Tootpaste Formulation of Telang Flower (Clitoria Ternatea L.) Ethanol Extract as an Antibacterial for Streptococcus Mutans and Antifungal for Candida Albicans in the Oral Cavity

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Abstract

Caries and candidiasis are still high and require preventive management. Telang flowers (Clitoria ternatea L.) have the potential to inhibit the growth of bacteria and fungi in the oral cavity. This study aims to determine the correct formulation of telang flower toothpaste ethanol extract as an oral antibacterial and antifungal to inhibit Streptococcus mutans and Candida albicans.

The study used 32 samples of ethanol extract of telang flower toothpaste with concentrations of 50%, 60%, 65%, and 70% to test antibacterial activity, while concentrations of 20%, 30%, 40%, and 50% to test antifungal activity. The inhibition zone testing method used is diffusion with holes in agar media.

The results of One Way Anova analysis show p < 0.05, so it can be concluded that there is a difference in the effectiveness of antibacterial activity against Streptococcus mutans in the toothpaste group containing ethanol extract of telang flowers. There is also a difference in the effectiveness of antifungal activity against Candida albicans in the toothpaste group.

The conclusion is the telang flower ethanol extract toothpaste has an antibacterial effect against Streptococcus mutans; concentrations of 50%, 60%, and 65% have antibacterial effectiveness in the weak category, while a concentration of 70% is in the medium category. Telang ethanol extract toothpaste has antifungal activity against Candida albicans; concentrations of 20%, 30%, and 40% have the same effectiveness statistically.

Experimental article (J Int Dent Med Res 2023; 16(4): 1578-1584) Keywords: Toothpaste, telang flower (Clitoria ternatea L.), Streptococcus mutans, Candida albicans.

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Introduction

Oral health is essential to general body health because it can affect a person's quality of life, such as speech function, chewing process, and self-confidence. Poor dental and oral hygiene can cause various kinds of problems. One of the dental and oral health problems that many Indonesians experience is dental caries and candidiasis. According to the 2018 National Basic Health Research, the percentage of dental

*Corresponding author: Dendy Murdiyanto Department of Dental Materials Science Faculty of Dentistry Universitas Muhammadiyah Surakarta Surakarta 57162, Indonesia E-mail: <u>dm124@ums.ac.id</u> and oral health problems in Indonesia reached 57.6%, with the highest proportion being caries at 45.3%, while the prevalence of oral candidiasis reached 84% in 2009.¹ The most common microorganism that causes oral candidiasis is Candida albicans, including on denture bases.²

Caries is a disease that occurs in the hard tissue of teeth, including enamel, dentin, and cementum. The caries process can occur due to an increase in the concentration of lactic acid caused by the bacteria Streptococcus mutans. Streptococcus mutans bacteria can ferment carbohydrates into lactic acid. Increased lactic acid concentrations will trigger enamel demineralization.³ Dental caries can be prevented by mechanical measures such as toothbrushing and toothpaste, effectively eliminating caries-causing microorganisms. The main ingredient in toothpaste that is often used to

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prevent caries is fluoride, but if the fluoride level is excessive, it can cause fluorosis of the enamel and other side effects. Toothpaste can be overcome by adding natural ingredients to toothpaste.⁴

Candidiasis is an infection caused by Candida albicans. The fungus Candida albicans is normal flora but will become pathogenic when present in excessive amounts in the body, which can cause opportunistic infections with supporting factors such as immunosuppression, malignancy, use of broad-spectrum antibiotics, use of prostheses, smoking, and xerostomia. The number of Candida albicans in the oral cavity is 100-500 colonies per millimeter of saliva. Candida albicans colonization in the oral cavity is greatly influenced by human hygiene, namely lifestyle and environmental cleanliness.⁵ Preventing Candida albicans colonization is by avoiding the cause, maintaining cleanliness, checking dentures regularly, or the most straightforward way is by maintaining oral hygiene by brushing teeth with toothpaste. The American Dental Association (ADA) and the Indonesian National Standard (SNI) state that herbal ingredients have relatively few side effects, so it is highly recommended to use toothpaste with natural ingredients because they have relatively no side effects and are also not difficult to find ⁶

One of the natural ingredients beneficial for health is the telang flower (Clitoria ternatea L.). This antioxidant anti-inflammatory inhibits the growth of bacteria, including gram-positive and gram-negative bacteria and fungi, such as Streptococcus mutans. , Lactobacillus casei, and Staphylococcus aureus, where these bacteria cause tooth decay.⁷ Rezaldi and friends (2022) also reported that telang flowers could inhibit the growth of Candida albicans, Malasezia furfur, Pitosprorum ovale, and Aspergilus fumigatus.⁸

Telang flower extract contains phytochemicals such as alkaloids, flavonoids, tannins, saponins, and several other aromatic compounds, which are helpful as antibacterials against the growth of microorganisms and insects. Alkaloids have antibacterial properties, which can inhibit the work of enzymes to synthesize bacterial proteins. The bacteria's metabolism is disrupted, resulting in insufficient energy requirements; as a result, bacterial cells are permanently damaged.^{9,10}

Research on the formulation of ethanol extract of

telang flower toothpaste as an antibacterial and antifungal in the oral cavity needs to be carried out because telang flower has several advantages that have the potential to inhibit Streptococcus mutans and Candida albicans, which cause oral disease.

Materials and methods

1. Telang Flower Extract

Using the maceration method, two kilograms of telang flower simplicia powder was extracted with 20 liters of 70% ethanol solvent. The powder is put into a vessel, then added with 70% ethanol solvent, covered, and left for 3 x 24 hours at room temperature while repeatedly stirring to extract the active substance completely. After three days, the extract was filtered, and then the residue was extracted again with ethanol solvent. The vessel was closed, left at room temperature, protected from light for 3 x 24 hours, and filtered. The extract was concentrated using a vacuum rotary evaporator to separate the solvent from the active substance and then evaporated over a water bath until a thick extract of telang flower was obtained. The extract is then weighed to determine the weight and percentage of the extract.



Figure 1. Fresh telang flower (Clitoria ternatea L.), powder, and paste.⁷

2. Toothpaste preparations

Weigh the active ingredients of telang flower ethanol extract with varying concentrations

of 20%, 30%, 40%, 50%, 60%, 65%, and 70%, as well as additional ingredients of calcium carbonate, glycerin, sodium carboxymethyl cellulose, sodium lauryl sulfate, saccharin, methylparaben, peppermint oil, and distilled water.

In anodianta (9/)	Function	Formula							
ingrealents (%)	Function	F0	F1	F2	F3	F4	F5	F6	F7
Telang flower ethanol extract	Active Ingredient	0	20	30	40	50	60	65	70
Calcium carbonate	Abrasive	45	45	45	45	45	45	45	45
Glycerin	Humectant	25	25	25	25	25	25	25	25
Sodium carboxylmethylcellulose (Na CMC)	Binder	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium lauryl sulphate	Surfactant	1	1	1	1	1	1	1	1
Sodium benzoate	Preservative	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Saccharin sodium	Sweetener	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Menthol	Flavouring	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Aquadest	Solvent	ad 100							

Table 1. Formula of Toothpaste with EthanolExtract of telang Flower Extract.

Description:

 ${\sf F0}$: Toothpaste without telang flower ethanol extract (negative control)

F1 : Toothpaste with 20% telang flower ethanol extract.

F2 : Toothpaste with 30% telang flower ethanol extract

F3 : Toothpaste with 40% telang flower ethanol extract

F4 : Toothpaste with 50% telang flower ethanol extract

F5: Toothpaste with 60% telang flower ethanol extract F6: Toothpaste with 65% telang flower ethanol extract

F7 : Toothpaste with 70% telang flower ethanol extract

3. Antibacterial Test

The test bacteria, Streptococcus mutans, which came from pure culture, were taken one dose each, then inoculated by streaking on slanted Nutrient Agar (NA) media, incubated at 37° C for 24 hours. The bacterial culture was taken with a sterile tube needle and then suspended in a test tube containing 10 ml of 0.9% NaCl solution until the turbidity of the bacterial suspension was the same as the standard Mc Farland turbidity of 0.5. The antibacterial power test in this study used the diffusion method using wells. The test was carried out by inserting toothpaste with various concentrations of 0.1 g each into the wells; then, the petri dishes were incubated for 24 hours at 37°C. Measurements were carried out on the clear zone formed around the well, which shows the zone of inhibition of bacterial growth with a caliper.

4. Antifungal Test

Candida albicans two ose mixed with 10 mL of 0.9% NaCl in a test tube. The candida suspension was shaken for about 15 seconds to homogenize and poured into a 7 ml cuvette. Place the cuvette in a spectrophotometer and

measure the turbidity at a wavelength of 530 nm and an absorbance of 0.5-0.6, which means, according to Mc Farland, 0.5 (1x106-5x106cells/mL). The fungal suspension is smeared on the media and waited for 5-15 minutes for the fungal suspension to absorb into the media. Antifungal activity was tested using the diffusion method in wells with a diameter of 6 mm, into which toothpaste was inserted with various concentrations of 0.1 g each; incubation was carried out for 48 hours at $37^{\circ}C$.

5. Measurement of the inhibition zone

The zone of inhibition is marked by a clear zone that forms around the well and is then measured using a caliper to an accuracy of 0.1 mm.



Figure 2. Measurement of the resistance of the diffusion method and zone of inhibition formula. Dv: Vertical diameter; Dh: Horizontal diameter; Dc: Well diameter ¹¹

The ethanol extract of telang flower toothpaste that has been made is also tested for quality to determine its suitability when used in the oral cavity. Toothpaste preparations have pharmaceutical requirements by Indonesian National Standards by carrying out several tests such as organoleptic, homogeneity, pH, spreadability, foam height, and stability tests.¹²

Results

1. Stability Test Results

The results of the physical stability test of the preparation carried out using a cycling test can be seen in Table 2. The preparation of ethanol extract of telang flower (Clitoria Ternatea L.) toothpaste was stored at a temperature of 4 degrees Celsius for 24 hours, then continued with storage at 40° C (1 cycle). The test was carried out over six cycles, where each cycle observed changes in the paste's physical properties, including pH, homogeneity, and organoleptic tests.¹³

Cycle	рН	Homogeneity	Organoleptic (Consistency, color, smell)
1	6.51	homogeneous	paste, blue-black, mint
2	6.49	homogeneous	paste, blue-black, mint
3	6.15	homogeneous	paste, blue-black, mint
4	5.95	homogeneous	paste, blue-black, mint
5	6.07	homogeneous	paste, blue-black, mint
6	5.79	homogeneous	paste, blue-black, mint

Table 2. Stability test results of ethanol extract of telang flower toothpaste.

2. Spreadability Test Results

The results of observations of the spreadability test of the ethanol extract toothpaste preparation of telang flower (Clitoria Ternatea L.) can be seen in Table 3. The spreadability test of the toothpaste preparation is intended to determine the ability of the toothpaste to spread when applied to the teeth. Spreadability is an essential characteristic in formulations because it influences the transfer of active ingredients to the target area in the correct dose, ease of use, the pressure required to get out of the package, and consumer acceptance.¹⁴

3. Foam Height Test Results

The results of testing the foam height of the ethanol extract toothpaste preparation of telang flower (Clitoria Ternatea L.) can be seen in Table 3.

Concentration of Toothpaste	Load	Spreadability	Initial Foam Height	Final Foam High
	63.4067 gr	2.8 mm	45 cm	44 cm
50%	50 gr	3 mm		
	100 gr	3.1 mm		
	63.4067 gr	1.86 cm	42 cm	41 cm
70%	50 gr	2.06 cm		
	100 gr	2.2 cm		

Table 3. Spreadability and foam height test results of ethanol extract of telang flower toothpaste.

The foam formation test aims to see the amount of foam produced by toothpaste to remove dirt and clean the mouth when brushing teeth. The foam produced from a toothpaste preparation is generally influenced by the concentration of detergent used. SLS (Sodium lauryl sulfate) is used as a detergent in this toothpaste base. SLS is an anionic surfactant with high cleaning power.¹⁵

4. Antibacterial Activity Test Results

The results of measuring the diameter of the inhibition zone of toothpaste with ethanol

extract of telang flowers (Clitoria Ternatea L.) against Streptococcus mutans were carried out using a caliper in millimeters.



Figure 3. Results of the inhibition zone for Streptococcus mutans bacteria.

Petri dish	Concentrati (Clitoria Te	Negative Control			
	50%	60%	65%	70%	-
I	3.95	4.43	5.13	6.78	0
Ш	4.05	4.76	4.80	6.15	0
111	4.22	4.75	5.50	6.06	0
IV	4.10	4.45	4.88	5.81	0
Average	4 08	4 59	5 07	6 21	0

Table 4. Average Growth Inhibition Zone forStreptococcus mutans bacteria.

Based on Table 4, the inhibitory zone of telang flower ethanol extract toothpaste at a concentration of 50% has an average of 4.08 mm, at a concentration of 60% has an average of 4.59 mm, at a concentration of 65% has an average of 5.07 mm and the largest inhibition zone was at a concentration of 70% with an average of 6.21 mm.

5. Antifungal Activity Test Results

The results of the antifungal activity test can be seen in Figure 4 and Table 5.



Figure 4. Results of Candida albicans inhibition zone.

Petri dish	Concentration of ethanol extract of telang flower (Clitoria Ternatea L.) in paste preparation tooth				Negative Control
-	20%	30%	40%	50%	-
I	4.20	5.98	3.20	5.06	0
11	3.01	3.60	4.14	5.40	0
111	3.78	3.14	3.85	5.72	0
IV	3.00	4.41	4.55	6.11	0
Average	4.2	4.3	3.9	5.15	0

Table 5. Results of Candida albicans GrowthInhibition Zones.

Based on Table 5, the inhibitory zone of telang flower ethanol extract toothpaste at a concentration of 20% has an average of 4.2 mm, at a concentration of 30% has an average of 4.3 mm, at a concentration of 40% has an average of 3.9 mm and the largest inhibition zone was at a concentration of 50% with an average of 5.15 mm.

6. Data analysis

The statistical data analysis used in this research is One-way ANOVA. The normality test used in this research was the Shapiro-Wilk test, obtaining a p-value> 0.05, and it was concluded that all data were normal. The homogeneity test shows a result of p > 0.05, so it can be concluded that the data comes from a population with the same variance.

The results of the One-Way Anova analysis show p < 0.05, so it can be concluded that there is a difference in the effectiveness of antibacterial power against Streptococcus mutans in the group of toothpaste containing ethanol extract of telang flowers. Besides that, there is also a difference in the effectiveness of antifungal power against Candida albicans in the toothpaste containing ethanol extract of telang flower.

The results of the LSD (Least Significant Difference) Post Hoc Test are used to determine whether groups have significant differences from other groups with a significance value of p<0.05. The antibacterial inhibition zone of toothpaste with ethanol extract of telang flowers at concentrations of 20%, 30%, and 40% using the LSD Post Hoc Test obtained a p-value> 0.05, meaning there was no significant difference in concentration values. Meanwhile, the the antifungal inhibition zone of the ethanol extract of telang flower toothpaste showed the results of the Post Hoc LSD test analysis with a p-value> 0.05 in all groups, meaning that all groups had significant differences.

Discussion

Antibacterial testing of ethanol extract of telang flower toothpaste was carried out to determine the inhibitory power of ethanol extract of telang flower formulated in toothpaste preparations. The bacteria used in this research are Streptococcus mutants. telang flower extract is stated to inhibit three bacteria that cause tooth decay: Streptococcus mutans, Staphylococcus aureus, and Lactobacillus casei. telang flowers contain several phytochemical compounds, such as alkaloids, flavonoids, tannins, and saponins, which are helpful as antibacterials against the growth of many microorganisms and insects. Alkaloids can inhibit protein synthesis using antibacterial agents that attach to a specific receptor protein on microbial ribosomes and then inhibit the formation of peptide synthesis, resulting in the formation of non-functional proteins that cannot be synthesized. This proteindenaturing activity damages cells irreversibly, causing cell death.¹⁶

The results of testing the antibacterial activity of ethanol extract of telang flower toothpaste against Streptococcus Mutans. Concentrations of 50%, 60%, 65%, and 70% showed antibacterial activity with a clear zone around the well; the 70% concentration had the largest zone of inhibition. The results of Jeffrey research in 2023 have different effective concentrations from this research, where the

preparation telang flower kombucha of fermentation solution has the potential to prevent the growth of mutant Streptococcus.¹⁷ The this research stated results of that а concentration of 40% mouthwash was the best concentration.¹⁸ This can be caused by using different methods to obtain the active substance. The inhibitory ability of an antibacterial is divided into four categories based on the diameter of the inhibition, namely weak (< 5 mm), medium (6-10 mm), strong (11-20 mm), and very strong (> 21mm).¹⁹ Based on these categories, in this study, the ethanol extract toothpaste of telang flowers with concentrations of 50%, 60%, and 65% was a weak category of antibacterial, and the ethanol extract toothpaste of telang flowers with a concentration of 70% was an antibacterial in the medium category against Streptococcus mutans.

Based on the results of identifying the antifungal activity of the ethanol extract of telang flower toothpaste at concentrations of 20%, 30%, 40%, and 50%, it shows that the ethanol extract toothpaste of telang flower has the antifungal ability, namely inhibiting the development of Candida albicans which is characterized by the formation of an inhibitory zone arranged in along the excellent hole. Each concentration in the preparation provides an antifungal effect against Candida albicans, but the 50% concentration shows the most significant effect with the largest average zone of inhibition, 5.15 mm. The conclusion can be drawn that the greater the concentration of the active ingredients contained, the greater the inhibitory power formed because the ethanol extract of telang flowers as an active ingredient contains antifungal effects that can inhibit Candida albicans' development.^{20,21}

The antifungal activity of the ethanol extract of telang flower toothpaste is due to several inaredients in telana flowers. Various phytochemicals such as kaempferol, guercetin and myricetin glycosides as well as anthocyanins have been isolated from C. ternatea flowers. These anthocyanins inhibit fungal growth by binding to proteins and fungal cell walls, damaging the fungal cell membrane. Alkaloids penetrate cell membranes, which flavonoids will follow by breaking down cell membranes. At the same time, saponins will suppress the decay of cell walls, which results in damage, so that they can prevent pathogenic fungi's growth. If we look at the increase in telang flower ethanol extract

concentration in toothpaste, the greater the inhibition zone produced. ^{22,23}

The use of dental materials in the human mouth is not possible except by relying on physiological, biological, pathological studies and the study of physical, mechanical, and chemical properties.²⁴ Physical tests of toothpaste at the concentration that produces the most extraordinary inhibitory power were carried out to determine organoleptic parameters, spreadability, foaming, homogeneity, pH, and viscosity. The results of the physical test concluded that the ethanol extract of telang flower toothpaste met the requirements of toothpaste.²⁵

The weakness of this research is that the ethanol extract toothpaste formula of telang flower has a limited spreading capacity and lacks foaming. Future research should replace the Streptococcus Mutans and Candida albicans tests with bacterial and fungal specimens taken directly from the mouth. Cytotoxicity tests also need to be carried out to determine the effect of ingredients on the condition of oral cavity cells.

Conclusion

Based on this research, it can be concluded that:

1. The ethanol extract of telang flower toothpaste has an antibacterial effect against Streptococcus mutans at concentrations of 50%, 60%, 65% and 70%. Concentrations of 50%, 60%, and 65% have antibacterial effectiveness in the weak category, while concentrations of 70% are in the medium category.

2. The ethanol extract of telang flower toothpaste has antifungal activity against Candida albicans at concentrations of 20%, 30%, 40%, and 50%. Concentrations of 20%, 30%, and 40% statistically have the same antifungal effectiveness.

3. The concentration of 50% ethanol extract of telang flowers as toothpaste can inhibit the growth of Streptococcus mutans and Candida albicans.

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Declaration of Interest

The authors report no conflict of interest.

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