

Clove Oil (*Syzygium aromaticum*) Edible Film Formulation and Antibacterial Activity Test against *Streptococcus mutans*

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Abstract

Clove oil contains eugenol as an antibacterial. Meanwhile, products containing clove oil have been widely used as toothpaste and mouthwash. In this study, clove oil was formulated in the form of edible film because it is practical, easy to use, and could be used without water like other oral hygiene preparations. The edible film is a thin layer film made of consumable materials used as a carrier of antibacterial compounds. Clove oil edible film was then formulated with clove oil concentrations of 1%, 1.5%, and 2% and determined for its antimicrobial activity against *Streptococcus mutans*. Clove oil edible film preparations were evaluated under their physical properties, including friability, drying shrinkage, pH, thickness, and swelling ability. Antibacterial activity testing of clove oil edible film was conducted, employing the blood agar diffusion method against *Streptococcus mutans*. The physical evaluation of the clove oil edible film showed almost the same physical properties as the comparison (GF). Clove oil edible film test results revealed the greatest inhibition at F₁ of 18.6 mm ± 0.577, F₂ of 22.3 mm ± 2.081, and F₃ of 25.3 mm ± 1.527. According to David and Stout, the inhibition activity of bacteria on F₃ was categorized as a very strong group inhibition response. In addition, ANOVA test analysis results uncovered that the concentration of clove oil affected the inhibition of the *Streptococcus mutans* bacteria with a significance value of 0.000 (p < 0.05). Also, Duncan's test exhibited that each concentration of clove oil had a significant difference in the inhibition of *Streptococcus mutans* bacteria.

Keywords: *antibacterial*, edible film, eugenol, agar diffusion, *clove oil*

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INTRODUCTION

Clove oil has secondary metabolites in the form of essential oil with the highest eugenol content so that it has biological activity as an antiseptic and analgesia in the treatment of teeth and mouth,

antifungal, antibacterial, antioxidant, and anticarcinogenic¹. Clove oil can be isolated from the leaves (1-4%), stems (5-10%), and clove flowers (10-20%). Previous research was about the bactericidal effect of clove oil on *Streptococcus mutans* and *Streptococcus pyogenes* bacteria, which

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were tested on mouthwash preparations at 0.5%, 0.75%, and 1% clove oil concentrations. It was shown that there was no minimum bactericidal concentration (MBC) at a concentration of 0.5%, so it was concluded that it could inhibit bacterial growth (bacteriostatic). Meanwhile, at concentrations of 0.75% and 1%, clove oil had an MBC value, so that it was denoted to have bacteria-killing activity (bactericidal)².

Moreover, oral hygiene preparations that have been widely circulated are in the form of toothpaste, mouthwash, and edible film as mouth fresheners containing menthol. The edible film is a thin layer made of safe-for-consumption materials and used as a wrapper to prevent the process of fat oxidation, organoleptic changes, microbial growth, or absorption of moisture. In addition, edible films function as a barrier against the mass transfer of solutes, as a carrier for additives, and improving the handling of food³. The formation of edible films can also prevent the evaporation of eugenol in clove oil since one of its functions is as a barrier to mass transfer. Many studies on edible films have been conducted, including the use of betel leaf extract as an anti-halitosis formulated in the form of edible films⁴ and areca seed extract edible films as antibacterial⁵.

On the other hand, *Streptococcus mutans* is a bacterium capable of attaching to the tooth surface that produces glucosyltransferase enzyme. These enzymes produce insoluble glucans in water and play a role in causing plaque and colonies on the tooth surface⁶. The growth of *Streptococcus mutans* must be inhibited so that it does not become pathogenic and cause caries by giving antibacterial agents. One way to prevent caries is to limit the plaque formation on the tooth surface,

either by preventing its formation or regularly cleaning plaque. Plaque control can be done by mechanical and chemical cleaning of plaque containing antibacterial ingredients, suppressing *Streptococcus mutans* growth. Based on the description above, the formulation of clove oil in the form of edible film was carried out, which could prevent the evaporation of clove oil compared to the dosage form of toothpaste and mouthwash and tested its antibacterial activity against *Streptococcus mutans*.

METHOD

Materials and Tools

1. Materials

Clove oil (PT. Lansida), corn starch (Maizenaku), HPMC (Brataco), sorbitol (Brataco), Na-saccharin, mint oil, menthol (Brataco), sodium metabisulfite (Brataco), nipagin (Brataco), nipasol (Brataco), and aqua dest

Culture of *Streptococcus mutans* ATCC 31987, 70% ethanol (Brataco), crystal violet solution, Lugol's solution, safarin solution, physiological NaCl solution (Widatra), Blood agar media, Comparative (GF®), and Dimethylsulfoxide (Merck).

2. Tools

The tools used were digital scale (Shimadzu), refrigerator (Panasonic), hot plate and magnetic stirrer (Boeco), oven (Mettler), desiccator, modified edible film printing equipment, caliper (Kenmaster), pH meter (Ohaus), Roche friabilator (Amtast CS-2), incubator (Mettler), LAF (Laminar Air Flow) (Robust), autoclave (Mettler), and micropipette (Socorex).

Clove Oil (*Syzygium aromaticum*) Edible Film Formula

The edible film formula used referred to the formula made by Harmely et al. (2014) because this formula gave good results in previous studies.

Table 1. Formula Edible Film

Composition (%)	Formula			
	F ₀	F ₁	F ₂	F ₃
Clove oil	0	1	1.5	2
Corn starch	6	6	6	6
HPMC	4	4	4	4
Sorbitol 70%	4	4	4	4
Na Saccharin	0.25	0.25	0.25	0.25
Menthol	0.1	0.1	0.1	0.1
Candy Oil	1	1	1	1
Nipagin	0.18	0.18	0.18	0.18
Nipasol	0.02	0.02	0.02	0.02
Na Metabisulfite	0.02	0.02	0.02	0.02
Distilled water up to	100	100	100	100

Description:

F₁: Clove oil concentration of 0%

F₂: Clove oil concentration of 1%

F₃: Clove oil concentration of 1.5%

F₄: Clove oil concentration of 2 %

Corn starch was dispersed into 20 parts of aqua dest, heated at $\pm 60^{\circ}\text{C}$, and stirred until a clear gel was formed. HPMC was developed in distilled water, which had added sorbitol and sodium saccharin, and then was stirred at a temperature maintained at $\pm 60^{\circ}\text{C}$. The two gels were mixed at a temperature of $\pm 60^{\circ}\text{C}$ by adding other additives (nipagin, nipasol, menthol, mint oil, Na metabisulfite, and clove oil) at room temperature; the mixture was stirred homogeneously, then was poured and leveled on the mold (26 x 20 cm). The preparation was dried in the oven at a temperature of 40-50 $^{\circ}\text{C}$ for 24 hours. Then, the edible film formed was released from the mold and cut into pieces with 2.2 x 3.2 cm.

Edible Film Evaluation

1. Organoleptic Examination

The organoleptic examination included visual observations using the five senses on the edible film's shape, smell, taste, and color. This examination was carried out at room temperature ($15\text{-}30^{\circ}\text{C}$)⁷.

2. Edible Film Friability Examination

The friability of the edible film was carried out according to the friability test of the tablet,^{8,9} utilizing a Roche Friabilator. First, 20 sheets of edible film free of dust (W_1) were weighed, then put into the Roche Friabilator. The tool was run for four minutes with a rotation speed of 25 rpm. The 20 edible films were cleaned of dust and weighed again (W_2). Edible film friability can be calculated by the formula:

$$\text{Friability} = \frac{W_1 - W_2}{W_1} \times 100\%$$

3. Drying Shrinkage Examination

The porcelain dish was dried in an oven at 105°C until a constant weight (A) was obtained. Edible film weighed 2g in a porcelain dish (B), then dried in an oven for 2-5 hours until a constant weight was obtained (C). The drying shrinkage was then determined in percent by weight of the sample used¹⁰.

$$\% \text{ Drying shrinkage} = \frac{(B-A) - (C-A)}{B-A} \times 100 \%$$

4. pH Examination

This examination was performed using the Inolab pH meter. First, this instrument was calibrated employing a buffer of pH 4 and pH 7. The electrodes were rinsed with distilled water and dried. The pH measurement of the clove oil edible film was carried out by dissolving 1g of the edible film in distilled water up to 10 ml. The electrode was immersed in the

container, and the number on the pH meter represented the pH value of the clove oil edible film.⁷

5. Edible Film Thickness Examination¹¹

The thickness of the edible film produced was measured utilizing a micrometer with an instrument accuracy of 1 m. Measurements were made at five different places.

6. Swelling Test

The swelling test was carried out by inserting one sheet of edible film into a glass beaker, then expanded with 10 mL of distilled water. Then, it was determined how long it took the preparation to expand.¹²

Antimicrobial Activity Test by Diffusion Method (Kirby Bauer Method)

This method was used to determine the activity of antimicrobial agents. A plate containing an antimicrobial agent was placed on an agar medium on which microorganisms had been grown, which would diffuse into the agar medium. Then, it was incubated at 37°C for 18-24 hours. The clear zone indicated the presence of inhibition of the growth of microorganisms on the surface of the agar medium¹³.

Clove Oil Antibacterial Activity Test

1. Sterilization of tools and materials

The tools utilized were washed and dried before being sterilized. Some tools, such as Petri dishes, were wrapped in newspaper, while the mouthpieces, test tubes, and droppers were covered with cotton and then wrapped one by one with newspaper. All tools were sterilized in an autoclave at 121°C for 15 minutes. Laminar Air Flow was cleaned and then sprayed with 70% ethanol. After that, it was

sterilized by turning on a UV lamp for five minutes.

2. Making Blood Agar Media

The media powder of blood agar base was weighed as much as 4 grams, then dissolved with 100 ml of distilled water and heated to boiling while stirring. Then, it was sterilized by autoclave at 121°C for 15 minutes. The sterile media was cooled to a temperature of 45-50°C. Then, 10 mL of fresh sheep blood was added and stirred until smooth. Then, it was poured into a petri dish and waited for it to solidify. Once frozen, the media was ready to use.

3. Preparation of Test Microbial Suspension

Bacterial colonies were suspended in sterile physiological NaCl solution in sterile test tubes and homogenized by the vortex. Then, the turbidity of the equivalent suspension was measured by standard turbidity UV-Vis Spectrophotometry. Thus, a suspension with a transmittance of 25% at a wavelength of 580nm was obtained¹³.

4. Clove Oil Antibacterial Activity Testing

The sterilized blood agar medium was poured into a ±20mL petri dish. After the media solidified, a sterile cotton swab was dipped into the bacterial suspension and then smeared evenly over the media. Sterile disc paper that had been previously dripped with clove oil with concentrations of 1%, 1.5%, 2%, and as a negative control dimethyl sulfoxide (DMSO) was taken using a 10µL micropipette. It was incubated for 24 hours at 37°C. Then, it was observed for bacterial growth. The inhibition area was also measured, indicated by the appearance of a clear area around the disc utilizing a caliper.

5. Edible Film Antibacterial Activity Testing

Blood agar media sterilized was poured into a petri dish as much as ± 20 mL. After the media solidified, a 5 mm diameter film sheet was placed on the agar medium and then incubated at 37°C for ± 24 hours. The growth of bacteria was observed, and the inhibition area diameter was measured, indicated by the presence of an area not overgrown by bacteria. Tests were carried out on F₁, F₂, F₃ preparations. F₀ was used as a negative control based on the edible film, while (GF[®]) was employed as a comparison¹⁴.

Data Analysis

The data on the antibacterial activity of clove oil in edible film preparations were processed by one-way analysis of variation (ANOVA) utilizing the SPSS program. The results will be meaningful if the comparison of inhibition in each formula gives a real and meaningful difference.

RESULTS AND DISCUSSION

This study aimed to formulate a preparation of clove oil (*Syzygium aromaticum*) in the form of an edible film as a deodorizer and determine its antibacterial activity against *Streptococcus mutans*. Clove oil contains eugenol as much as 78-98%. The substance is produced from the oil glands on the surface of the clove flower body. In addition, clove oil also contains eugenol

acetate, caryophyllene, and other minor compounds, in small amounts¹⁵.

Edible clove oil film was made by casting method on a mold (26 x 20 cm). The edible film formed was removed from the mold and cut into pieces with 2.2 x 3.2 cm (Figure 1).



Figure 1. Preparation of clove oil edible film

Description:

P = Comparative Preparation Formula "GF[®]"

F₀ = Edible film base formula with 0% clove oil concentration

F₁ = Edible film base formula with 1% clove oil concentration

F₂ = Edible film base formula with 1.5% clove oil concentration

F₃ = Edible film base formula with 2% clove oil concentration

The edible clove oil film formed was evaluated for physical properties and activities against *Streptococcus mutans*. Evaluation of the clove oil edible film physical properties was then compared to the circulating physical properties (GF).

Table 2. Evaluation Results of Clove Oil Edible Film

Evaluation	Observation				
	Fo	F1	F2	F3	P
Description					
-Shape	PLT	PLT	PLT	PLT	PLT
-Smell	KM	KMmc	KMmc	KMmc	KM
-Taste	Mm	MAPm	MAPm	MAPm	Mm
-Color	PT	PT	PT	PT	U
Friability (%)	0.98 ± 0.004	0.94 ±0.004	1.02 ±0.003	1.17 ±0.007	1.06 ±0.006
Drying shrinkage (%)	13.91 ±0.016	13.23 ±0.013	12.98 ±0.014	12.69 ±0.013	14.32 ±0.017
pH	5.98 ±0.232	6.33 ±0.156	5.96 ±0.167	5.81 ±0.143	6.23 ±0.186
Thickness (mm)	0.035 ±0.0037	0.0392 ±0.0037	0.044 ±0.0038	0.050 ±0.0060	0.010 ±0
Swelling test (second)	08.48	10.31	12.22	14.15	05.93
Antibacterial activity (mm)	10 ± 0.5	18.6 ± 0.577	22.3 ± 2.081	25.3 ± 1.527	10.5 ± 0.5

Description

PLT : Thin layer solid
 KM : Typical mint
 KMmc : Typical mint clove oil

Mm : Refreshing sweet
 MAPm: Sweet but a bit refreshing bitter
 PT : Transparent white
 U : Purple

The organoleptic examination of the clove oil edible film was carried out for six weeks. Every week, the organoleptic examination results of edible films showed no change in shape, smell, taste, and color. It indicates that the edible film preparation was stable during storage. In addition, the friability examination of edible films utilizing the "Roche Friabilator" friability test equipment did not appear to have changed shape or broken the edible film during the test. However, there was a reduction in the dosage weight caused by the edible film experiencing friction during testing. The % friability test results of the edible film also revealed that the % friability of edible film F3 (1.17%) was higher than the comparison (1.06%). This friability test describes the resistance of the preparation surface to the friction experienced during packaging, shipping, and storage.¹⁶

The results of the edible film drying shrinkage examination uncovered that the highest drying shrinkage was shown in the formula Fo, which was 13.91%, whereas the lowest was displayed in the comparison preparation, which was 12.69%. Meanwhile, according to previous research, the percentage of good drying shrinkage for edible films was less than 9.29%.¹⁷

Moreover, the pH evaluation of the edible film was performed for six weeks. The test results exposed that the pH changed every week but still met the physiological pH range of the mouth. Weekly changes in pH values might be caused by environmental factors, such as temperature, storage, and the sensitivity of the pH meter. The pH value of the resulting edible film must be in the pH range of the oral cavity, which ranges from 5.5 to 7.918¹⁸, so as not to

irritate the oral mucosa when the preparation is consumed.

Next, the thickness of the edible film was examined employing a screw micrometer with an accuracy of 0.01 mm at five different places, and the results were then averaged. The thickness difference occurred due to clove oil concentration differences in each preparation, where F₃ was the thickest compared to F₁, F₂, and F₀. The measuring results of the edible film thickness in all formulas met the requirements of a good thickness of the edible film, which was <0.25 mm.¹⁹ This thickness test was carried out to see that the edible film produced is thin but not easily torn, which is an indicator of uniformity and quality control of the edible film.

Further, in the swelling test, the mean swelling time results of the edible film were obtained. The measurement results disclosed that F₁, F₂, and F₃ required a longer time for the edible film to swell because it contained clove oil, which is difficult to dissolve in water with different concentrations. It is suspected that the mechanism of film destruction is through swelling and wicking mechanisms. The swelling process is that after the film is in contact with water, water penetration occurs due to capillarization (wicking) so that the film swells. Meanwhile, the wicking mechanism is the presence of water drawn by the disintegrant, where water will penetrate the film pores. As a result, the bonds between the particles become weak, and the film swells.¹¹

Furthermore, the antibacterial activity testing of clove oil edible film against *Streptococcus mutans* bacteria was conducted using the agar diffusion method. This method is most commonly used to determine the activity of the test

material against bacteria. The 24-hour incubation was carried out to give time for bacteria to multiply rapidly and carry out metabolic activities. The results showed that after 24 hours of incubation, all clove oil groups' concentrations could inhibit the bacteria *Streptococcus mutans* growth. The higher the concentration, the higher the zone of inhibition. It aligns with Pelcer and Chan's opinion that the higher the concentration of an antibacterial agent, the stronger the antibacterial activity.²⁰

In this study, the test bacteria were suspended in a 0.9% physiological NaCl solution because the physiological NaCl solution was an isotonic environment for the test bacteria. The suspension was homogenized by vortex, and the turbidity was measured utilizing a UV-Vis spectrophotometer with a wavelength of 580 nm and transmitter 25%, describing the optimal cell density for antibacterial activity testing.¹³

From the antibacterial activity test analysis, homogeneous and normal data were obtained, which showed that the data obtained were parametric, followed by one-way ANOVA statistics utilizing SPSS 16 and Duncan's test. The results of the one-way ANOVA statistical test revealed the P-value (<0.005). It signifies a significant difference between all formulas. The difference in the concentration of clove oil at a 2% concentration did not show a significant difference with a 1.5% concentration but showed greater inhibition at a 2% concentration. In the edible film preparation, between the comparisons of the GF® edible film, a significant difference was shown with the edible film preparation in varying concentrations. However, on 1.5% clove oil edible film, it did not show a significant difference with 1% and 2% edible film, but 2% clove oil

edible film had greater antibacterial inhibition.

Based on the response table of bacterial growth inhibition according to Davis and Stout, the inhibition classification was divided into four categories: very strong = 20 mm, strong = 10-20 mm, moderate = 5-10 mm, and weak = 5 mm. From the results obtained for each clove oil edible film formula, F₁ = 18.6 mm, F₂ = 22.3 mm, and F₃ = 25.3 mm. It was categorized into a very strong bacterial growth inhibition response. According to a study,²¹ factors that affect the size of the inhibition zone include the organism sensitivity, the culture medium, the incubation conditions, and the rate of agar diffusion. Meanwhile, the factors that influence the rate of agar diffusion consist of the microorganism concentration, the medium composition, the incubation temperature, the incubation time, and the pH value of the medium. In this regard, some antibacterials work well under acidic conditions and others under alkaline conditions.

CONCLUSION

Clove oil with a concentration of 1% (F₁), 1.5% (F₂), and 2% (F₃) can be formulated in the form of edible film. From the physical evaluation results, almost the same results as the comparison of GF[®] in the form of edible film containing menthol were obtained. In addition, the antibacterial activity test results showed significant differences between F₁, F₂, and F₃. Of the three formulas containing clove oil, F₃ gave the greatest inhibitory power of 25.3mm ($p < 0.05$) and was included in the very strong bacterial growth inhibition response.

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