

# Effectiveness of Herbal-based Toothpaste Extract of Wundu Watu (*Alpinia Monopleura*) against Plaque-Causing Bacteria and Dental Caries

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## Abstract

Zingiberaceae has high biological activity in treating various diseases. One of the largest genera of Zingiberaceae is *Alpinia*, found in tropical and subtropical regions—some of the newly discovered generations of Zingiberaceae such as the genus *Alpinia* and *Meistera*. The *Alpinia* genus found in Southeast Sulawesi is the Wundu Watu plant (*Alpinia monopleura*). *Alpinia monopleura* contains secondary metabolites such as lignin, flavonoids, and diterpenoids that have antibacterial activity. The purpose of this study was to prepare a herbal toothpaste formulation of wundu watu extract with a concentration of 30%. The research design used is the maceration method using 96% ethanol solvent, this research design uses experimental methods in the laboratory. The preparation formulation was evaluated by organoleptic test, homogeneity test, physical stability test, liking test, pH test, viscosity test, foam formation, bacterial activity, and a decrease in the plaque index causing dental caries. The results showed a statistical test of Paired Sample T-Test significant  $p < (0.05)$  toothpaste containing Wundu Watu extract (*Alpinia Monopleura*) has effectiveness against bacteria that cause plaque and dental caries at a concentration of 30%. The toothpaste formulation of Wundu Watu extract provides servant power against *Streptococcus mutans*.

**Keywords:** Wundu Watu; Zingiberaceae; *Streptococcus Mutans*; Dental Caries; Plaque.

## I. INTRODUCTION

Dental caries is a disease due to damage to the enamel layer which can spread to the dental nerve caused by bacterial activity in the mouth. Caries are caused by several factors, namely dental factors, microorganisms, substrates, and time. *Streptococcus mutans* are cariogenic bacterium that can quickly form acids from fermentable carbonic acid and cause the formation of caries. They can thrive in an acidic environment and can stick to the tooth surface because of their ability to produce polysaccharide cell extracts [1].

One of the dental health problems is dental caries. This condition occurs when food particles stick to the teeth and are fermented by bacteria in the mouth, causing plaque formation. As a result of dental caries, teeth are calcified so that they become porous and can cause holes or even fractures [2].

*Streptococcus mutans* is a type of acidogenic bacteria that produces acid that causes dental caries by causing decalcification, or loss of calcium, along with erosion of the tooth surface. Plaque buildup not only changes the appearance of teeth but can also result in the development of dental caries and gingivitis if plaque builds up persistently [3].

The main causes of dental caries are cariogenic foods, microorganisms, teeth, and the element of time. Carbohydrates, in particular, are cariogenic and play an important role in developing dental caries. One way to clean teeth is by using toothpaste. Apart from playing a role in maintaining oral hygiene, toothpaste selection can also prevent the proliferation of bacteria that cause dental caries, including *Streptococcus mutans* [3].

Colonization of *Streptococcus mutans* bacteria occurs after caries and if left unchecked, colonies will form in the teeth, so tooth decay is inevitable. However, if the formation of bacterial colonies can be avoided, the cause of tooth decay will be reduced or even not occur. One alternative to preventing and breeding bacteria and reducing the plaque index is using natural ingredients, which need to be developed to make healthy toothpaste preparations. One of the plants that can be utilized is the wundu watu (*Alpinia monopoleura*) extract [4].

Nowadays, many toothpastes contain fluoride, which is intended to help prevent tooth decay. However, studies have revealed that excessive use of fluoride over time can lead to irreversible enamel fluorosis, weak bones, porous teeth, premature aging, miscarriage, and potential cancer risk [5]. In this case, the researcher is interested in researching herbal toothpaste preparations from the plant extract of wundu watu extract (*Alpinia monopoleura*) which is abundant in southeast Sulawesi, Indonesia.

*Alpinia* has about 250 species distributed in tropical and subtropical regions and is the genus with the largest species in Zingiberaceae. Several pharmacological studies of *Alpinia* have been reported, and they have various bioactivities such as anticancer activity, antioxidant activity, antimicrobial activity, antiviral activity, anti-inflammatory activity, and nociceptive activity, antiparasitic activity and neuroprotective activity, antihypertensive activity and analgesic activity [6].

The wundu watu plant contains alkaloids, saponins, flavonoids, and steroids. Phenolic compounds and flavonoids have significant biological activities, such as anti-tumor ability, anti-inflammatory, and antioxidant properties that are beneficial to human health [7].

This plant is endemic to Southeast Sulawesi, its distribution is wide and abundant, and it is utilized by the community. In this context, the utilization of local plants in Southeast Sulawesi can be developed into something interesting in the scope of oral health to be further researched and explored as one of the innovations in the development of herbal ingredients, especially herbal products. In the form of toothpaste, it can prevent dental caries and reduce plaque index so that it can be utilized by people in Southeast Sulawesi to reduce the prevalence of dental caries and towards golden Indonesia.

## II. METHODOLOGY

The design of this research is a Quasy Experiment with a Randomized Pretest-Posttest Design. This

experimental research will be conducted in the microbiology laboratory, pharmacy laboratory, and oral health care laboratory. The minimum sample size is calculated based on the Federer formula. Samples of 30% concentration group, as many as 32 respondents using disclosing agent.

Tools used Glassware, Autoclave, Stirring rod, Maceration vessel, Blender, Bunsen, Petri dish, Porcelain cup, Cover glass, Hot plate, Incubator, ose needle, Laminar air flow, mortar and pestle, Analytical balance, Oven, Ruler, Microliter pipette, Drip pipette, pH-meter, Vacuum rotary evaporator, Horn spoon, Brookfield viscometer, Hallway, Toothpaste container, Oral diagnostic tool, toothbrush.

Bacterial strains derived from dental caries samples were streaked onto a special growth medium known as NA to facilitate the isolation of *Streptococcus mutans*. Afterwards, the petri dishes were placed in an environmentally controlled environment for 48 hours at 37°C, all under anaerobic conditions in wax bottles. This process was repeated several times until a completely pure culture was achieved [8].

Antibacterial test NA media was placed in a Petri dish and then allowed to stand until half hardened. A suspension of *Streptococcus mutans* bacteria was taken using a micropipette as much as 1000 µL. The suspension was streaked evenly on NA media. Next, pits (wells) were made using a cork drill on a Petri dish media that had a diameter of + 8 mm. each toothpaste formula wundu watu extract Petri dish, Then the dosage formulation of wundu watu extract was inserted + 0.5 grams. After that, the Petri dish was put into an incubator at 37 ° C for 24 hours. An inhibition zone will form around the well that is clean or free of bacteria. Calculated the diameter of the inhibition zone [3][5].

➤ Calculation of Extract Yield:

$$\text{Yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of extracted simplistic}} \times 100\%$$

Toothpaste Formulation 25 g of toothpaste was prepared. Na CMC concentration was determined using trial and error method. The formula that showed the maximum physical examination results and met the requirements was the best formula. Toothpaste ingredients and formulas can be seen in Table 1. Toothpaste formula Herbal Toothpaste Formulation Wundu Watu Rhizome Extract [9], [10], [11].

Table 1: Toothpaste Formula Herbal Toothpaste Formulation of Wundu Watu Rhizome Extract.

Name	Function	Concentration F I
Extract alpinia monopoleura	Active ingredients	30%
Na CMC	Paste base	2%
Sorbitol	Humectants	15%
Menthol	Scent	0,5%
Sodium benzoate	Preservatives	0,5%
Sodium lauryl sulfate	Surfactants	1%
Sodium saccharin	Sweetener	0,1%

Calcium carbonate	Abrasives	30%
Ethanol 96%	Solvent	3%
Aquadest ad	Solvent	ad 30 ml

### III. PHYSICAL EVALUATION OF PREPARATIONS

#### ➤ Organoleptic Test

Testing is done qualitatively by looking at the texture, aroma, and color of the toothpaste preparation. The preparation is said to be good if it meets the national standard for toothpaste based on SNI No. 12-3524-1995, namely soft, homogeneous, with no air bubbles, lumps, or separated particles [12].

#### ➤ Homogeneity Test

The test was carried out by applying toothpaste 3 times from 3 different parts, namely the top, middle, and bottom of the glass object, and then making visual observations of whether the preparation was homogeneous or not. The preparation is said to be good if it meets the toothpaste quality standards based on SNI No.12- 3524-1995, namely homogeneous, with no air bubbles, clots, and separated particles [13].

#### ➤ pH test

The test was carried out by measuring the preparation directly using a pH meter 3 times and then taking the average. The pH of the preparation is said to be good if it meets the quality standards of toothpaste based on SNI No.12-3524-1995, which is 4.5 - 10.5 [14].

#### ➤ Spreadability Test

A sample weighing 1 g was placed between 2 glasses. Then a load of 50 g was placed on the glass and the diameter was measured before and after 1 minute of loading. A load of 50 g was added, and the wait was 1 minute. Then the diameter was measured again. Diameter measurements were taken at 3 different points and the average was taken. A good toothpaste has a spreadability range by market toothpaste preparations, which is 2.61 - 5.32 cm [15].

#### ➤ Viscosity Test

Aims to determine how viscous the toothpaste produced is, where the viscosity value states the strength of a liquid to flow—different concentrations of Na.CMC

affects the viscosity value of the three toothpaste formulas made. The higher the concentration of Na.CMC, the greater the viscosity value. Na.CMC works through the development process by trapping or binding the existing water so that the water molecules will be close to each other and an attractive force occurs. In a scale below 10, the speed (rpm) of the spindle must be increased, and if the scale is more than 100, the spindle is replaced with a larger spindle number [15].

#### ➤ Foam Height Test

The foam formation test was carried out by making a 1% solution of 30% concentration of wundu watu extract toothpaste in water. Then put into a measuring cup with a lid, then shake for 1 minute. Then measure the height of the foam formed [16].

#### ➤ Stability Testing

The cycle testing method is used to check stability. Store toothpaste at 4°C for 24 hours and gel toothpaste at 40°C for 24 hours. The test was conducted for 3 cycles, and the physical changes in the formulation were observed from the beginning to the end of each cycle. Observations included sensory testing, viscosity testing, and pH testing using the cycling test method [16].

### IV. RESULT AND DISCUSSION

The maceration process is carried out by maceration, namely, the pulp is macerated again with the same ratio of sample and solvent until the extract is concentrated. The purpose of the repeated maceration process is to get more filtrate with greater active compounds. In this study, maceration was carried out 2 times which was marked by the filtrate obtained was the same color as the solvent color, meaning that no more metabolites could be drawn by the solvent (100% extraction). The results of maceration were then filtered and obtained filtrate which was then evaporated to remove the solvent in the filtrate using a Rotary Vacuum Evaporator tool and received a thick and concentrated extract [10], [11].

Table 2: Average Results of Organoleptic Test

Formula	Parameters	Organoleptic Test			
		Week to			
		I	II	III	IV
Concentration 30%	Shape	Semi-solid	Semi-solid	Semi-solid	Semi-solid
	Color	brown	brown	brown	brown
	Aroma	typical active substance	typical active substance	typical active substance	typical active substance

The results of the organoleptic test showed that the concentration of toothpaste extract wundu watu NA. CMC does not affect the physical preparation of toothpaste produced. This is shown in the color, aroma, and texture of

the formula is the same. Wundu watu extract which is dark brownish in color does not affect the final result of toothpaste preparation, besides that the toothpaste formula also has a minty aroma caused by the addition of OMP1

drops with a soft paste texture. The results of this organoleptic test prove that the wundu watu extract toothpaste made is stable, indicated by no change in color,

aroma, and texture during 4 weeks of storage at room temperature.

Table 3: Average Results of Homogeneity Test, pH Test, Spreadability Test, Foam Height Test

Homogeneity Test				
Week to				
Formula	I	II	III	IV
Concentration 30%	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH Test				
Week to				
Formula	I	II	III	IV
Concentration 30%	7,7	7,6	7,2	7,68
Spreadability Test				
Week to				
Formula	I	II	III	IV
Concentration 30%	3,6	3,7	3,5	3,6
Foam Height Test				
Week to				
Formula	I	II	III	IV
Concentration 30%	3,9	3,7	3,6	3,7

Homogeneity testing of 30% concentration herbal toothpaste did not show any coarse particles resulting in a homogeneous toothpaste preparation. This shows that all additives and extracts as active substances used in making toothpaste are evenly mixed. Wundu watu extract toothpaste has good physical properties in various concentrations with homogeneity remaining good for 4 weeks of storage at room temperature [17].

pH testing during 4 weeks of storage, the pH value of 30% concentration toothpaste is still within the pH requirements for toothpaste, which ranges from 4.5 to 10.5. Testing the pH of wundu watu extract toothpaste shows that the higher the concentration of extract in toothpaste, the lower the pH of wundu watu extract toothpaste. This is because the chemical compounds contained in wundu watu extract such as flavonoids have an acidic pH so that wundu watu extract tends to have an acidic pH. The pH value of wundu watu extract toothpaste during 4 weeks of storage has changed pH, but it is still within the standard requirements of toothpaste pH based on SNI. Changes in pH value are caused by environmental factors such as changes in temperature because storage is carried out at room temperature and storage containers that are less impermeable so that air can enter [18], [19].

The spreadability test is intended to determine the spreadability of toothpaste when applied to a toothbrush. The ability to spread is an important characteristic because it affects the transfer of active ingredients to the target with the right dose, ease of use, the pressure needed to get out of the package, and acceptance by consumers. Based on the results of the spreadability test in table 3, the formula is in

the range of 3.5 -3.6 cm. So that the results are still categorized as relatively safe because the spreadability obtained is in accordance with the requirements for good spreadability for toothpaste preparations, namely 2.61 - 5.32 cm, which shows a semi-solid consistency that is very comfortable in use [5], [19].

Foam formation tests show that the higher the concentration of wundu watu extract used in toothpaste, the less foam is produced and the lower the concentration of extract used, the more foam is produced, due to the concentration of sodium lauryl sulfate used as a surfactant remains the same, namely 1% so that the amount of natrum lauryl sulfate is not enough to emulsify the extract. The foam height test aims to see the amount of foam formed from toothpaste preparations due to the presence of foam-forming ingredients. Based on Table 3 test results, wundu watu extract toothpaste forms foam when used [20].

Table 4: Average Results of Viscosity Test

Formula	Average viscosity test
	cps
Concentration 30%	22000

Paste viscosity test, from the results of the study obtained toothpaste has a viscosity value between 22,000 - 23,000 (Cps) on toothpaste extract wundu watu concentration of 30%, these results are in accordance with SNI (12-3524-1995) the viscosity value of toothpaste ranges from 20,000-50,000 (Cps). The viscosity test aims to determine how thick the toothpaste produced is, where the viscosity value states the strength of a liquid to flow [20], [21].

Table 5: Average Results of Favorability Test

Number of Panelists		
Parameters	Concentration 30%	%
Color	6	20%
Aroma	10	33%
Tekstur	14	47%

Evaluation of the favorability test is important in toothpaste preparations because it will affect the value of public acceptance. The results of the liking test in the form of aroma, color, and consistency of Wundu Watu extract toothpaste preparations for 30 respondents had the highest value of 47%. It can be concluded that panelists like the texture of herbal toothpaste from Wundu Watu extract.

Table 6: Average Results of Stability Test (Cycling Tets)

Test Parameters	Formula	Before Cycling Test	After Cycling Tets
<b>Organoleptic test</b>	Concentration 30%	Semi-solid, brown in color, characteristic aroma of active substances	Semi-solid, brown in color, characteristic aroma of active substances
<b>Homogeneity test</b>	Concentration 30%	Homogeneous	Homogeneous
<b>pH Test</b>	Concentration 30%	7,67	7,87
<b>Spreadability Test</b>	Concentration 30%	3,5	3,7
<b>Foam Height Test</b>	Concentration 30%	4,5	4,9

Stability test The Cycling test method was used to check the stability. The toothpaste was stored for 24 hours at 4°C, and then the gel toothpaste was stored for 24 hours at 40°C. The test was conducted in 3 cycles and during each cycle the physical changes in the preparation were observed from the beginning to the end. Observations included an organoleptic test, viscosity test, and pH test [20].

#### V. INHIBITION TEST OF *STREPTOCOCCUS MUTANS* BACTERIA CAUSING DENTAL CARIES

Bacteria in the mouth are linked to oral health and tooth decay. Dental caries is an oral disease whose prevalence is still high in Indonesia. Dental caries is an infectious disease of the hard tissues of the teeth (namely enamel, dentin, and cementum). Dental caries is caused by the activity of microorganisms on fermented carbohydrates. Dental caries is characterized by the demineralization of dental hard tissues followed by the breakdown of organic matter. This can lead to bacterial invasion and damage to the pulp tissue and the spread of infection to the periapical tissues. The main factor in the occurrence of dental caries is the presence of *Streptococcus mutans* colonies on the tooth surface biofilm. Biofilm formation depends on carbohydrates and some enzymes produced by *Streptococcus mutans*. This increases the acidity of the oral environment and causes tooth demineralization [3],[22].

Table 7: The results of measuring the diameter of the inhibition zone of wundu watu leaf extract against *Streptococcus mutans* bacteria.

Treatment	Inhibition Zone Diameter (mm)			Average (mm)	Inhibition Response
	I	II	III		
Concentrasion FII A 30%	12,17	16,05	12,3	12,9 mm	Strong
Concentrasion FII B 30%	14,8	15,75	10,35	11,99 mm	Strong
Concentrasion FII C 30%	19,95	17,9	15,1	16,6 mm	Strong

In table 7. above, the inhibition zone test was carried out three times during the preparation. The results showed that the toothpaste containing wundu water leaf extract had a positive effect on inhibiting the growth of *Streptococcus mutans* bacteria. The inhibition zone was included in the strong category with the highest average value, namely the concentration of FII C 30% in the third replication.

The antibacterial potential test of herbal toothpaste containing Wundu watu leaf extract was carried out by measuring the inhibition zone produced after the culture medium containing *Streptococcus mutans* was incubated in an incubator at 370°C for 24 hours. Bacterial growth is observed away from the wells that have been made so that an inhibition zone is formed in the wells that are dripped with wundu watu leaf extract. A caliper is used to measure the horizontal, vertical, and diagonal diameters in millimeters (mm) of the inhibition zone formed around the hole [7], [4].

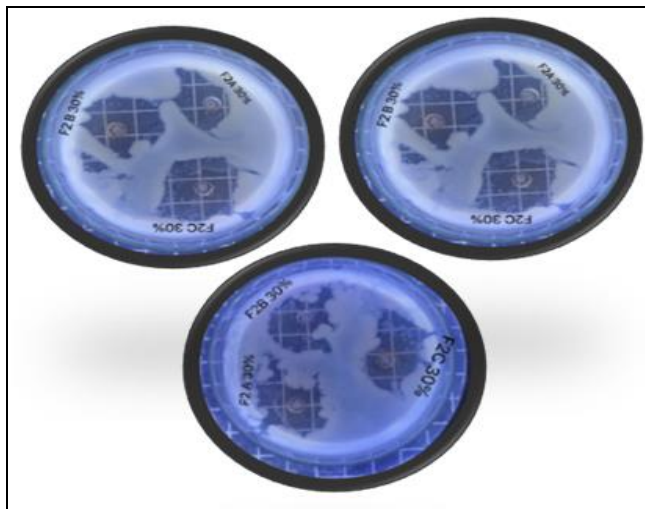


Fig 1: Formulation Repeat Testing of Wundu Watu Toothpaste on Streptococcus Mutans A, B, C

Table 8: Effectiveness of Herbal-based Toothpaste Extract of Wundu Watu (*Alpinia Monopleura*) against Plaque-Causing Bacteria and Dental Caries 30% Concentration

Variables	Mean $\pm$ SD Pre - Test	Mean $\pm$ SD Post - Test	Sig.
30% Concentration	2,800 $\pm$ 7694	1,983 $\pm$ 7055	0,002

Table 8 above presents, that the results of the Paired Sample T-Test test obtained a p-value of  $p (<0.05)$ , which means that toothpaste containing Wundu Watu extract (*Alpinia Monopleura*) has effectiveness against bacteria that cause plaque and dental caries at a concentration of 30%.

Zingiberaceae has high biological activity to treat various diseases. One of the largest genus of Zingiberaceae is *Alpinia* which is widely found in tropical and subtropical regions—some of the newly discovered generations of Zingiberaceae such as the genus *Alpinia* and *Meistera*. One of the *Alpinia* genus species found in Southeast Sulawesi is the Wundu Watu plant (*Alpinia monopleura*). Previous research suggests that *Alpinia monopleura* contains secondary metabolites such as lignin, flavonoids, diterpenoids, phenylpropanoids, and essential oils and has antioxidant and antibacterial activity [4], [23].

The main active ingredients in the Wundu watu plant, such as flavonoids, play an important role in its ability to reduce plaque. In addition, flavonoids have antimicrobial properties that inhibit the growth of bacteria and other microorganisms involved in plaque formation on teeth and gums. These compounds help reduce gum inflammation and fight infection-causing bacteria [6].

Dental plaque is a soft deposit that is tightly attached to the surface of the teeth, consisting of microorganisms that multiply in an intercellular matrix if a person neglects dental and oral hygiene. The role of toothpaste is to reduce plaque formation, strengthen teeth against caries, clean and polish the tooth surface, eliminate or reduce bad breath, and provide a fresh taste in the mouth. Caries or cavities are a disease due to damage to the enamel layer which can spread to the dental nerve caused by bacterial activity in the mouth [24].

Caries is caused by several factors, namely dental factors, microorganisms, substrates, and time *Streptococcus mutans* is a cariogenic bacterium that can quickly form acids from carbonic acid which can be fermented and cause the formation of caries and can thrive in an acidic environment and can stick to the tooth surface because of its ability to produce polysaccharide cell extracts [25].

Plaque and dental caries caused by *Streptococcus mutans* bacteria can be prevented through the use of chemical compounds and mechanical actions, such as brushing teeth [26]. One effective solution is the use of toothpaste made from herbs with Wundu Watu (*Alpinia monopleura*) extract. Research shows that this extract is able to reduce the dental plaque index and has antibacterial properties against *Streptococcus mutans*, the main cause of dental caries. This effectiveness comes from the plant content of Wundu Watu, which has antioxidant and antibacterial activities.

*Alpinia Monopleura* essential oil is an oil from the terpenoid group, the mechanism of terpenoids as antibacterial is a sensor with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong polymer bond resulting in damage to the porin. Damage to porins, which are gateway compounds in and out, will reduce the permeability of the bacterial cell wall which will result in a lack of nutrients in the bacterial cell, resulting in inhibited bacterial growth [6].

Flavonoids have properties and structures that contribute significantly to antibacterial activity. Various studies have shown that these chemical compounds have antimicrobial effects. Flavonoids can inhibit the activity of cyclooxygenase and lipooxygenase enzymes, as well as reduce the accumulation of leukocytes in certain locations, making them potential antibacterial agents. In addition, flavonoids can cause lysis and inhibit bacterial cell wall formation. This mechanism allows flavonoids to kill or inhibit bacterial growth. Flavonoids are also known to damage bacterial cell membranes by interacting with lipopolysaccharides, which then damage the phosphate groups of the membrane. This leads to phospholipid degradation, membrane leakage, and ultimately, bacterial death [4].

Dental plaque is a mixture of bacteria, food debris, and saliva that forms naturally in the mouth. The bacteria in plaque produce acids that can damage tooth enamel, leading to cavities. The process of plaque formation begins when bacteria in the mouth attach to the smooth surfaces of the teeth, multiply, and form a layer of plaque. If not cleaned regularly, plaque will continue to thicken. In the early stages, plaque is pale yellow, but if left to harden, the color will change to dark yellow or brown. In addition, dental plaque can also be the cause of bad breath [27].

Dental plaque can also be the cause of bad breath. Plaque is a soft layer formed due to bacterial growth that develops in the matrix, especially when oral hygiene is not maintained properly. Therefore, it is important to brush your teeth regularly using toothpaste that has antibacterial activity [14], such as toothpaste made from wundu watu (*Alpinia monopleura*) extract.





Fig 2: Wundu Watu (*Alpinia Monopleura*) Extract Toothpaste 30% Concentration

## VI. CONCLUSION

Based on the results obtained, it can be concluded that the toothpaste of Wundu Watu extract (*Alpinia Monopleura*) at a concentration of 30% has antibacterial activity against *Streptococcus Mutans*, the cause of dental plaque and caries, which produces an inhibition diameter of 16.6 mm. The results of physical-chemical quality testing showed that all kinds of toothpaste of Wundu Watu (*Alpinia Monopleura*) extract met the physical-chemical quality of toothpaste.

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