

## Antibacterial Activity Test of Gel Toothpaste from Cinnamon Leaf Essential Oil (*Cinnamomum burmanni* Blume.) Against *Staphylococcus aureus* Bacteria

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### ABSTRACT

Cinnamon leaf essential oil can inhibit the growth of *Staphylococcus aureus* bacteria because it contains cinnamaldehyde and eugenol compounds, which function as antibacterials. *Staphylococcus aureus* is a bacteria involved in various infections in the mouth and human body. This research aims to formulate cinnamon leaf essential oil into a gel toothpaste preparation by differentiating the concentration of cinnamon leaf essential oil as an active ingredient and testing its antibacterial activity against *Staphylococcus aureus* bacteria. The concentration of active ingredients in formula I is 0.5%, in formula II, 1%, and in formula III, 1.5%. Evaluation of the physical properties of gel toothpaste preparations includes organoleptic, homogeneity, spreadability, pH, viscosity, foam ability, stickiness, and antibacterial activity test against *S. aureus* using the well diffusion method. The results of the organoleptic and homogeneity evaluations were analyzed descriptively. In contrast, the spreadability, pH, viscosity, foam height, adhesion, stability, and antibacterial activity tests were analyzed statistically using One-way ANOVA. Organoleptic test results showed yellowish white (FI), light yellow (FII), and yellow (FIII); all three formulas had a characteristic smell of essential oils and were semisolid and homogeneous. The spreadability test results ranged from 5-5.90 cm, pH value from 5.05-5.47, viscosity value from 31,980-43,900 Cp, foam ability from 9-14 mm and adhesion value from 05.84-08.83 seconds. The antibacterial activity test results for formulas I and II formed an inhibition zone in the strong category, while formula III formed an inhibition zone in the powerful category.

## INTRODUCTION

Dental and oral health problems can seriously disrupt bodily health, so these problems must receive the best possible attention (Putra et al., 2017). Toothpaste is an ingredient commonly used daily by the general public. The use of toothpaste is significant in achieving the goal of minimizing dental and oral hygiene. Toothpaste is an ingredient that helps to clean teeth from food debris mechanically, then removes plaque on teeth and eliminates unpleasant odours in the mouth (Saputro et al., 2020).

Toothpaste is a semisolid preparation used to clean and protect teeth from damage caused by oral bacteria. It has seven essential claims, including removing plaque and stains and leaving the mouth feeling clean. There are two types of toothpaste on the market: paste formulations and transparent gel formulations. Transparent gel toothpaste is more comfortable to use because it is odourless, easily removed with water, and contains more moisturizer, maintaining the stability of the gel toothpaste preparation (Ayuningtyas et al., 2022; Lestari et al., 2022).

Cinnamon leaves can be processed into essential oil with economic value and health benefits. Cinnamon leaves contain around 0.5-0.7% cinnamaldehyde, 70-95% eugenol, and cinnamylacetate 3-4% (Khasanah et al., 2014). Eugenol is the most significant active compound found in cinnamon leaf essential oil, and its function is to be an antibacterial and antioxidant (Utami et al., 2018).

One of the most common microorganisms in the oral cavity is *Staphylococcus aureus*. *Staphylococcus aureus* is a bacteria involved in various infections in the mouth and human body. This bacteria is also one of the leading causes of the formation of dental caries agents (Jesumirhewe & Ayomide Olayinka Ariyo, 2023). *Staphylococcus aureus* is the second largest bacteria that can cause inflammation in the oral cavity. Examples of inflammation in the oral cavity caused by *Staphylococcus aureus* are *parotitis*, *angular cheilitis*, and *Djais periodontal abscess*. *Staphylococcus aureus* is essential in causing periodontal disease by forming biofilms on dental plaque and exacerbating periodontal disease by releasing various pathogenic factors (Amalia et al., 2023).

This research aims to formulate a gel toothpaste using cinnamon leaf essential oil and evaluate its antibacterial activities.

## METHODS

### Material

The material used for the research was cinnamon leaf essential oil obtained from the

Karya Forest Farmers Group, a business supported by the Unit 1 Production Forest Management Unit, Kerinci Regency, Jambi. Na-CMC; glycerin; sodium lauryl sulfate; peppermint oil (*Pharmaceutical Grade of other mints*) was obtained from P.T. Kisbiokim Medika Laboratori Padang; distilled water from Medika Laborta; culture of *Staphylococcus aureus* bacteria; MHA and NA media; NaCl 0.9%; Mc—Farland standard 0.5.

### Equipment

The equipment used in this research was a digital scale (Mettler Toledo-Switzerland), dropper pipette, beaker glass (Pyrex-Japan), measuring cup (Pyrex-Japan), Erlenmeyer (Pyrex-Japan), petri dish (Pyrex-Japan), stir stick (Pyrex-Japan), Hot Plate (Torrey Pines Scientific-United States), watch glass (Pyrex-Japan), mortar and stamper (Onehealth, Indonesia), spatula, parchment paper, slide, pH meter (Hanna Instrument-Indonesia), viscometer (Brookfield DV2T-United States), micropipette, tube needle, tweezers, laminar air flow, aluminium foil, sterile cotton and gel pot.

### Plant Determination

Plant determination was carried out to ensure that the samples used in this study were the correct species, namely *Cinnamomum burmanni*. This plant was determined at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Biology Department, FMIPA UNPAD.

**Table 1. The formula of Cinnamomum leaf essential oil gel toothpaste**

Material	Formula (%)		
	I	II	III
Cinnamomum essential oil	0.5	1	1.5
Na-CMC	1.5	1.5	1.5
Glycerin	20	20	20
Methyl Paraben	0.3	0.3	0.3
Xylitol	1	1	1
Sodium Lauryl Sulphate	0.3	0.3	0.3
Peppermint oil	0.3	0.3	0.3
Aquadest ad	100	100	100

## Evaluation of Characteristics of Cinnamon Leaf Essential Oil

### Specific Gravity

The specific gravity of essential oils is determined using a pycnometer. Pycnometer with a capacity of 25 ml that has been cleaned and dried, then filled with Cinnamomum leaf essential oil, closed, and weighed again. The specific gravity obtained the mass value by subtracting the weight of the pycnometer containing Cinnamomum leaf essential oil from the weight of the empty pycnometer (Anggraini et al., 2018).

$$\text{Specific gravity} = \frac{\text{Mass}}{\text{Volume}} \dots\dots\dots(1)$$

### Colour test

Based on SNI 06-3734:2006, the colour analysis uses UV-Vis spectrophotometry and visual (subjective) colour perception using the sense of sight. Measure 3 ml of essential oil using a dropper pipette and then put it into a test tube to avoid the formation of air bubbles. The test tube containing the cinnamon leaf oil sample is leaned against white cardboard. Next, observe the colour directly with the eye. The colour perception distance of cinnamon leaf essential oil is 30 cm (Badan Standardisasi Nasional, 2006).

### Optical Rotation

The method is based on SNI 06-3734:2006, measuring the angle of the plane of polarised light. Turn on the light source and wait until a full glow is produced. The polarimeter macrometer was rotated and observed the formation of a light-dark circle on the right and left sides, and then the macrometer was adjusted until a bright circle was obtained between the two light-dark circles that existed when turning it to the right and left. The macrometer is placed in a colourful circle, and then the dextro optical rotation value (+) or (-) of the sample is read on a scale provided on another lens. Then, the results of monitoring the optical rotation values read on the polarimeter scale are recorded. Optical rotation is expressed in degrees of circumference to approximately  $0.01^\circ$ . Dextro optical rotation must be given a positive sign (+), and levo optical rotation must be given a negative sign (-) (Badan Standardisasi Nasional, 2006).

## Formulation Gel Toothpaste

The formulation of toothpaste gel is shown in **Table 1**. Na-CMC was dispersed in heated distilled water (Mixture A). Then, sodium lauryl sulfate is added to the beaker glass with distilled water (Mixture B). Next, methylparaben is added with glycerin (Mixture C). Then, add mixture C to mixture A and stir at medium stirring speed. Add xylitol, stir until homogeneous, add mixture B, and stir gently until homogeneous so it does not foam. After that, add peppermint oil and stir until homogeneous. After the preparation is homogeneous, add cinnamon leaf essential oil, stir until homogeneous, and add 100 ml distilled water.

### Test the Physical Properties of Gel Toothpaste

#### Organoleptic Test

During the manufacture of gel toothpaste, visual observations were made of the gel paste preparation regarding the colour, aroma, and shape. The gel is usually consistent in colour and semisolid (Warnida et al., 2016).

#### Homogeneity Test

This homogeneity test is carried out by placing a small sample of the gel toothpaste on a piece of glass or other material suitable for homogeneity testing (Hidayati et al., 2022). Then, whether there are coarse particles or inhomogeneities in the preparation is observed. A preparation with good homogeneity must show results that there are no coarse grains and no air bubbles or lumps during this homogeneity test (Warnida et al., 2016).

#### Spreadability Test

The gel toothpaste was weighed 0.5 g, the sample was placed on a petri dish, weights weighing 50 and 100 g were on top of the petri dish, and then waited 1 minute for each addition of material (Egam et al., 2023). With each addition of load material, the distribution diameter is then measured, but when the diameter is measured and the results obtained are constant, the load material is stopped (Warnida et al., 2016).

#### pH evaluation

The pH of the gel toothpaste preparation was measured using a pH meter. The resulting pH value meets the quality requirements of SNI 12-

3524-1995, namely 4.5-10.5 (Warnida et al., 2016).

### Viscosity Test

The gel toothpaste preparation of 100 mL was taken and then placed into a 250 ml beaker, after which the viscosity was measured with an appropriate spindle using a viscometer (Nitasari et al., 2022). According to the quality requirements in SNI 12-3524-1995, the viscosity value is 20,000 to 50,000 Cp (Achsia et al., 2021).

### The Test of Foam ability

Mix 1 gram of toothpaste with aqua distillate, then put it in a 25 mL measuring cup. Next, shake the measuring cup containing the sample and aqua distillation for 20 seconds by turning the measuring cup evenly (Gunawan & Rahayu, 2021). Observe the height of the foam that forms after leaving it for 1 minute. Then, measure the height of the foam using a ruler (Warnida et al., 2016).

### Adhesion Test

Take 0.5 grams of the preparation, apply it to a glass object whose area has been determined (2x2 cm), place another glass object, and press it with a weight of 1 kg for 5 minutes (Fernandes et al., 2016). The equipment is installed on the test equipment, a load weighing 80 grams is released, and the time is recorded until the two glass objects are released (Ayuningtyas et al., 2021)

### Stability Test

Accelerated stability testing is used by centrifugation. This test is carried out by inserting the gel toothpaste preparation into a centrifuge tube and a sample centrifuge. This test was carried out at a speed of 5000 rpm for 30 minutes (Rini et al., 2022).

### Antibacterial Activity Test

The antibacterial activity test was carried out using the Well Diffusion method. The pure culture of *Staphylococcus aureus* bacteria was inoculated evenly by dipping the tip of a sterile cotton bud in a liquid nutrient medium and then scratching it on the surface of the NA plate medium until it was even. Then, a well is made using a spore with a diameter of 6 mm. For the negative control, use a gel toothpaste base without cinnamon leaf essential oil, and for the positive control, use the "P" brand gel toothpaste. Drop the test sample into the well. It

was incubated at a temperature of 37 °C for 1x24 hours. After 24 hours, a clear zone will form around the well, indicating that the test compound can inhibit bacteria. After that, the diameter of the inhibition zone was measured using a calliper (Magvirah et al., 2020).

### Data analysis

The data is usually distributed, and the variance is homogeneous. Therefore, the quantitative analysis was carried out using the One-way ANOVA method with a confidence level of 95%, and the Duncan test was continued.

## RESULT AND DISCUSSION

Cinnamon plant samples determined include all parts of the plant. The determination results (No.59/HB/01/2024) showed that the plant used for this research sample was a cinnamon plant (*Cinnamomum burmanni* Blume.), which belongs to the *Lauraceae* family. Thus, the species of the samples used by researchers corresponds to the desired species.



**Figure 1. The result of the antibacterial activity of Cinnamomum leaf essential oil gel toothpaste**

The SNI 06-3734-2006 states that the specific gravity value for cinnamon leaf essential oil is 1.008-1.030. The results of particular gravity testing on cinnamon leaf essential oil showed a value of 1.021 g/ml, and the results obtained entered the range of specific gravity values for cinnamon leaf essential oil (Utami et al., 2018).

Based on the research results that have been carried out and compared with the quality standards for essential oils that comply with SNI

06-3734-2006, the colour of cinnamon leaf essential oil is light yellow-light brown (Jailani et al., 2018). This shows that the essential oil obtained is following predetermined standards. The yellow colour produced in this oil is because the leaves used are fresh and old.

preparation and showed that the mechanism for mixing the ingredients used for each formula was appropriate. A declared homogeneous preparation can be characterized by the absence of coarse particles and no lumps, the gel toothpaste being mixed evenly, and the uniform

**Table 2. The result of Cinnamomum leaf essential oil gel toothpaste**

Formula	Formula		
	I	II	III
<b>Spread test (cm)</b>	5.28±0.41	5.42± 0.47	5.91±0.06
<b>pH test</b>	5.31 ± 0.17	5.18 ±0.17	5.17±0.14
<b>Viscosity (cPs)</b>	33100±1364.69	35780 ±2057.86	36573.3 ±6389.25
<b>Foam ability (cm)</b>	11.66±2.08	10.00±1.00	10.33±1.53
<b>Adhesion test (second)</b>	8.21±0.59	6.61±0.76	7.15±0.70

The results obtained from the optical rotation test were -1.950°. The minimum standard for the optical rotation value of cinnamon leaf essential oil, according to SNI 06-3734-2006, is -5.0° (Utami et al., 2018). So, the results of the optical rotation show that it meets the standards.

Organoleptic testing aims to determine the appearance of cinnamon leaf essential oil gel toothpaste. This observation uses the five senses to describe smell, colour, and dosage form (Yauri, 2017). All formulas made have a distinctive smell, namely cinnamon leaf essential oil. The colour resulting from the observation is yellow. All the formulas have a semisolid dosage form.

The results of the homogeneity test showed that the three formulas produced were homogeneous; this was indicated by the absence of coarse particles in the gel toothpaste

colour visible in the preparation.

The spreadability results of the gel toothpaste preparation ranged from 5.28-5.91 cm (**Table 2**). The requirement for good spreadability of a gel toothpaste preparation is 5-7 cm (Warnida et al., 2016). So, the results show that the preparation has met the spreadability test standards. The diameter test results indicate that the greater the diameter of the spreading power, the greater the surface area the toothpaste preparation can cover (Lestari et al., 2022).

The pH results of the gel toothpaste preparation ranged from 5.17 to 5.31 (**Table 2**). The pH requirement for an excellent gel toothpaste, according to SNI 12-3524-1995, is 4.5-10.5 (Warnida et al., 2016). So, the results show that the preparation has met the pH test standards for toothpaste preparations. pH will

**Table 3. Result of the inhibition diameter zone**

Formula	Inhibition diameter zone ± SD	Category of antibacterial activity
F I	15.60±2.26 <sup>ab</sup>	Strong
F II	18.29±3.897 <sup>b</sup>	Strong
F III	23.75±1.65 <sup>c</sup>	Powerful
Control (+)	18.70±3.97 <sup>ab</sup>	Strong
Control (-)	12.74±1.38 <sup>a</sup>	Strong

be related to the effectiveness and comfort of the active substance when the preparation is used. If the resulting pH is acidic, it will irritate after use.

The viscosity results of the gel toothpaste preparation ranged from 33,100-36,573.3 cPs (**Table 2**). The viscosity requirement of an excellent gel toothpaste, according to SNI 12-3525-1995, is 20,000-50,000 cPs (Lestari et al., 2022). So, the results show that the preparation has met good viscosity test standards for toothpaste preparations. The viscosity of a preparation can be influenced by several factors, one of which is mixing or stirring during the preparation process (Hudairiah et al., 2021).

The resulting foam ability from gel toothpaste preparations ranges from 10-11.66 mm (**Table 2**). The maximum value of the foam height test is 15 mm (Marlina & Rosalini, 2017). So, the results show that the preparation has met the test standards for good foam height from toothpaste preparations.

The adhesion test results of the gel toothpaste preparation ranged from 6.61 to 8.21 (**Table 2**). The adhesion test does not have definite parameters, but it is stated that the results of this adhesion test are at least 4 seconds (Ayuningtyas et al., 2021). So, the results from the table above show that the adhesion strength value is not less than 4 seconds, and it can be interpreted that the foam height results have met the standard.

Results stability show that FI, FII, and FIII with three replications are physically stable. No phase separation, such as differences in colour and sediment in the centrifuge tube, characterizes this. The effect of this centrifugal force is equivalent to the gravitational force received by the test preparation for one year (Suryani et al., 2019).

Test results of antibacterial activity produce an inhibition diameter zone. However, negative control results produce an inhibitory zone, whereas, in general, this negative control does not create an inhibitory zone. The inhibition zone is formed because the ingredients used in the base consist of sodium lauryl sulfate, methylparaben, and peppermint oil (Chumpitazi et al., 2018; Dhurhania, 2019;

Mayasari & Kusuma, 2023). These three ingredients are effective as antibacterials, so the negative control results produce an inhibition zone. However, the inhibition zone value produced in the negative control was not more significant than the FI, FII, and FIII values (**Table 3** and **Figure 1**). FIII is the best performer due to its significantly greater inhibitory power than other groups. FIII demonstrates a powerful inhibition of bacteria.

## CONCLUSIONS

Formulating gel toothpaste with cinnamon leaf essential oil concentrations at FI 0.5%, FII 1% and FIII 1.5% obtained physical properties following standards. The gel toothpaste formulation with cinnamon leaf essential oil concentrations at FI, FII, and FIII has antibacterial activity against *Staphylococcus aureus* bacteria. In FI and FII, the resulting inhibition zone is in a strong category; the resulting diameter is 15.60 mm and 18.29 mm, and in F3, the resulting inhibition zone is 23.75mm; this inhibition zone category is powerful.

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## AUTHORS' CONTRIBUTIONS

Indri Maharini did the conception and study design; all authors provided administrative technical/logistic support and conducted data collection and assembly; data analysis and interpretation were made by Chintya Dwiyunia Putri, Elisma, Indri Maharini; Indri Maharini and Elisma did a critical revision of the article for important intellectual content; all authors contributed to article drafting and approved final version.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

## ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, double publication, etc) have been completely observed by the authors.

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