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Evaluation of Virgin Coconut Oil Concentration Variations in Emulgel Formulations of Garlic (*Allium sativum* L.) Extract as an Antibacterial Hand Sanitizer

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ABSTRACT

One of the effective ways to stay healthy is maintaining cleanliness, including hand hygiene. Various diseases can be easily transmitted through the hands. However, modern developments have changed the habit of handwashing with water to using hand antiseptics. Hand sanitizer is an alternative to overcome this problem. Unfortunately, some hand sanitizer gels on the market still contain alcohol as an antibacterial ingredient. It is necessary to innovate in making hand sanitizers using non-alcoholic ingredients as antibacterials, for example, natural ingredients like garlic. Garlic contains *allicin* that functions as an antibacterial. Meanwhile, VCO contains high lauric acid with antibiotic properties that function as antiviral, antibacterial, and antiprotozoal. It also contains capric acid, with antiviral and antibacterial functions. This study aims to examine the effect of concentration variation of VCO on the physical stability of garlic extract emulgel preparation and antibacterial effectiveness against the growth of *Staphylococcus aureus*. The concentration variation of VCO in this study was 3%, 5%, and 7% for 3 types of formulas. The results showed that the concentration variation of VCO in emulgel preparations gave differences in the physical properties of the gel, including pH, viscosity, adhesive strength, and spreadability. It can be concluded that the higher the VCO concentration, the higher the pH, viscosity, and adhesive power, and the lower the spreadability and antibacterial inhibition power against *Staphylococcus aureus*. The best result from the evaluation of physicochemical properties and antibacterial tests was Formula 1, although it was still in the weak inhibitory power category.

***Keywords*:** Emulgel; Garlic; Hand sanitizer; *Staphylococcus aureus*; VCO

INTRODUCTION

Health is an important aspect that affects the quality of life of an individual. One of the effective ways to stay healthy is maintaining cleanliness, including hand hygiene. Various diseases can be easily transmitted through the hands. Viruses, bacteria, and fungi get onto the hands when someone does an activity. One of the bacteria that most often contaminates the skin of the hands is *Staphylococcus aureus*. *Staphylococcus aureus* is most often transmitted from hand to hand.[1] Health is an important aspect that affects the quality of life of an individual. One of the effective ways to stay healthy is maintaining cleanliness, including hand hygiene. Various diseases can be easily transmitted through the hands. Viruses, bacteria, and fungi get onto the hands when someone does an activity.

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One of the bacteria that most often contaminates the skin of the hands is *Staphylococcus aureus*. *Staphylococcus aureus* is most often transmitted from hand to hand.[1] One of the easiest, simplest, most effective, and most common preventions is handwashing with soap under running water. Handwashing with soap prevents the spread of diseases transmitted through hands, such as diarrhea, cholera, and worms.[2] Bacteria can become pathogenic and dangerous to humans if their numbers exceed the limit. Excessive bacteria are caused by, among other things, the lack of hand washing habits. In certain conditions, the availability of water and soap becomes an obstacle since there are no facilities for washing hands.

Modern developments have changed the habit of handwashing with water to using hand antiseptic.[3] Hand sanitizer is an alternative to overcome this problem. Unfortunately, some types of hand sanitizer gels on the market still contain alcohol as an antibacterial ingredient. Using alcohol as a hand sanitizer is considered harmful to health since it can dissolve the layer of fat on the skin that functions as a protector against microorganism infection, and repeated use of alcohol can cause dryness and irritation of the skin. It is necessary to innovate in making hand sanitizers using non-alcoholic ingredients as antibacterials, for example, natural ingredients like garlic. Garlic contains *allicin* that functions as an antibacterial. *Allicin* is a substance in garlic that protects against bacterial attacks.[4] *Allicin* is an active substance in garlic that is effective in killing microbes, such as germs, that cause infections (flu, gastroenteritis, and fever). Various diseases and infections are often caused by bacteria entering the body through contaminated food, such as *Staphylococcus aureus* bacteria.[5] Adding VCO is expected to increase the effectiveness of hand sanitizer since VCO contains lauric acid and capric acid, which have antibiotic properties functioning as antivirals, antibacterials, and antiprotozoals.

Emulgel is chosen as a preparation because it has good spreadability on the skin, provides a cooling effect due to the slow evaporation of water on the skin, and is easy to wash off with water.[6] Hand sanitizer preparations are made in emulgel form since it consists of two systems, namely the emulsion and gel systems. The emulsion system functions as an emollient. Emulsions consist of an oil phase that can make hand sanitizer preparations not easily rinsed with water, providing long-term protection. However, it is still comfortable to apply because the gel system provides a cooling sensation and reduces the oily feeling of the emulsion.

Emulgel hand sanitizer preparation uses a virgin coconut oil variation. Virgin Coconut Oil or VCO, is a fatty oil [8] that can be used as a cream base. VCO is a natural moisturizer that can prevent tissue damage and protect the skin.[5] VCO used in emulgel preparations has several benefits for the skin, which can be used as an emollient and moisturizer to soften and moisturize the skin, and can reduce its diffusion resistance.[5] This development is one of the innovations of non-alcoholic hand sanitizer products. Until now, garlic extract has not been widely used in hand sanitizer formulations in Indonesia.

METHODS

Equipment and Materials

The tools used included analytical scales (*precisa*®), beakers (*pyrex*®), rotary evaporators (*cole parmer*®), pH meters (*ohaus*®), viscometers (*rion viscotester* vt-04®), incubators (*memmert*®), microscopes (*Olympus cx 21*®), waterbath (*Grant*), and refrigerators (*Phillips*).

The materials used were dried garlic powder purchased at Gede Market (Surakarta); 96% ethanol (*Merck, Germany*); *Staphylococcus aureus* culture bacteria obtained from the Microbiology Laboratory of the Faculty of Medicine, UNS; MHA media (Muller Hinton Agar), aquadest (Merck, Germany), Chloramphenicol (Kimia Farma), Nutrient

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Agar medium (*Merck, Germany*), MHA media (*Merck, Germany*), 0.9% NaCl (Otsuka), FeCl3 (smartlab), HCl 2N (smartlab), VCO (smartlab), Tween 80 (*Duchefa, Netherlands*), Span 80 (*TPC, India*), Isopropanol (*Merck, Germany*), HPMC (Agung Jaya), and Propylene Glycol (*Merck, Germany*).

Work Procedures

Extraction Process

As much as 1000 g of dried garlic powder was added with 7500 mL of 96% ethanol (1:7.5) and left for ± 5 days while stirring occasionally.[9] It was then filtered using filter paper. The filtration results were evaporated using a rotary evaporator at a temperature of ± 50ºC to obtain a thick extract.

Measurement of Extraction Yield

The extract yield was calculated by dividing the weight of the extract obtained (g) by the weight of the initial simplicial (g), and the result is expressed as a percentage (%). Extract yield calculation formula [7]:

$$\%\ \text{Yield} = \frac{weight\ of\ the\ extact}{weight\ of\ the\ simplicial} X\ 100\%$$

Determination of Water Content

The water content of the extract was calculated using a Moisture Analyzer. A total of 1 g of extract ampule was placed on the aluminum foil previously placed on the tool and tared. Furthermore, the water content was measured by pressing the start button to obtain the percentage of water content.

Extract Stickiness Test

A total of 100 mg of thick extract was placed on a glass object, then covered with another glass object, and given a load weighing 1 kg for 5 minutes, the two glass objects were separated by pulling them using a pulley system with a certain weight assisted by clamps. The duration until the two glass objects were released must be recorded. [10, 11]

Emulgel Formulation of Garlic extract (*Allium sativum* L.)

Table 1. Emulsion formula for emulgel preparation of garlic extract

Name of Material	Function	F1 % (w/v)	F2 % (w/v)	F3 % (w/v)
Garlic extract	Active Ingredients	20	20	20
VCO	Moisturizer	3	5	7
Tween 80	Emulgator	23,1	23,1	23,1
Span 80	Emulgator	3,8	3,8	3,8
Isopropanol	Solvent	12,5	12,5	12,5
Aquadest ad	Solvent	100	100	100

Table 2. Gel formula for emulgel preparation of garlic extract

Name of Material	Function	Formula % (w/v)
HPMC	*Gelling Agent*	2
Propylene glycol	Humectant	5
Aquadest ad	Solvent	100

The making process of garlic extract emulgel (*Allium sativum* L.)

In making the emulsion, garlic extract was added with Span 80, VCO, and, finally, isopropanol as the oil phase. Tween 80 and water were then mixed as the water phase. The oil phase was added to the water phase little by little while being stirred slowly and constantly until an emulsion was formed.

The next stage was making a gel by dispersing HPMC in hot water at 20x the weight of HPMC and stirring until a gel base was formed. Propylene glycol was mixed in a gel base, and then the remaining water was added little by little. Next, the emulgel was made by mixing the emulsion and gel with a concentration ratio of 20:80 using a homogenizer at a speed of 700 rpm for 45 minutes until the emulgel was formed.

Physical testing of garlic extract emulgel (*Allium sativum* L.)

Organoleptic test of emulgel

Organoleptic observations were carried out using the five senses to determine the visual appearance of the emulgel preparation. Organoleptic

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observations included shape, odor, and color.

Emulgel homogeneity

Emulgel was weighed 0.1 g and then spread evenly and thinly on transparent glass; the preparation must show a homogeneous composition and no coarse grains are visible.[11]

Emulgel pH measurement

The emulgel was weighed 1 g, and dissolved in 10 mL of water, and its pH was measured using a PH-meter.[11]

Emulgel Spreading Test

A total of 0.5 g of emulgel was placed on a graduated round glass, covered with another round glass, and then left for 1 minute. It was given a load of 150 g, and the spreading diameter was recorded on 3 sides using a ruler. [13]

Emulgel adhesion test

An amount of 0.5 g of emulgel was placed on a glass object with a predetermined area. Then, another glass object was placed on top. The glass object was then mounted on the test equipment and given a load of 1 kg for 5 minutes. Then, it was released with a load of 80 g. The time was recorded until the two glass objects were released. [13]

Viscosity Measurement of Emulgel

The viscosity test was carried out using Rotor No. 2 of the Rion Viscosimeter VT-04 which was dipped into the emulgel. The viscosity was determined by observing the movement of the viscosity indicator needle pointed to a certain number. [12,14,15]

Antibacterial Activity Test

An antibacterial activity test was conducted on *Staphylococcus aureus* bacteria using the agar diffusion method with wells. The clear zone formed around the well was measured using a caliper. *Staphylococcus aureus* suspension was applied with sterile cotton buds on the surface of the media in a zigzag manner, covering all parts of the media. After that, it was incubated for 1 x 24 hours in an incubator at 37°C, and the clear zone formed was measured. Antibacterial activity testing uses the antibacterial strength classification guidelines according to Davis and Stout (1971) [12, 15] which are shown in Table 3.

Table 3. Antibacterial Power Category Based on Davis and Stout

Antibacterial Inhibitory Power	Antibacterial Inhibitory Power Category
≥ 21 mm	Very strong
11-20 mm	Strong
6-10 mm	Moderate
≤ 5 mm	Weak

RESULTS AND DISCUSSION

Extract Yield Testing

Yield is the ratio between the dry weight of the obtained product and the weight of the raw material. [16,17] The results of the study showed that the extract yield was 26%. The requirement for determining the yield is not less than 10%.[18] The yield of an extract is directly proportional to the bioactive compounds it contains, which is the higher the yield, the greater the content of bioactive compounds in the extract.[10]

Water Content Testing of the Extract

Determining the water content is necessary to provide a minimum limit or range related to the amount of water content in the extract. The higher the water content in the extract, the higher the possibility that the extract will be damaged since the high level of water content in the extract will become a medium for the growth of microorganisms, such as fungi and mold, reducing the biological activity of the extract during storage.[19] According to the literature, the water content for the extract should be less than 10%. The observation results obtained a water content of 4%. Based on these results, the water content of garlic extract is in a good category since the water content is <10%. [18]

Stickiness Testing of the Extract

The results of the observation of the viscosity test showed that the stickiness of the thick garlic extract was 75 s. The

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stickiness in the extract is caused by its resin content. The more polar content in the solvent used, the more resin is extracted, so the resulting extract is stickier.

Emulgel Organoleptic Test

Organoleptic testing aims to examine the preparation forms visually through the five human senses.[13] The results of organoleptic observations of the emulgel are presented in Table 4. Based on the results of the organoleptic test, it can be concluded that the concentration variation of VCO does not affect the color and odor of the emulgel preparation, all of which have a light brown color and a distinctive garlic odor. However, it affects the viscosity of the emulgel preparation: the viscosity of formula 1 is slightly thicker but tends to be more liquid than formulas 2 and 3.

Table 4. Results of organoleptic testing of garlic extract emulgel

Formula	Organoleptic parameters	Observation results
F1	Color	Light brown
	Odor	Distinctive garlic odor
	Viscosity	Slightly thick but tends to be liquid
F2	Color	Light brown
	Odor	Distinctive garlic odor
	Viscosity	Slightly thick
F3	Color	Light brown
	Odor	Distinctive garlic odor
	Viscosity	Slightly thick

Homogeneity test

The homogeneity test aims to ensure that the garlic extract emulgel is visually homogeneous. The observation results show that there are no coarse grains on the transparent glass, so the emulgel can be said to be homogeneous.

pH Test

The pH test aims to determine the pH of a preparation. The normal pH of the skin is 4.5-6.5.[20] The results of the pH test of the preparation are presented in Table 4.

Table 5. Table of pH testing of garlic extract emulgel

Formula	pH ± SD
1	5,80 ± 0,01
2	5,84 ± 0,01
3	5,98 ± 0,01

Differences in VCO concentrations produce different pH levels in the preparations, but as shown in Table 5, the pH of all preparations still meets the normal skin pH standard, which is 4.5 - 6.5, so this emulgel is safe to use.

Spreadability

The spreadability test is conducted to examine the spreadability on the skin surface when applied. In addition, the active ingredients spread on the skin more evenly so that the effect is more optimal.[18] The difference in spreadability highly influences the speed of diffusion of active substances through the membrane. The wider the membrane where the preparation is spread, the greater the diffusion coefficient, increasing the diffusion of the drug, meaning the greater the spreading power of a preparation, the better. The results of the spreadability test are presented in Table 6.

Table 6. Results of the spreadability test of garlic extract emulgel

Formula	Average (cm) ± SD
1	6,8 ± 2,02
2	5,6 ± 0,87
3	4,6 ± 1,18

The expected spreadability results for emulgel preparations range from 5 - 7 cm; this value means that the emulgel can be used well.[19]

Adhesion test

The adhesion test aims to examine how long the preparation can adhere to the skin. Good adhesion for topical preparations is more than 4 seconds.[20] Good adhesion will provide a maximum effect because the longer the preparation adheres to the skin, the more ideal the time for the drug to be released into and absorbed by the skin. The results of the adhesion test are presented in Table 7.

Table 7. Results of the adhesion test of garlic extract emulgel

Formula	Average (s) ± SD
1	1,72 ± 0,17
2	3.83 ± 0,17
3	5,11 ± 0,17

It can be seen that the higher the viscosity of the preparation, the greater the impact on the adhesive strength of the preparation. The force between the gel-forming particles is getting stronger. The adhesive power is directly proportional to the viscosity; the lower the viscosity, the shorter the time of the adhesive strength of the preparation.

Viscosity Measurement

The viscosity test is used to examine the thickness of a preparation. The test results are presented in Table 8.

Table 8. Results of the viscosity test of garlic extract emulgel

Formula	Viscosity (dPa.s) ± SD
1	112,22 ± 0,01
2	124,41 ± 0,01
3	166,60 ± 0,05

Based on the results of the viscosity test, there is a difference in viscosity in each formula: the higher the concentration of VCO preparation, the higher the viscosity. Viscosity is a measure of the resistance to flow of a system; thus, the thicker a fluid, the greater the force required for the fluid to flow.

Antibacterial Test

The antibacterial test aims to test the antibacterial activity of ethanol extract of garlic (*Allium sativum* L.) in emulgel preparation against the growth of *Staphylococcus aureus* bacteria. Ethanol extract of garlic (*Allium sativum* L.) contains secondary metabolite compounds, such as tannins, alkaloids, and saponins. Tannins can shrink cell membranes or cell walls that may disrupt bacterial cell permeability. Alkaloids can disrupt the peptidoglycan component in bacterial cells, so the cell wall is not formed perfectly. Saponins can damage the cytoplasmic membrane which leads to the cell membrane leaking.

Antibacterial testing uses samples F1, F2, and F3, each of which has a different concentration of VCO. Positive control uses chlorampHenicol and negative control uses emulgel formulation containing only gel base without any extract content.

The results of the antibacterial test showed that the diameter of the inhibition zone of the garlic extract emulgel preparation provided the highest inhibition power in Formula 1. The antibacterial activity test of *Staphylococcus aureus* showed that the diameter of the inhibition zone in all cases, based on the classification of inhibition strength according to Davis and Stout (1971), was included in the weak category because it was in the range of less than 5 mm.[14] The results of the antibacterial test for *Staphylococcus aureus* can be presented in Table 9.

Table 8. Antibacterial test results of garlic extract emulgel

Test Parameters	Inhibition power (mm) ± SD	Category
F1	4,11 ± 0,06	Weak
F2	2, 10 ± 0,06	Weak
F3	1, 04 ± 0,07	Weak
Control (+)	27,04 ± 0,02	Very strong
Control (-)	0	No activity

CONCLUSION

The concentration variation of VCO in emulgel preparations affects the difference in the physical properties of the gel, including PH, viscosity, adhesive strength, and spreadability, where the higher the VCO concentration, the higher the PH, the higher the viscosity and adhesive strength, the lower the spreadability, and the lower the antibacterial inhibitory power against

Staphylococcus aureus. The best result of the evaluation of physical and chemical properties is F1. The results of the antibacterial test showed that the diameter of the inhibition zone of the garlic extract emulgel preparation provided the highest inhibition power in Formula 1 but in the weak category.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by the authors.

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