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The Evaluation of Clove oil Concentration on Physicochemical and Antimicrobial Activity in the Laponite Gel Delivery System

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Abstract: Recurrent Aphthous Stomatitis (SAR) is inflammation in the oral mucosa. One of the factors that can cause SAR is bacterial and fungal infections of the oral mucosa. Clove oil is an essential oil that contains eugenol and has antimicrobial, analgesic, anti-inflammatory, and antioxidant activities. Clove oil in this study resulted from steam distillation with an eugenol content of 71.06%. Clove oil as an antimicrobial topical requires a drug delivery system that is easy and comfortable to apply. Laponite is a synthetic hydrophilic layered silicate that can hydrate and expand when water is added, is compatible with the properties of active ingredients, and is without an emulsifier. Please state the objective of this research. This research aims to determine the effect of variations in the concentration of clove oil incorporated in laponite on the gel's physicochemical properties and antimicrobial activity. Laponite with a concentration of 2.5% was developed in water and then added to clove oil with a concentration of 2%, 4% and 6%. Physicochemical properties test of oral gel preparations includes organoleptic tests, homogeneity, pH, adhesion, and spreadability tests. Antimicrobial activity test to *Streptococcus mutans* bacteria and *Candida albicans* fungi. The research showed that a gel preparation using laponite with a concentration of 2.5% was semi-solid with a clove oil odor. Clove oil, with an eugenol content of 71.06%, has potent antimicrobial activity. The higher concentration of clove oil increases the viscosity and sticky time but decreases the pH and spreadability. The adhesive time and pH of the gel are suitable for application to the oral mucosa with mouth ulcers, and 2.5% laponite can form a gel preparation that meets the gel's physicochemical properties and antimicrobial activity.

Keywords: clove oil; SAR; eugenol; antimicrobial; hydrogel

1. INTRODUCTION

Mouth ulcer or Recurrent Aphthous Stomatitis (SAR) is inflammation that occurs on the oral mucosa, such as the tongue, lips, cheeks, gums, and floor of the mouth. The prevalence of SAR on the oral mucosa is estimated to reach more than 25% of the population worldwide [1]. Based on size, the clinical form of SAR is divided into three, namely minor aphthous ulcers (90% of cases), major aphthous ulcers (8% of cases), and heptiform ulcers (2%) [2]. Most fungal infection in human is 75% caused by *Candida albicans*. About 40% of healthy adults carry this species in the oral cavity [3]. Oral

Candida species can lead to oral candidiasis and denture stomatitis [4]. Oral candidiasis, commonly known as oral thrush, appears as creamy white or yellowish, crusty, curd-like patches with cracks in the corners of the mouth, lips, tongue, palate and buccal cheeks.

Medicines for thrush circulating in the community mostly contain antibiotics and policresulen. However, inappropriate use of topical antibiotics will cause side effects such as rash, fever, chills, and urticaria [5] and policresulen can cause burning and tissue death in the mucosa mouth [6]. People usually treat SAR with drugs that contain policresulen, but this active substance had been withdrawn from market by Indonesian Drug and Food Administration (BPOM RI) since February 2018 [7]. Policresulen was reported to have side effects in 38 cases, two of them are enlargement of SAR and oral mucosal injury that causes infection, also 6 cases reported that the drugs which contain policresulen cause burn on the oral mucosa [8]. Therefore, it is necessary to develop alternative antibacterial agents that are safe and have minimal side effects, one of which is agents from natural ingredients, namely essential oils. Herbal extracts are used in dentistry for treatment of various dental disorders. The natural photochemical could offer an effective alternative to antibiotics and represent a promising approach to prevention and therapeutic strategies for various oral infections.

High eugenol concentration is founded in Clove which has antimicrobial activity for gram-negative and gram-positive bacteria, one of them is *Staphylococcus aureus*. Clove oil has been traditionally used as dental care, analgesic, and antiseptic. Eugenol was shown to have antifungal activity against *Candida albican* [9]. Eugenol of clove have strong antibacterial activity against Cariogenic (MBC 0.2-1.6μg/mL; MIC 0.1-0.8 μg/mL) and against periodontal pathogens (MIC 0.1-1.6 μg/mL) [10]. Clove oil is a group of essential oils containing the main component, eugenol, and is known to have antimicrobial, analgesic, anti-inflammatory, and antioxidant activity. Clove oil contains >70% eugenol, which can destroy proteins and react with phospholipids from cell membranes [11]. Eugenol of clove oil has been confirmed to be effective in combating some pathogenic bacteria including *Pseudomonas aeruginosa S. aureus*, *E. coli*, *Proteus mirabilis,* and *Streptococcus mutans* [12]. Crude clove oil that the use of 10 % could irritate to the skin [13]. Clove oil can be treated by administering painkillers and antiseptics, which are available in various forms, such as lozenges, sprays, mouthwash, and ointments. The gel is a semi-solid preparation used on the oral mucosa as a drug carrier material. It has high adhesion, provides a cooling effect or sensation and good drug release, and can make drug application easier [14].

Laponite® is a synthetic smectite clay that already has many important technological applications, which go beyond the conventional uses of clays in pharmaceutics and cosmetics. In biomedical applications, particularly in nanomedicine, this material holds great potential [15]. Laponite is a clay-based material composed of synthetic disk-shaped crystalline nanoparticles with highly ionic, large surface area. These characteristics enable the intercalation and dissolution of biomolecules in Laponite-based drug delivery systems. Furthermore, Laponite's innate physicochemical properties and architecture enable the development of tunable pH-responsive drug delivery systems. Laponite's coagulation capacity and cation exchangeability determine its exchange capabilities, drug encapsulation efficiency, and release profile [16].

Synthetic magnesium lithium silicate or laponite is a synthetic smectite with a structure and composition similar to the natural clay mineral hectorite in the form of a fine, dense white powder with a bulk density of around 1g/cm^3. *Laponite* is a synthetic hydrophilic layered silicate insoluble in

water but hydrates and expands when water is added. Laponite has advantages in terms of productivity, purity, and efficient availability. This makes laponite suitable for application as a filler and thickener for aqueous preparations in the industrial sector [17]. Laponite can disperse active ingredients that have both lipophilic and hydrophilic properties without additional emulsifiers. In this research, we will observe the effect of variations in the concentration of clove oil incorporated into the laponite system on the physicochemical properties of oral gel preparations. We hope to find out the best (there is no optimation step in the methods, only orientation step to get the best concentration) concentration that produces an excellent oral gel preparation and reduces clove oil's bitter and hot taste when applied to the oral mucosa area because it is incorporated into the laponite system. Laponite provides a relaxed feeling and can release active ingredients periodically for a long time, thereby reducing the frequency of drug use and patients more compliant in using medicines. In this study, the influence of the concentration of clove oil incorporated in laponite gel will evaluate its physicochemical properties and antimicrobial activity.

2. MATERIALS AND METHODS

Laponite (LABTEC, German), aquadest (repackaged by PT. Agung Jaya), citric acid (repackaged by Cipta Kimia), phenoxyethanol (repackaged by Cipta Kimia), propylene glycol (DOW), clove oil (UMKM Surya Wulan Kulonprogo), bacterial culture *Streptococcus mutans* ATCC 2517 (Thermo Scientific), fungus *Candida albicans* ATCC 14053 (Thermo Scientific), antibiotic amoxicillin, antifungal ketoconazole, PDA powder (Merck), human blood type "O" media, and BHI powder (Merck). Instrument: glassware (Pyrex, England), analytical scales (Ohaus CP214, America), stir bar (Pyrex, England), Petri dish (Pyrex, England), pH meter (Lutron-PH-207, Taiwan), magnetic stirrer (IKA C-MAG HS 7, Germany), spinbar, stopwatch, Biosafety Cabinet (BIOBASE BSC-110011A2, China.

2.1. Sample preparation

Clove leaves were steam distilled at 5 am, the oil samples were separated, and the eugenol content of clove oil was tested at LPPT Gadjah Mada University Yogyakarta using the GC method by taking 0.1 mL of the sample and diluting it using ethanol to 1.0 mL volume, then the solution was homogenized. They were using vortex. A standard eugenol solution was prepared with series concentrations, then 1.0 μL of the sample solution, and standard eugenol was injected into the GC machine, and the eugenol concentration was obtained in the Suryawulan UMKM essential oil sample.

2.2. Laponite delivery system of Clove oil

Table 1. Clove oil gel formula, incorporate in laponite delivery system

Ingredients	Concentration (%)				Function
	Base formula	Formula 1	Formula 2	Formula 3	
Clove oil	-	2.0	4.0	6.0	Active substance
Laponite	2.5	2.5	2.5	2.5	Gelling agent
Propylene glycol	0.5	0.5	0.5	0.5	Co-solvent
Citric acid	qs	qs	qs	qs	pH adjusting
Water	Add 100	Add 100	Add 100	Add 100	Solvent

Laponite is dissolved in distilled water to 100 mL, stirred using a magnetic stirrer at 450 rpm for ± 4 hours at room temperature, and placed in the refrigerator until thickened. Then, propylene glycol, and clove oil were gradually added, and the pH-adjusting citric acid solution was added (Table 1).

2.3. Clove oil Hydrogel physicochemical evaluation

Organoleptics were observe directly by the senses of the gel's shape, color, and smell. The gel is usually evident in color with a semi-solid consistency. The electrode on the Lutron-PH-207 pH meter is calibrated with buffer pH 4 and pH 7, then washed with distilled water, pressed on the calibration button, and adjusted. The electrode is then dipped into the gel and waited until stable. The gel preparation's pH must match the mouth's pH of 6.5-7.5. A total of 0.5 g of gel was placed on a glass object, another was placed on top for 1 minute, and its diameter was measured. After that, 200 g of additional load was added, and the constant diameter was measured. A total of 0.5 g of the gel preparation was placed on an object glass, attached to a rope, then covered with another object glass, given a load of 1 kg for 3 minutes, and released. A weight of 80 g is attached to the string, and while the stopwatch is turned on, the time required for the two glass objects to come off is recorded. The criteria for adhesion time of gel preparations is more than 10 seconds [15].

2.4. Antimicrobial Test

Antibacterial blood media: BHI powder was weighed 9.3 grams, dissolved in 250 ml water, and heated while homogenized using a magnetic stirrer for 15 minutes. The solution was incubated for 15 minutes; after it solidifies, weigh 5 grams of agar medium and dissolve it into 125 ml water, then heat it while stirring using a magnetic stirrer for 15 minutes; finally, add five cc of human blood type "O." The solution was incubated for 15 minutes and then poured into sterile Petri dishes [15]. *Streptococcus mutans* bacteria, available in BHI-B media, are put into an anaerobic jar and incubated at 37 ºC for 24 hours. Make seven holes in the agar blood media using a tip punch (7 mm), then fill each hole with clove oil as an oil control, the antibiotic amoxicillin as an antibacterial control, water as negative control, base formula as base control, and F1, F2, and F3 as test samples. The experiment was repeated three times, then incubated for 3x24 hours at 30°C [15].

Mueler Hinton Agar media weighed 3.9 grams, was dissolved in 100 mL of water, then sterilized in an autoclave at 121°C for 15 minutes. Fungal cultivation: The Candida albicans fungus suspension was then suspended with 10 mL of physiological NaCl, then mixed and adjusted to the turbidity to be the same as the Mc Farland solution [16]. Make seven holes in the MHA media using a tip punch (7 mm), then fill each hole with clove oil as an oil control, the ketoconazole as an antifungal control, water as negative control, base formula as base control, and F1, F2, and F3 as test samples. The experiment was repeated three times, then incubated for 3x24 hours at 30°C [15].

2.5. Statistical Analysis

The data obtained from the test results is then compared with parameters from several sources or libraries. The data obtained was then analyzed statistically using IBM SPSS statistics 25 software, which uses the One-Way Anova method if the sig. <0.05. The results of the statistical tests have not yet been explained in the results and discussion section.

3. RESULTS AND DISCUSSION

Active substance content test in clove oil as a quality parameter that the clove oil from steam distillation of UMKM Suryawulan Kulonprogo meets the quality requirements for eugenol content, which has antibacterial and antifungal activity using the gas chromatography method. Eugenol content testing at the Integrated Research and Testing Laboratory (LPPT Gadjah Mada University, Yogyakarta) showed that the eugenol content in clove oil was 71.6%. The main component of clove oil is eugenol, which is 70 – 80% [20]. The eugenol as the major constituent of clove oil, followed by

eugenol acetate. The mass spectral data for eugenol acetate showed the molecular ion peak at 206 corresponding to the relative molecular mass of eugenol acetate, the peak at 164. Eugenol is a phenolic compound (Figure 1). Phenols are known to have antiseptic properties, which is consistent with the antimicrobial data obtained for these compounds. Generally, eugenol (4-allyl-2-methoxyphenol) accounts for 60%–90% of the total composition of clove oil and is the source of the antiseptic property of clove oil [12]. Clove oil was mainly composed of three terpenic compounds in which eugenol was the most abundant, accounting for 78.55%, followed by caryophyllene (15.75%) and humulene (4.28%). Meanwhile, two minor constituents, methyleugenol (0.27%) and epizonarene (0.92%) [13]. So, the eugenol content in clove oil producted by UMKM Suryawulan Kulonprogo, Indonesia meets the requirements for eugenol content.

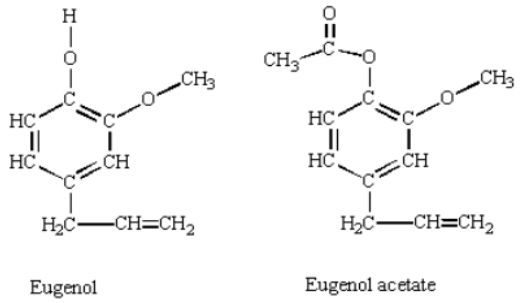


Figure 1. Chemical structures of the components of clove oil [x]

Table 2. Antimicrobial activity of crude clove oil producted by UMKM Suryowulan Yogyakarta compare with clove oil brand product

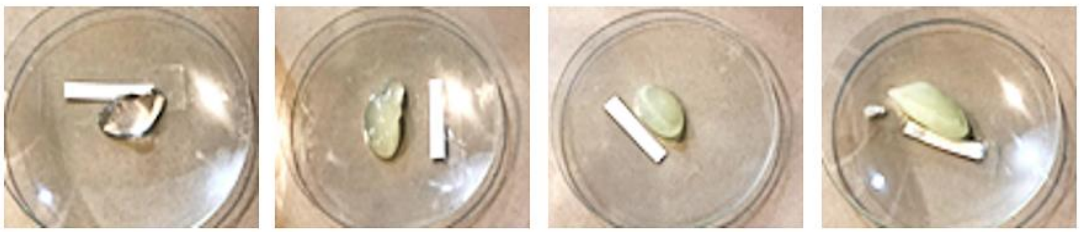
Microbes Species	Inhibitor Zone Diameter (mm)			
	Clove oil UMKM	**Clove oil Brand**	**Control Antibiotics/anti-fungal**	**Control Negatif**
Escherichia coli	10.30	9.70	27.90	0.00
Staphylococcus aureus	29.30	23.40	23.70	0.00
Candida albicans	32.90	24.60	30.40	0.00

Based on the results of antimicrobial testing (Table 2), clove oil produced by UMKM Suryowulan Yogyakarta has better antimicrobial activity than clove oil sold on the market under leading trademarks. *Staphylococcus aureus* was found to be the most sensitive to clove extract with an inhibition zone diameter (IZD) of 29.30 mm and 23.40, respectively. The modes of action by which microorganisms are inhibited by essential oil and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents [18].

3.1. Physicochemical Properties

The previous orientation of clove oil concentrations in 0.5%, 1%, and 2%. However, there is no change in the physical properties of the hydrogel, and it does not have antibacterial and antifungal activity, so we increased the concentration to 2%, 4%, and 6%. Based on previous research, the lowest concentration was 2.5%, so we used a concentration of 2% as the lowest dose. Research by Gupta et al., 2009 [19] indicated that 5% clove oil had a diameter of inhibition of 18 mm to *S. aureus*. In this

research, we want to know If the 4-6% concentration of clove oil is higher, lower, or equal to the previous research by Gupta. The oral gel preparation has a semisolid consistency and a characteristic clove oil aroma. The aroma becomes stronger, and the color becomes cloudier as the concentration of oil used increases (Figure 2). The base formula of laponite gel is clear and odorless. The clove oil hydrogel was pale yellow in F1, F2, and F3.



(a) (b) (c) (d)

Figure 2. Citronella oil laponite hydrogel using various concentration of clove oil where (**a**) base formula of clove oil; (**b**) 2% of clove oil hydrogel; (c) 4% of clove oil hydrogel; (d) 6% of clove oil hydrogel. The hydrogel appearance becomes cloudy with increasing concentration of clove oil

The viscosity of the gel preparation is influenced by several factors, namely the type and concentration of the gelling agent, the mixing time, and material incompatibility [11]. The pH value test aims to see if the pH of the gel preparation is suitable and can be accepted by the skin and oral mucous membranes. The higher the concentration of clove oil, the greater the pH value. The pH measurement results were obtained from 6.6-7.7 (Table 3). The pH value of laponite based on the MSDS has a basic pH of 9.8, while clove oil has an acidic pH of 4. However, the pH of the resulting clove oil gel preparation has been added to a citric acid solution, a pH-adjusting solution. In the Laponite MSDS, citric acid is the recommended agent for lowering pH. The citric acid solution is added little by little according to the expected pH value. The results of the homogeneity test using the Levene test are seen based on the mean being 0.178, which shows that the data is homogeneous because it has a significant value≥ 0.05. The ANOVA test results show a Sig value. 0.000 shows that the data is significant because it has a Sig value. <0.005. The results of this analysis show that there are differences. The concentration of clove oil has a significant effect on the pH value of the gel preparation.

The wider the distribution capacity of the preparation, the better the distribution of the active substance of the preparation. The increase in the diameter of the spreadability will be inversely proportional to the increase in the viscosity of the preparation [2]. This is based on research [18] which states that increasing the concentration of laponite will cause the gel to become viscous, where the consistency of the gel will become thicker. The viscosity will be greater, causing the spreadability of the gel to decrease. The results of the homogeneity test using the Levene test are seen based on the mean being 0.182, which shows that the data is homogeneous because it has a significant value≥ 0.05. The ANOVA test results show a Sig value. 0.000 shows that the data is significant because it has a Sig value. <0.005. The results of this analysis show that there are differences. The concentration of clove oil has a significant effect on the value spreadability of the gel preparation.

The sticking power of a gel is directly proportional to viscosity. The higher the viscosity, the higher the sticking power. The ability of the gel to stick will influence the therapeutic effect. The longer the gel can stick to the skin, and the skin absorbs the more active substances, the longer the gel can have a therapeutic effect and the more effective its use will be. If it is too weak, the therapeutic

effect will not be achieved [23]. The standard adhesion time for an excellent topical preparation is 4.0 seconds [24]. The higher the concentration of laponite in the gel preparation, the greater the spreading power, thereby reducing the adhesive power. The results of the homogeneity test using the Levene test are seen based on the mean being 0.836, which shows that the data is homogeneous because it has a significant value≥ 0.05. The ANOVA test results show a Sig value. 0.000 shows that the data is significant because it has a Sig value. <0.005. The results of this analysis show that there are differences. The concentration of clove oil has a significant effect on the value adhesion of the gel preparation.

Viscosity testing aims to determine the viscosity of flowing liquid; the higher the viscosity value, the higher the viscosity of the preparation [25]. The gel viscosity results meet the viscosity standards for good gel preparations, namely the 3000-50,000 cps (SNI, 1996). Viscosity can be influenced by temperature, electrolyte, and pH level. The results of the homogeneity test using the Levene test are seen based on the mean being 0.021, which shows that the data is homogeneous because it has a significant value≥ 0.05. The ANOVA test results show a Sig value. 0.000 shows that the data is significant because it has a Sig value. <0.005. The results of this analysis show that there are differences. The concentration of clove oil has a significant effect on the viscosity value of the gel preparation.

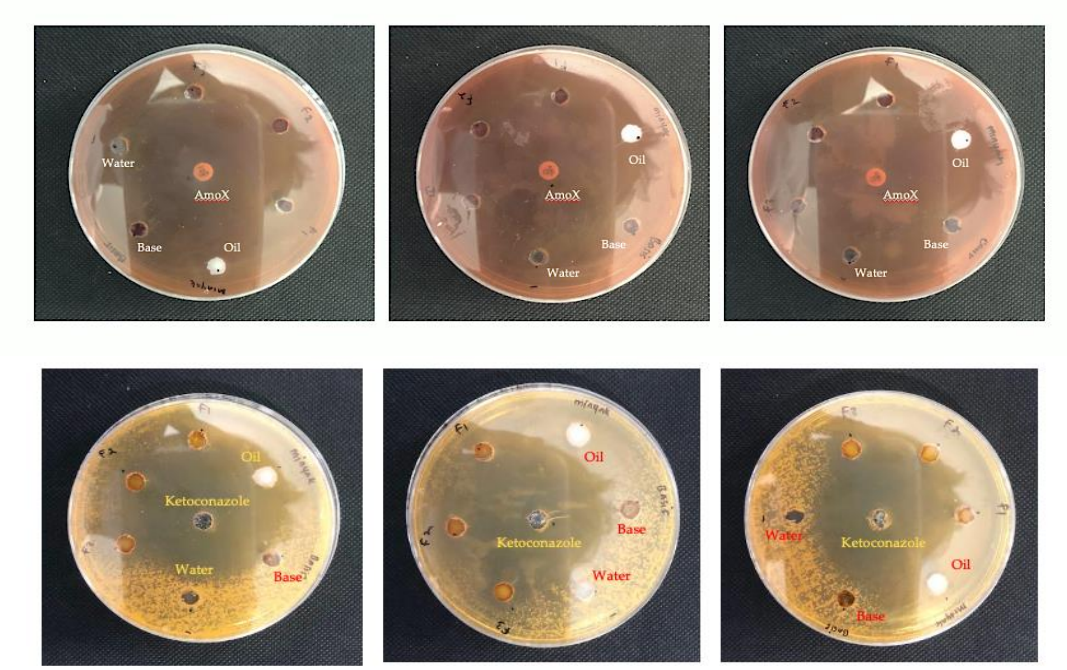


Figure 3. The results of diameter inhibitor zone of antibacterial activity to *Streptococcus mutans* and antifungal activity to *Candida albicans*.

Table 3. The physicochemical test result of clove oil hydrogel using various concentration of clove oil

Formulas	Physicochemical properties			
	pH	Viscosity (cps)	Spreadability (cm)	Sticky time (s)
Base formula	7.70±0.03	1541±6.92	5.00±0.15	0.91±0.02
F1	7.50±0.07	3420±7.53	5.72±0.10	0.98±0.06
F2	6.80±0.01	4617±5.50	5.60±0.15	1.03±0.07
F3	6.60±0.04	6389±6.35	5.41±0.10	1.25±0.04

Table 4. The physicochemical test result of clove oil hydrogel using various concentration of clove oil

Formulas	Diameter of Inhibitor Zone (mm)	
	Antibacterial	**Antifungal**
Base formula	0.00±0.00	0.00±0.00
F1	13.84±0.10	13.35±1.03
F2	18.96±0.11	14.08±0.50
F3	19.05±0.05	14.07±0.35
Citronella oil	22.47±0.10	17.06±1.00
Antibiotics agent	36.80±0.00	-
Antifungal agent	-	14.72±0.00
Water	0.00±0.00	0.00±0.00

Based on the classification of antimicrobial inhibitory categories, both antibacterial and antifungal, an inhibitory zone diameter of >20 mm is a very strong inhibitory; an inhibition zone diameter of 10 mm-20 mm is categorized as strong; an inhibition zone diameter of 5 mm-10 mm is categorized as medium; and the diameter of the inhibition zone is <5 mm, it is categorized as weak. The results of the antimicrobial activity test, both antibacterial and antifungal, in Table 4 show that the diameter of the inhibition zone is largest in F3 with a strong inhibition category, whereas. This shows that the greater the concentration of clove oil used, the greater the inhibitory power, which is indicated by the greater the diameter of the inhibition zone. The results of the antimicrobial activity test showed that clove flower essential oil in the oral gel preparation had inhibitory power against the growth of *Streptococcus mutans* bacteria and the fungus *Candida albicans* [26].

In this study, eugenol was confirmed as the main component of 78% clove oil. Eugenol of clove oil showed obviously antibacterial activities against *S. aureus* and *C.albicans*. The antibacterial mechanism of eugenol against *S. aureus* was probably related to the damage of cell wall and membrane, the inhibition on biofilm formation, the oxidative stress-mediated apoptosis and the disruption of DNA synthesis. These results indicated that clove oil and eugenol may be used as substitutes for antibiotics and synthetic antimicrobial agents [13]. Based on test results using crude clove oil sample showed that the use of 10 % crude clove oil could irritate to the skin [12]. The concentrations of *Syzygium aromaticum* oil were 2,5% (FI), 5% (FII) and 10% (FIII), respectively, variation of essential oil did not influence the irritation index of the ointment. The irritation index of the ointment was negligible [29]. Based on the previous research, where the clove oil topical preparation used various concentrations, our clove oil concentration used was under the previous research's clove oil concentration. It can be concluded that the concentration used is safe even though we did not conduct the anti-irritation test [13].

4. CONCLUSION

The research showed that a gel preparation using laponite with a concentration of 2.5% was semi-solid with a clove oil odor. Clove oil, with an eugenol content of 71.06%, has potent antimicrobial activity. The higher concentration of clove oil increases the viscosity and sticky time but decreases the pH and spreadability. The adhesive time and pH of the gel are suitable for application to the oral mucosa with mouth ulcers, and 2.5% laponite (Table 1 state 2.5% laponite) can form a gel preparation that meets the gel's physicochemical properties and antimicrobial activity. Please ensure that the conclusion aligns with the objectives stated in the background.

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Conflicts of interest: "The authors declare no conflict of interest."

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