

Garlic Mixed Olive Oil Cream Formulation and Its Activity against A Clinical Isolate of *Staphylococcus aureus*

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Abstract. Garlic or *Allium sativum* is known containing of organosulfur compounds. These compounds are known have potential as antimicrobial, antiviral, and anticancer. The purpose of this study is to determine the activity of garlic mixed olive oil (GMO2) cream against the clinical isolate of *Staphylococcus aureus* and its evaluation by observing the organoleptic, homogeneity, pH, spread ability, stickiness, and viscosity. The result show that GMO2 was able to inhibit the growth of *Staphylococcus aureus* bacteria at concentrations of 25 and 50 mg/ml. Meanwhile, the formulation of GMO2 cream at concentration 25 mg/ml was done by poisoned food method show no activity against clinical isolate of *Staphylococcus aureus*. In this study, vanishing cream was used as a negative control. The evaluation of GMO2 cream at concentration of 25 mg/mL has stability in organoleptic and homogeneity after the cycling test. The pH value of GMO2 cream was approximately equal to vanishing cream. The results of spread ability and stickiness tests indicated that GMO2 cream had the spreading and sticking strength which satisfied with the standard cream in range 5-7 and > 4 respectively. The viscosity value of GMO2 cream were decreased after the cycling test. The stability test result show that of GMO2 cream was stable after the cycling test.

Keywords: *Allium sativum*, garlic mixed olive oil (GMO2), cream formulation, *Staphylococcus aureus*

INTRODUCTION

Garlic (*Allium sativum*) is the plant that has been widely used to prevent and treat various diseases including antimicrobial [1]. Harris reported that the main organosulfur compound contained in garlic such as allicin. Allicin is believed to have the effect of treating various diseases and has antibacterial, antiviral, and anticancer properties. The mechanism action of allicin as an antibacterial is by destroying the bacterial proteins that cause disease so that the bacteria become lysis and died [2]. Ankri and Mirelman reported that allicin in garlic can inhibit the growth of *Staphylococcus aureus* with LD₅₀ value of 12 µg /ml [3]. Allicin is a volatile compound, very unstable and easily decomposes into dialyldisulfide (DADS), diallytrisulfide (DATS) and diallyltetrasulfide (DATTS) [4,5].

Garlic oil, has been known to have effective antifungal, antibacterial and anti-inflammatory activities [6,7,8]. Diallyltrisulfide (DATS) and diallyldisulfide (DADS) are the most abundant volatile organosulfur compound (VOSs) in garlic oil [9,10]. Currently, there are three of garlic products are available in the market, i.e. garlic powder, garlic extract, and garlic oil. The previous study was reported that garlic oil was able to inhibit the growth of several Gram positive bacteria such as *Bacillus cereus*, *Listeria monocytogenes* and *Streptococcus sp.* with a minimum inhibitory concentration (MIC) value of <1 mg/mL. Li et al also reported that garlic oil has antifungal effect against *Candida albicans* with a MIC value of 0.35 µg/mL.

Olive oil is a liquid fat obtained by pressing whole fruit of olives (*Olea europeae* L). Olive also known as lilac, is a taxonomic family of flowering shrubs, trees, and a few lianas in the order Lamiales. Olive oil contains 90-99% of the glycerol fraction which consists of polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA). The previous study reported that almost 70-80% of fatty acid compounds in olive oil was oleic acid. Oleic acid has been reported possess antimicrobial activity against bacteria and fungi [11]. The phenolic compounds contained in olive

oil are believed play an important role in the antimicrobial activity of the olive oil. The phenolic compounds (such as ydroxytyrosol, oleuropein, caffeic acid, coumaric acid, vanillic, and tirosol) isolated from virgin olive oil can inhibit the growth of various bacteria including *Staphylococcus aureus*. Tripoli also mentioned that olive oil also contains of α -tocopherol, squalene, chlorophyll (color pigment) and β -carotene compounds, and these compounds were known possesses antioxidant activity. However, based our best knowledge, there is very limited study regarding the formulation of garlic oil mixed olive oil (GMO2) cream especially for topical using. Therefore, the objective of this study is to formulate garlic oil mixed olive oil (GMO2) cream and evaluate the antibacterial activity of the formula against a clinical isolate of *Staphylococcus aureus*.

METHODOLOGY

Materials

Garlic (*Allium sativum*) bulbs were purchased from the traditional market. Olive oil and commercial garlic oil (CGO) were purchased from the local medicinal market, Banda Aceh, (Indonesia). A clinical isolate of *Staphylococcus aureus* provided by Faculty of Medicine, Syiah Kuala University, and maintained in our laboratory before experiments. Nutrient Agar (NA) and Mueller-Hinton Agar (MHA) were purchased from Merck. All solvents and reagents were of analytical grade and used directly without further purification. Aquadest, stearic acid, cera alba, vaseline album, propylene glycol, butyl hyroxy toluene (BHT), methyl paraben (nipagin), and triethanolamine (TEA) were provided by our laboratory at Department of Pharmacy, Universitas Syiah Kuala.

Preparation of Garlic Mixed Olive Oil (GMO2)

The garlic mixed olive oil (GMO2) was prepared as described by Ryther [12]. Briefly, 20 g of garlic cloves peeled into a beaker containing 40 g of olive oil (1:2). Then, the garlic were heated using a hot plate for 10 minutes at 80-90 °C. After cooling, the GMO2 was then sterilized with a filter syringe (0.45 μ m) and stored in a sterile vial until used.

Antibacterial activity: Poisoned food method

The antibacterial activity of garlic mixed olive oil (GMO2) and commercial garlic oil (CGO) against clinical isolate of *Staphylococcus aureus* were done by poisoned food method. In this assay, antibiotic of ciprofloxacin and olive oil were used as positive and negative control respectively. Briefly, a sterile GMO2 (at various concentrations of 1; 5; 10; 25; and 50 mg/mL) was pour into a measuring cup, then a sterile MHA media were mixed up to 10 mL. The mixture was then removed aseptically into a petri dish, and allowed to solidify. To every MHA plate was inoculated with approximately 10^5 CFU/mL of *Staphylococcus aureus*, the plate then sealed and incubated at 37°C for 24 hr. The inhibition of the mycelial growth of the test bacteria by garlic mixed olive oil (GMO2) and commercial garlic oil (CGO) was observed descriptively.

Preparation of Cream Formulation

The cream formulation were performed in this study were listed in Table 1. The active concentration of GMO2 against a clinical isolates of *Staphylococcus aureus* was used to prepare of cream formulation.

Table 1. The vanishing cream formulation of GMO2

No.	Ingredients	Cream Formulation (%)	
		F ₀	F ₁
1.	GMO2	-	2,5
2.	Stearic acid	14,2	14,2
3.	Cera alba	2	2
4.	Vaseline album	8	8
5.	Propylene glycol	8	8
6.	Triethanolamine (TEA)	2	2
7.	Methyl paraban (nipagin)	0,18	0,18
8.	Buthyl hydroxy toluene (BHT)	0,1	0,1
9.	Aquadest	ad. 100	ad. 100

Briefly, the stearic acid and water soluble ingredients (aquadest and methyl paraben) were heated up to 80°C. Other oil soluble components such as cera alba and vaseline album were heated separately. To the oil phase then added a butyl hydroxyl toluene (BHT) gradually with constant stirred. Propylene glycol and triethanolamine (TEA) were added gradually while being constantly

stirred. Furthermore, to the oil soluble ingredients then added oil soluble components gradually while being constantly stirred to form a basis cream formulation (F_0). Finally, 2.5 % of GMO2, an active ingredient was added constantly until homogenous to form F_1 cream formulation. The cream formulations (F_0 and F_1) then stored for further analysis.

Evaluation of The Cream Formula: Cycling test

The cream formulations (F_0 and F_1) obtained were stored at cold condition ($4^{\circ}\text{C}\pm 2^{\circ}\text{C}$) for 24 h, then stored again into the oven ($40^{\circ}\text{C}\pm 2^{\circ}\text{C}$) for the another 24 h (one cycle). This experiment was repeated for 6 cycles¹¹. After a cycling test, analyzed the physical condition before and after the cycling test including organoleptic, homogeneity, pH, spread ability, sticking strength, and viscosity.

Antibacterial activity: disc diffusion method

The antibacterial activity of formulated creams were determined using disc diffusion method. The suspension of a clinical isolate of *Staphylococcus aureus* were inoculated on the plate containing Mueller Hinton Agar by swap method used sterile cotton bud. The dilution of formulated creams (F_0 and F_1) were placed on disc paper on the plates of MHA media. The plates were placed in to the incubator and are incubated at 37°C for 24 h. Antibiotic ciprofloxacin were used as positive control. After the incubation period, the diameter inhibition zone of *Staphylococcus aureus* were measured.

RESULTS AND DISCUSSION

Garlic Mixed Olive Oil (GMO2)

In this study we used two kinds of garlic oils; garlic mixed olive oil (GMO2) and commercial garlic oil (CGO) (Fig.1). These two type of garlic oils was used to determine the antibacterial activity of the oils against a clinical isolate of *Staphylococcus aureus*.

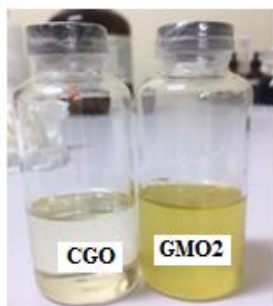


Figure 1. Commercial garlic oil (CGO) and garlic mixed olive oil (GMO2)

Antibacterial activity

The activity of GMO2 and CGO against clinical isolate of *Staphylococcus aureus* were done by poisoned food method. The concentrations of GMO2 and CGO were used in this study were 1; 5; 10; 25; and 50 mg/mL, a ciprofloxacin and olive oil were used as positive and negative controls. The activities of GMO2 and CGO against clinical isolate of *Staphylococcus aureus* is presented in Fig. 2.

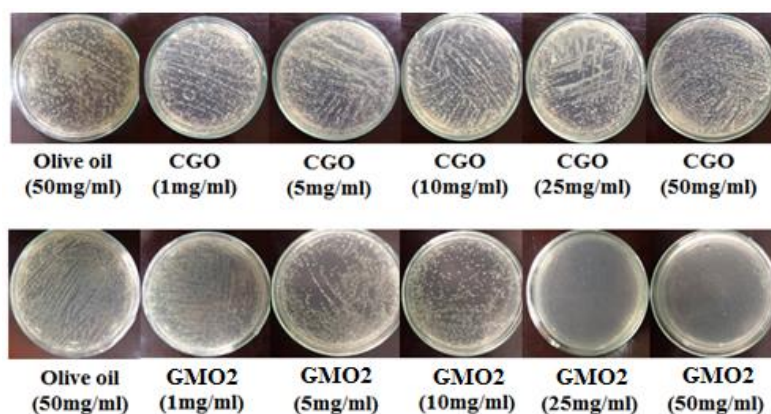


Figure 2. The antimicrobial activity of commercial garlic oil (CGO) and garlic mixed olive oil (GMO2) and on clinical isolate of *Staphylococcus aureus* by poisoned food method.

The results showed that the GMO2 at concentration of 25 mg/ml has begun to inhibit the growth of *Staphylococcus aureus*. While at concentration of 50 mg/ml GMO2 showed stronger inhibition to *Staphylococcus aureus*. GMO2 exhibited strong antibacterial activity against clinical isolate of *Staphylococcus aureus* at concentrations of 25 to 50 mg/ml. O'gara stated that garlic oil, which is extracted from garlic, has been known to have effective antibacterial activity [6]. Mossa mentioned that the most prominent organosulphur compounds found were diallyl sulfide, diallyl disulfide, dimethyl tetrasulfide, trisulfide, di-2-propenyl, tetrasulfide, di-2-propenyl, disulfide, methyl 2-propenyl, and trisulfide. These compounds about 80.42% were found of total garlic oil. Other organosulphur compounds such as 1,2-dithiolane, diallyl disulfide, 1,1'-thiobis[3-(methylthio)-, and 3-vinyl-[4H]-1,2-dithiin were also founded [13]. However, olive oil at concentration of 50 mg/ml used as negative control showed no inhibition activity against this bacteria. Interestingly, the result showed that the commercial garlic oil (CGO) has no inhibition effect on a clinical isolate of *Staphylococcus aureus* at all concentrations used.

Preparation of Cream Formulation

The cream formulation of F_0 (a vanishing cream formula) and F_1 (a vanishing cream formula with 2.5% of GMO2 as an active ingredient) are presented in Figure 3. A vanishing cream formula is an oil in water type cream (O/W). The O/W type cream is contains fewer oil components, not sticky and more comfortable to use [14].



Figure 3. Cream formulation; a. Cream formulation of F_0 (basis cream); B. Cream formulation of F_1 (added GMO2).

Cycling Test

The stability of the cream formulations in this study were performed by using the cycling test method. The cycling test method was conducted to provide a simulation changes in environmental temperature (cold and hot) with 6 cycles [15]. The evaluation of cream stability was measured by measuring 6 physical parameters of the cream, such as organoleptic, homogeneity, pH, spread ability, sticking strength and viscosity tests. The results of cream evaluation by cycling test method are presented in Table 2 below.

Table 2. Evaluation of cream formulations (F₀ and F₁) before and after cycling test

Cream Evaluation	Standard value	Cycling test	Cream Formulation	
			F ₀	F ₁
Organoleptic		Before	White color, soft, wax odor, unsticking	White color, soft, wax odor, unsticking
		After	White color, soft, wax odor, unsticking	White color, soft, wax odor, unsticking
Homogeneity	Homogeneity	Before	Homogeneity	Homogeneity
		After	Homogeneity	Homogeneity
pH	4.5-6.5	Before	7.97±0.06	8.00±0.10
		After	8.10±0.10	8.13±0.12
Spread ability (cm)	5.0-7.0	Before	5.32 ± 0.08	4.83 ± 0.38
		After	6.43 ± 0.21	5.73 ± 0.31
Sticking strength (second)	<4.0	Before	6.03±0.41	5.82±0.61
		After	4.30±0.24	4.70±0.40
Viscosity (mPa)	<4000	Before	3483.92	4029.06
		After	1420.37	2049.54

The results showed that F₀ and F₁ formulas were white color, soft, wax odor, and unsticking, the formulas no phase separation occurs. All formulas have no significant difference during storage condition (cold and hot). The aim of homogeneity test was to evaluate the materials distribution in the formula. The homogeneity test showed that the formulated cream had no any coarse grains found on the glass object, it shows that all the formula (F₀ and F₁) tested were homogeneous.

The pH of a topical cream should be within the pH range corresponding to the pH of the skin, in the range of 4.5–6.5. The results showed that the formulas was tended to be alkaline before and after cycling test i.e. 7.97-8.13 (Table 2). The sticking strength describes the length of time the cream is able to stick to the skin, the sticking strength of the cream formulation should be below 4 seconds [16]. In this study, the sticking strength of the formulated cream above 4 seconds before and after cycling test. The spread ability of the cream was also evaluated. In this assay, the standard weight applied to the upper plate was 50 g and 100 g. The results showed that F₀ and F₁ are according to the standard. Garg stated that the ideal value of the spread ability of the topical cream is in the range of 5-7 cm [17]. The spread ability of the cream formulated were presented in the Figure 4.

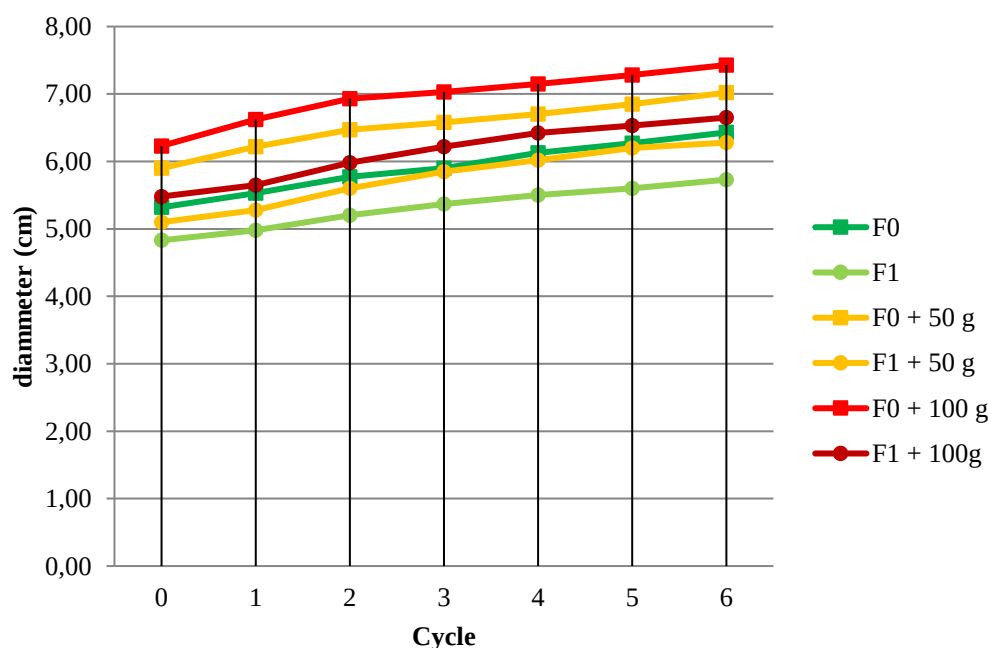


Figure 4. Spread ability of cream formulation (F₀ and F₁) using cycling test

The viscosity was prepared using Rheology International Viscometer with spindle No.4 and speed 200 rpm. The results showed that the cream viscosity decreases after the cycling test. The results indicate that F₀ formula more watery compare to F₁ because it has a smaller viscosity value. Viscosity values obtained from the multiplication of the dial reading and correction factors that exist in the Rheology International Viscometer.

3.5 Antibacterial Activity of Formulated Creams

The antibacterial activity of the formula F₀ and F₁ was performed by disc-diffusion method. Unfortunately, all the cream formulated tested show no antibacterial activity against a clinical isolate of *Staphylococcus aureus* (Fig. 5). This may be influenced due to base cream formula caused inhibit the release of active ingredients from GMO2 to the media. Another possibility is the interaction of the active substances between GMO2 with the base of the cream formula, caused the antibacterial action is not optimal.

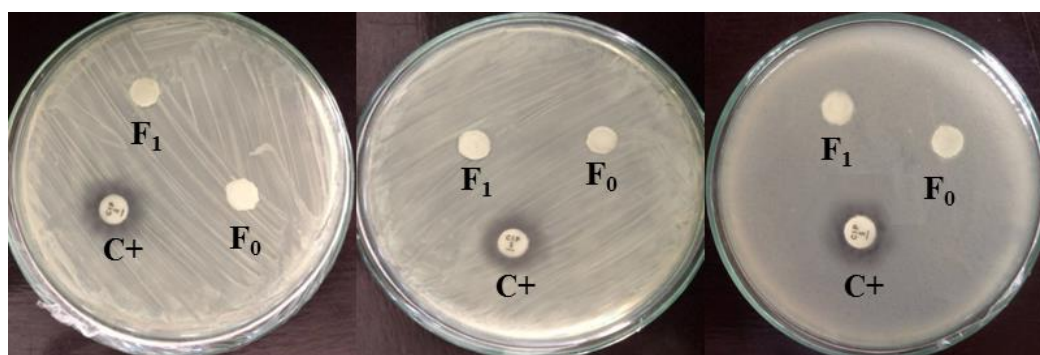


Figure 5. Antibacterial activity of formulated cream against clinical isolate of *Staphylococcus aureus*

CONCLUSION

Garlic mixed olive oil (GMO2) has antimicrobial activity against *Staphylococcus aureus* bacteria at concentrations of 25 and 50 mg/ml, while commercial garlic oil (CGO) has no antimicrobial activity against clinical isolate of *Staphylococcus aureus* at all of concentrations tested. The cream formulated of the F₀, F₁ were evaluated by cycling test method, and the results showed that all formula were according to standard values except for pH values, which tend to be more alkaline. The antibacterial activity of the formula (F₀ and F₁) showed no activity against clinical isolate of *Staphylococcus aureus*.

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