	EEDSM 75%	0.050
	50%	Polydent	0.050
		Aquadest	0.050
	EEDSM	Polydent	0.127
	75%	Aquadest	0.050
	Polydent	Aquadest	0.050
Candida albicans	EEDSM	EEDSM 25%	0.050
	10%	EEDSM 50%	0.050
		EEDSM 75%	0.050
		Polydent	0.050
		Aquadest	0.050
	EEDSM	EEDSM 50%	0.050
	25%	EEDSM 75%	0.050
		Polydent	0.050
		Aquadest	0.050
	EEDSM	EEDSM 75%	0.050
	50%	Polydent	0.050
		Aquadest	0.050
	EEDSM	Polydent	0.050
	75%	Aquadest	0.050
	Polydent	Aquadest	0.050

*Description: EEDSM = Red Betel Leaf Ethanol Extract

The Mann-Whitney test was conducted to determine the significant difference in the number of bacterial and fungal colonies between two different concentrations. As seen in table 4. There was a significant difference in the number of bacterial colonies between all groups because of the p=0.05 value. However, there was no significant difference in the number of colonies in the group of red betel leaf ethanol extract with a concentration of 75% with a Polydent because of the value of p=0.127.

Discussion

This study used test materials in the form of ethanol extract of red betel leaf with concentrations of 10%, 25%, 50% and 75% to determine or evaluate its chemical content as antibacterial and antifungal against microorganisms in biofilms in the form of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* in dentures made of hot curing acrylic.

The denture plates used in this study were made cylindrical with a size of 10 mm x 2 mm. The material used as the denture plate is hot curing acrylic or commonly known as hot polymerized acrylic resin (RAPP). Hot curing acrylic is a material that is often used as a base material because it is non-toxic, easy to obtain, relatively cheap, relatively simple application techniques, good physical and aesthetic properties and has been widely known (Nugrahini et al., 2022).

Microorganisms attached to the surface of dentures will proliferate to form denture plaque that affects the health of the oral cavity (Nugrahini et al., 2022). Bacterial adhesion on the surface of acrylic dentures can also be caused due to the porosity of the acrylic and the rough surface that is on the denture. The rough surface makes it easier for microorganisms to reproduce and retain organisms (Ayu & Pintadi, 2020).

The results showed that acrylic hot curing denture plates soaked in red betel leaf ethanol extract with concentrations of 10%, 25%, 50%, and 75% could significantly reduce the number of colonies of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*.

In line with Ratri's (2024) research which stated that red betel leaf extract with a concentration of 20% has the ability to inhibit the double biofilms of *S. mutans* and *C. albicans* equivalent to 0.2% chlorhexidine gluconate. And in the study. However, this study uses a different method, namely the liquid dilution method where the inhibition force is measured from the number of colonies at each given concentration.

The results of the study also show that the higher the concentration of extract, the lower the number of bacterial and fungal colonies. This is in line with (Nasution & Daulay (2022)who stated that this is because the higher the concentration of the test material, which means that the greater the number of active substances contained in the extract, the greater the ability of the test material to inhibit the growth of a bacterium.

Based on the results of phytochemical screening, it was found that the red betel leaf ethanol extract contains active compounds in the form of flavonoids, alkaloids, triterpenoids/steroids, glycosides, saponins and tannins. These active compounds work to inhibit the formation of cell walls from bacteria and fungi. Halim et al. (2023)explained that saponins and triterpenoids have antifungal effectiveness, tannins and glycosides as antioxidants, while flavonoids are effective as antibacterial.

Flavonoids are the largest group of phenolic compounds, phenolic compounds have the property of effectively inhibiting the growth of viruses, bacteria and

fungi. Flavonoid compounds and their derivatives have two specific physiological functions as chemicals to overcome disease attacks as antibacterial and antiviral for plants. The mechanism of action of flavonoids is by damaging the bacterial cell membrane in the phospholipid part, thereby reducing permeability which can result in bacterial damage (Alifah et al., 2023) Flavonoids are also effective in inhibiting fungal growth because flavonoids can denature fungal cell proteins, causing disturbances during the formation of fungal cells and even death in fungal cells (Khoman et al., 2024).

Alkaloids are biological chemical compounds that are active in heterocyclic form. The ability of the mechanism is to inhibit the components that make up peptidoglycan against bacterial cells which results in the cell wall layer not being able to form completely so that the cell will die. In addition, alkaloids can bind to DNA. Substances that are between DNA cause inhibition of replication so that it can result in cell death (Tyas et al., 2024).

Triterpenoids are components of plants that have an odor and can be isolated from vegetable materials by distillation as essential oils. The mechanism of triterpenoids as antibacterial is to react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymer bonds resulting in damage to the porin. Damage to porin, which is the entrance and exit of compounds, will reduce the permeability of the bacterial cell membrane which will result in bacterial cells will lack nutrients, so that bacterial growth is inhibited or dies (Afiff et al., 2017).

Based on research from Rinanda & Alga (2012) in (Januarti et al., 2019) explained that saponins have an antibacterial mechanism because they have hydrophilic molecules and molecules that can dilute lipids or lipophilics, thereby lowering surface cell pressure. In addition, saponins cause increased permeability, which results in the content of intracellular fluids such as enzymes, nutrients, metabolic substances, and proteins in the cell can escape and cause damage and death of fungal cells (Khoman et al., 2024)

Tannins are antibacterial by forming complex compounds with enzymes or substrates that interfere with cell membranes. The mechanism of action of tannin compounds as antibacterial is by causing bacterial cells to be lysed. This is because tannin compounds have a target for the polypeptide wall of the bacterial cell wall so that the formation of the cell wall becomes less complete, so that the bacterial cell will die. In addition, tannins have the ability to inactivate bacterial enzymes as well as interfere with the passage of proteins in the inner layers of cells (Amal et al., 2023).

Conclusion

Based on the results of the study, it can be concluded that there are chemical compounds in red betel leaves in the form of flavonoids, alkaloids, steroids, glycosides, saponins and tannins that are effective as antimicrobials against microorganisms in the biofilm on denture plates made of hot curing acrylic. There was an effectiveness of red betel leaf ethanol extract as an antimicrobial in biofilms on denture plates made of hot curing acrylic, especially at a concentration of 75% and in the group of *Lactobacillus acidophilus* bacteria. Thus, red betel leaf ethanol extract can be used as an antimicrobial in patients who use dentures made of hot curing acrylic.

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Conflict of Interest

The authors declare no conflict of interest.

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