

## POTENT ANTIBACTERIAL OF GEL KAWANG FRUITS (*LITOCARPUS CELEBICUS*, (MIQ.) REHDER) ETHANOL EXTRACT ORIGIN FROM PAPUA INDONESIA

SORAYA RATNAWULAN MITA<sup>1\*</sup>, SRI AGUNG FITRI KUSUMA<sup>2</sup>, NURAINI INSIYAH<sup>1</sup>

<sup>1</sup>Department of Pharmaceutic and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. <sup>2</sup>Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. Email: soraya@unpad.ac.id

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### ABSTRACT

**Objective:** Kawang fruit (*Lithocarpus celebicus* (Miq.) Rehder) is native plant of Boven Digoel, Papua, Indonesia, have flavonoids which has antibacterial activity. Antiseptic is a substance used to inhibit the growth or destroy microorganisms that live on the surface of the body. Their resistance to antiseptics on the market and it needed a new antiseptic-based natural ingredients that are relatively safer. The purpose of this study to make an antiseptic gel with extract ethanol of Kawang fruit and determine its effectiveness against *Staphylococcus aureus* ATCC 29 213 and *Escherichia coli* ATCC 25922.

**Methods:** Extraction, phytochemical screening, testing activities with the agar diffusion method, determination minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC), antiseptic gel formulation, and test preparation gel. The test results showed the Kawang fruit has activity against bacteria with value MIC and MBC on *S. aureus* ATCC 29213 is 5% (b/b) and 10% (b/b), and on *E. coli* ATCC 25922 is 10% and 20%. The extraction is formulated in the form of a gel with a concentration of 5%, 10%, and 20%. Formulations made using 2% hydroxy ethyl methyl cellulose.

**Results:** The test results show the effectiveness of antiseptic gel extracts have inhibitory better on *S. aureus* ATCC 29213 than *E. coli* ATCC 25922.

**Conclusion:** Antiseptic gel preparation of Kawang fruit ethanol extract at a concentration of 5% have shown their antibacterial effectivity. Its inhibition to *S. aureus* ATCC 29213 was better than *E. coli* ATCC 25 922.

**Keywords:** Antiseptic gel, *Lithocarpus celebicus* (Miq.) Rehder, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922.

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### INTRODUCTION

Skin is the most outer organ of human which can serve as the first defense from external attacks such as germs, bacteria, viruses, and so on. The more often skin is exposed by microorganisms can cause infections of the skin. If not taken seriously can be fatal, even microorganisms can enter systemically through the food on hand that have been infected [1]. The prevalence of skin diseases in Indonesia is 1.4% in adults and 0.2% in children [2].

As a prevention against infection from bacteria, antiseptic preparations can be used to kill bacteria on the surface of the hand. Antiseptic gel is already widely used in the community. Gel is a preparation that is commonly used as a topical drug carrier that is semisolid and used on the skin [3]. Increased use of antiseptic in community cause an increasing degree of resistance to antibacterial. The resistance can increase the severity of the infection and the risk of death [4]. Therefore, another antiseptic-based natural ingredients is needed to be used as an alternative to prevent infection.

Another research results showed that *Lithocarpus celebicus* (Miq.) Rehder contains flavonoids, polifenolat, alkaloids, quinones, and saponins that have antibacterial activity, so it can be new antiseptic candidate as the basis antibacterial development [5]. Kawang fruit can be found in the woods Boven Digoel, Papua. Papua has a forest with pristine habitat, so it has a huge potential to be used as a basic ingredient of drugs. However, the development of science is still very lack so underutilized by the general public [6].

Therefore, this research is aim to develop the science of Kawang fruit particularly the antibacterial activity. The bacterial that is used are Gram-positive bacteria *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 as Gram-negative bacteria. The use of both

bacteria is intended to determine the antibacterial action spectrum of the ethanol extract of the Kawang fruit and to test the effectiveness of antiseptic gel formulation Kawang fruit ethanol extract [4].

### MATERIALS AND METHODS

#### Plant collections and determination

Plant material used is the Kawang fruit dried at room temperature. Fruit is milled until smooth and then stored in a sealed container in a dry place. Determination of Kawang fruit is performed in the Laboratory of Herbarium Biotechnology Research Center - LIPI Cibinong, Bogor, West Java, Indonesia.

#### Extraction

Simplicia powder included in macerator, then add enough 70% ethanol and left for approximately 10 minutes so the ethanol is wetting the simplicia. Allowed to stand for 24 hrs while stirring occasionally. The extraction process is done 3×24 hrs. Liquid extract obtained is collected and concentrated by rotary evaporator at a temperature of 40°C to obtain a thick extract. Condensed extract heated over a water bath at a temperature of 50°C to remove residual solvent.

#### Examination parameter extract

Examination parameters that are conducted including physical appearance of extract using the senses to describe the shape, color, smell, and taste of the extract obtained. Rendement of extract amounts of viscous extract mass from the extraction process is divided initial weight of simplicia multiplied by 100%, with the following calculation. The density of extract is conducted by weigh empty pycnometer, then pycnometer is filled with water and weighed again. The density of water can be calculated. Then, the pycnometer is filled by extract to the full, and then weighed. The water content of extract is determined by water vapor distillation method using toluene. Condensed extract

5 g put in a clean and dry flask was added 200 ml of toluene, and then connected to a distillation apparatus. Flask is heated at a temperature of 60°C, allowed to boil and left for 1 hr. The water content is calculated in % w/v.

### Secondary metabolite screening

Screening of secondary metabolite content of alkaloids, flavonoids, quinones, monoterpenoid-sesquiterpenoid, saponin, steroid-triterpenoids, and tannin-polyphenols of ethanol extract in Kawang fruit.

### Identification of bacteria

Identification of bacteria were performed with Gram stain under a microscope and biochemical tests include motility test, carbohydrate fermentation test, indole test, triple sugar iron agar test, urease test, methyl red test, voges prokauer test, and citrate test.

### Activity test of Kawang fruit ethanol extract

Kawang fruit extracts antibacterial activity test was performed by the agar diffusion method with perforation technique. Some extracts dissolved in Dimethyl sulfoxide to obtain a concentration variation of as much as 50%, 40%, 30%, 20%, and 10%. A total of 20 µl bacterial suspension are included in two sterile Petri dish diameter of 10 cm, MHA poured as much as 20 ml and allowed to solidify.

Medium in each Petri dish is divided into four zones are then made holes by using the perforator. Into each hole, put Kawang fruit ethanol extract as much as 50 µl and then incubated for 18-24 hrs at a temperature of 37°C. The antibacterial activity is indicated by the formation of inhibition zone around the hole in the test medium. Inhibition zone diameter was measured using a caliper.

A total of two sterile Petri dish diameter of 5 cm was prepared and included 20 ml MHA is still liquid. First as a Petri dish containing MHA negative control, a second Petri dish containing medium as a positive control and added 20 µl of bacterial suspension. After that both Petri dish shaken gently until homogeneous, and the medium allowed to solidify.

### Determination of minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) of Kawang fruit extract

Determination of MIC was carried out by microdilution method in 96 wells microtiterplates. The first column in microtiterplates 96 wells filled with a solution of 100 µl of the extract and MHB media as a negative control. The second column is filled with MHB as a negative control. The third column until eleven were filled with 100 µl of MHB media. Twelfth column was filled with 100 µl of MHB and the bacterial suspension. The third column was added 100 µl of the extract with a concentration of 40%. Dilution process done by pipette 100 µl of stock solution of the third column to the fourth column, from the fourth column to the fifth column, and so on until the eleventh column, then 100 µl solution of the eleventh column was discarded. After that, the suspension of test bacteria put into each well, except wells in the first column and the second column. Microtiter plate was covered and incubated for 18-24 hrs at 37°C.

MIC value was determined from the column with the smallest concentration of ethanol extract showed no growth of test bacteria that column provides the last clear and first turbid. Then, on the both tube, the MBC value was determined by inoculating 1 µl solution of each hole that does not show growth of test bacteria in a Petri dish containing 5 ml MHA then incubated for 18-24 hrs at 37°C. MBC ethanol extract of the test bacteria is determined from a Petri dish with the smallest concentration of the ethanol extract showed no bacterial growth test.

### Design and formulation antiseptic gel preparation

Design and antiseptic gel formulation is conducted by two stages of determining the orientation of the base formulation could be seen in Table 1.

- Orientation base  
The base used is hydroxy ethyl methyl cellulose (HPMC), Viscolam, and Carbopol.
- Gel formulation of ethanol extract Kawang fruit  
Gel formulations using HPMC base with variation extract concentration according MIC value of testing against bacteria. The formulation was done (Table 2).

### Effectiveness test of Kawang fruit antiseptic gel

Testing the antibacterial effectiveness of the Kawang fruit ethanol extract gel was conducted using cylinders method. Prepared ethanol extract antiseptic gel formulation to be tested. A total of MHA as much as 20 ml and allowed to solidify, put 20 µl bacterial suspension test and flattened with a split. Cylinder was cleaned using ethanol, then dried. Into each cylinder, ethanol extract gel Kawang fruit is inserted, then affixed to the surface of the media. Petri dishes were incubated for 18-24 hrs at a temperature of 37°C. The antibacterial effectiveness is shown by the formation of inhibition zone around the cylinder in the test medium. Inhibition zone diameter was measured using a caliper. A total of two sterile Petri dish diameter of 5 cm was prepared and included 20 ml MHA that is still liquid. First as a Petri dish containing MHA negative control, the second Petri dish containing medium as a positive control and added 20 µl of bacterial suspension. After that, both Petri dish shaken gently until homogeneous, and the medium allowed to solidify.

### Pharmaceutics evaluation of antiseptic gel preparations

Evaluation of antiseptic gel preparation was conducted for 28 days.

- Physical appearance  
Observations were conducted organoleptically to see changes in the shape, color, and smell of the gel. Observations were done during 28 days.
- Measurement of pH  
Preparations were observed changes in pH during storage at a temperature of 25°C. Observation pH changes made by dipping a special pH universal into stocks and then change of the color was compared to the color of the packaging. Measurements were made for 28 days.

Table 1: Orientation of base formulation

Compositional	Formula (% b/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
HPMC	1	2	2.5						
Glycerine	10	10	10				10	10	10
PPG	10	10	10				10	10	10
TEA	2	2	2	3	3	3	2	2	2
Viscolam SMC 20				20	15	25			
Carbophol 940							0.5	1	1.5
Aqua dest ad	100	100	100	100	100	100	100	100	100

PPG: Propileneglycol, HPMC: Hydroxypropyl methylcellulose,  
TEA: Triethanolamine, SMC 20: Steareth-20 methacrylic acid

Table 2: Formula of antiseptic gel Kawang fruit extract

Compositional	Formula (% b/b)		
	F1	F2	F3
Kawang fruit extract	5	10	20
HPMC	2	2	2
PPG	10	10	10
Glycerine	10	10	10
TEA	2	2	2
Sodium benzoat	0.02	0.02	0.02
Aquadest	100	100	100

F1: Concentration of extract from 1×MIC (5%), F2: Concentration of extract from 2×MIC (10%), F3: Concentration of extract from 4×MIC (20%),  
MIC: Minimum inhibition concentration, HPMC: Hydroxy ethyl methyl cellulose,  
*L. celebicus*: *Lithocarpus celebicus*, HPMC: Hydroxy ethyl methyl cellulose,  
PPG: Propileneglycol

## RESULTS AND DISCUSSION

### Determination of plant Kawang (*L. celebicus* (Miq.) Rehder)

Kawang fruit was obtained from Diegoel forests, Papua. The fruit has a brown skin with round shape and tapered at the end and also has white content inside. Determination Kawang plants were conducted at the Laboratory of Research Center for Biology LIPI, Cibinong-Bogor with the results stating the classification of Kawang fruit including in the kingdom *Plantae*, division *Spermatophyta*, subdivision *Angiosperms*, class *Dicotyledonae*, subclass *Hamamelidae*, Order *Fagales*, Family *Fagaceae*, Type *Lithocarpus* and species *L. celebicus* (Miq.) Rehder.

### Extraction and determination of parameter Kawang fruit ethanol extracts

Kawang fruit simplicia that was finely grounded was extracted for separating chemical components in simplicia by maceration method. This method was chosen to prevent damage to metabolites that are not heat resistant. According to the herbal pharmacopeia for dried plant extraction is good using 70% ethanol solvent which is a mixture of alcohol and water that have good power to attract natural compounds. During the extraction, the solvent was replaced continuously and collected.

The extraction product was brown liquid extract. The liquid extract was evaporated using evaporator with a temperature of 60°C with a speed of 70 rpm, then thickened using water bath so that ethanol evaporated and separated from the extract and viscous extract was formed. Ethanol has antibacterial activity that can inhibit the growth of bacteria that would negatively affect the test results of the extract.

The quality of the extract can be seen from the extract physical parameter such appearance, rendement, and water content of the extract [7]. Physical appearance of the Kawang fruit ethanol extract result are the extract has a brownish black color, with a characteristic odor and viscous form. Simplicia that was used was 200.99 g with viscous extract obtained was 8.41 g. Results of rendement viscous extract are 4.18% with density of the liquid extract was 0.89 g/ml. The outcome of this extract rendement will affect the content of compounds present in the extract. The water content of the extract is done with toluene distillation method with the result is 0.9%. The water content of the extract already meets the requirements of a standardized extract which extract water content must not exceed 10%. The water content should be <10% to avoid contamination from bacterial and fungal growth due to too much water.

### Secondary metabolite screening

Phytochemical screening is an early stage to identify the secondary metabolites contained in the extract. The result of phytochemical screening of the Kawang fruit ethanol extract (*L. celebicus*) can be seen in Table 3.

Phytochemical screening results showed the content of the metabolites in Kawang fruit extract including alkaloids, flavonoids, polyphenols, quinones, and saponins. The compounds above belongs to a class of phenolic compounds that have antibacterial activity [8].

Flavonoids can damage bacterial cell wall permeability, microsomes, and lysosomes and tend to inhibit the enzyme activity of microbes that ultimately disturb the metabolism of microbes. Polyphenols work by denaturation of proteins and interfere with the function of the cell membrane, thus becoming lysis [9]. Saponins can increase the permeability of the bacterial cell membrane and denature proteins capable of causing lysis [10].

### Identification of bacteria results

Observation of cell morphology was done by Gram staining method. Based on the results, bacteria *S. aureus* ATCC 29213 was included in the Gram-positive cocci characterized by purple shape. Gram-positive

bacteria are bacteria that retain the color of methyl violet during the gram stain so that it showed purple color under the light of microscope. *E. coli* ATCC 25922 is red rod-shaped. Gram-negative bacteria are bacteria that do not retain the purple color during staining, so it will be red under the microscope [11].

Biochemical test is performed to determine the interaction of metabolites produced by certain chemical substances to know the ability of bacteria using certain compounds as a source of carbon and energy source. The results of biochemical tests carried out on bacteria *S. aureus* ATCC 259213 and *E. coli* ATCC 25922 are listed in Table 4.

The results of biochemical tests of the bacteria *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 showed that the bacteria in accordance with the literature (Holt, 2005).

### Kawang fruit ethanol extract activity test

Antibacterial activity test was done to see their antibacterial activity of Kawang fruit extracts. Tests were performed by the Agar diffusion method with well was prepared to insert the sample. Samples will diffuse in the surrounding wells and will form a zone of inhibition. Their inhibition zone showed the presence of bacterial activity in the test. The result of the activity test is shown in Table 5.

Based on the results of the data in Table 5, it can be seen that the Kawang fruit extract has antibacterial activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. The higher the concentration, the greater the inhibition zone diameter. This shows the concentration of the sample is directly proportional to the antibacterial activity of the sample. The growth of bacteria in the sample can be compared to positive control visually.

**Table 3: Result of phytochemical screening of the Kawang fruit ethanol extract (*L. celebicus*)**

Test	Result
Alkaloids	+
Flavonoids	+
Polyphenol	+
Tannin	-
Monoterpenoid	-
Sesquiterpenoid	-
Steroid	-
Triterpenoid	-
Quinone	+
Saponin	+

+: Detected, -: Undetected, *L. celebicus*: *Lithocarpus celebicus*

**Table 4: Biochemical test *S. aureus* ATCC 259213 and *E. coli* ATCC 25922 bacteria results**

Test	<i>S. aureus</i> ATCC 29213		<i>E. coli</i> ATCC 25922	
	Result	Bergey's	Result	Bergey's
Motility	+	+	+	+
Glucose	+	+	+	+
Lactose	-	-	+	+
Manose	-	-	+	+
Maltose	+	+	+	+
Sacarose	-	-	+	+
Indol	+	+	+	+
TSIA	-	-	+	+
Urea	+	+	-	-
MR	+	+	+	+
VP	-	-	-	-
SC	+	+	-	-

MR: Metil red, VP: Voges-Proskauer, -: Unreacted, +: Reacted, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, TSIA: Triple sugar iron agar, SC: Simon Citrate

Based on the results of the data in Table 5, it can be seen that the Kawang fruit extract has antibacterial activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. The higher the concentration, the greater the inhibition zone diameter. This shows the concentration of the sample is directly proportional to the antibacterial activity of the sample. The growth of bacteria in the sample can be compared to positive control visually.

To find the highest antibacterial response from the ethanol extract of Kawang fruit can be seen in Fig. 1. Graph showing increased inhibition zone formed along with an increase in the concentration of Kawang fruit extracts. The antibacterial activity of Kawang fruit extracts provides a better response to *S. aureus* ATCC 29213 than with *E. coli* ATCC 25922.

#### Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Kawang fruit ethanol extract

Minimum inhibitory concentration of the Kawang fruit ethanol extract was done by microdilution method using micropipette well 96. MIC was known from the concentration of the last clear media and the first turbid. MIC results can be seen in Table 6.

Based on MIC observations of Kawang fruit ethanol extract (*L. celebicus* (Miq.) Rehder) in bacteria *S. aureus* ATCC 29213 contained at a concentration of 5-10%, while for the bacterium *E. coli* ATCC 25922 present in concentrations of 10-20%.

The MBC test results of the Kawang fruit ethanol extract were done by taking 1 µl of MIC value and grown in a dish with solid media (MHA). Determination of MBC is seen from the absence of bacterial growth in solid media. The results can be seen in Table 7.

Before the observations, calculations were done to see a minimal amount of colony forming unit (CFU) that is a prerequisite for the MBC. Based on the results of the calculation of the minimum number of CFU ranges that may grow on the medium is 0.01% of the bacteria are grown in the cup which means that  $0.25 < 1$ .

Terms of MBC value in this test is the absence of bacteria that may grow on the medium. Based on MBC observations of the Kawang fruit ethanol extract (*L. celebicus* (Miq.) Rehder), the absence of bacterial growth of the *S. aureus* ATCC 29213 at concentration of 10% while for the bacteria *E. coli* ATCC 25922 present in concentrations of 20%. Determination of the dose based on the smallest value of the MIC which is the smallest dose to inhibit the growth of bacteria. Then, the minimum dose used was 5%.

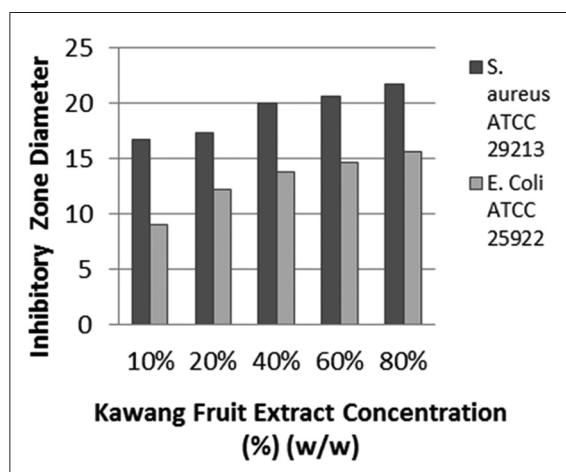


Fig. 1: Kawang fruit activity test against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922

#### Orientation gel base

Before creating formulations, orientation of base was done to determine which base that can be used. Orientation was done by physical evaluation such as physical appearance, pH and viscosity.

#### Kawang fruit ethanol extract (*L. celebicus* (Miq.) Rehder) antiseptic gel formulation

Selection of antiseptic gel formulations for the preparations was done after the base orientation for determining the appropriate gel base. Based on observations, the selected gel base was HPMC at a concentration of 2%. Preparation antiseptic gel with varying concentrations of Kawang fruit extracts. Formulation antiseptic gel in different extract concentration could be seen in Table 8.

Table 5: The average diameter of inhibitory zone Kawang fruit ethanol extract against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922

Extract concentration (w/w)	Average of inhibitory zone diameter (mm)	
	<i>S. aureus</i> ATCC 29213	<i>E. coli</i> ATCC 25922
80%	21.8	15.6
60%	20.7	14.7
40%	20.0	13.8
20%	17.4	12.2
10%	16.7	9.0
Control (+)	+	+
Control (-)	-	-

(+): Growing the bacteria in media, (-): Bacteria growth not detected on media, Well diameter=8 mm, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 6: Result of MIC Kawang fruit ethanol extract against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922

Extract concentration (% w/w)	<i>S. aureus</i> ATCC 29213	<i>E. coli</i> ATCC 25922
0.15625	+	+
0.3125	+	+
0.625	+	+
1.25	+	+
2.5	+	+
5	-	+
10	-	-
20	-	-
Extract	-	-
Positive control	+	+
Negative control	-	-

-: Clear (bacteria growth not detected), +: Turbid (growing bacteria), MIC: Minimum inhibition concentration, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 7: Results of MIC Kawang fruit ethanol extract against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922

Extract concentration (w/w %)	<i>S. aureus</i> ATCC 29213	<i>E. coli</i> ATCC 25922
20	-	-
10	-	+
5	+	++
2.5	++	+++
Extract	-	-
Positive control	+	+
Negative control	-	-

Positive (+) growing bacteria, Negative (-): Bacteria growth not detected, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

**Table 8: Formulation antiseptic gel with variation concentration of Kawang fruit extract (*Lithocarpus celebicus* (Miq.) Rehder)**

Composition	F0 (w/w %)	F1 (w/w %)	F2 (w/w %)	F3 (w/w %)
Extract	-	5	10	20
HPMC	2	2	2	2
Propyleneglycol	10	10	10	10
Glycerin	10	10	10	10
Tryethanolamin	2	2	2	2
Sodium benzoat	0.2	0.2	0.2	0.2
Aquadest ad	100	100	100	100

F0: Without Extract, F1: Extract 1×MIC (5%), F2: Extract 2×MIC (10%), F3: Extract 3×MIC (20%), MIC: Minimum inhibition concentration, HPMC: Hydroxypropyl methylcellulose, *L. celebicus*: *Lithocarpus celebicus*

#### Effectiveness test of antiseptic gel Kawang fruit ethanol extract against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 bacteria

The effectiveness test of the preparation on *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 bacteria were done using cylinder. The activity against bacteria indicated by their inhibition zone in bacteria. Observations were made at the beginning and end of the observation. The inhibitory zone diameters of effectiveness test result for *S. aureus* were demonstrated in Table 9.

Based on observations from both table can be seen that no significant change from the early observations to the last observation. At the F0 which base without active substance (Kawang fruit extract) did not show any inhibitory zone. This indicates that in the base preparation there is no substance that has antibacterial activity. In F1, F2, and F3 has shown a good antibacterial activity on the bacteria *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. The higher the concentration of the extract in the preparation the greater inhibitory diameter was formed. The values on Kawang fruit extract is greater than the gel preparation and it can happen due to extract is more liquid compared to gel so that can be easily diffuse at medium and killing bacteria around the hole bigger. While the inhibitory zone diameters of effectiveness test result for *E. coli* were demonstrated in Table 10.

#### Evaluation of Kawang fruit (*L. celebicus* (Miq.) Rehder) ethanol extract antiseptic gel

The evaluation of antiseptic preparation was done physically with physical appearance observation, pH measurement, and total lung capacity.

#### Physical appearance

Observations were done to see the shape, color, and smell of antiseptic gel formulation Kawang fruit extracts. This was done to see the stability of the appearance of preparation when stored at room temperature. The appearance of gel antiseptic on first day after formulation could be seen in Table 11.

#### pH measurement

pH measurements was carried out for 28 days. Observations can be seen in Fig. 2.

Gel is a topical dosage form where is used in the skin. Gel preparations must meet the skin's pH range that is 4.5-6.5. For formulas, F1 and F2 by variation of the concentration of Kawang fruit extracts 5% and 10% during the storage period they meet the desired pH range. Formula F3 during earlier storage they meet the desired pH range but after day 14 the pH continues to fall and did not meet the desired pH range. This can lead to decreased stability of the preparations. This can be seen in the physical appearance observation after 14 days gel preparation begin to form watery gel.

#### CONCLUSION

The result of this study suggested that antiseptic gel preparation of Kawang fruit ethanol extract at a concentration of 5% have shown their antibacterial effectivity. Its inhibition to *S. aureus* ATCC 29213 was better than *E. coli* ATCC 25922.

**Table 9: Effectiveness test of antiseptic gel formulation Kawang fruit extract against *S. aureus* ATCC 29213**

Concentration (%)	Average of inhibitory zone diameter (cm)			
	Beginning		End	
	Gel	Extract	Gel	Extract
0	-	-	-	-
5	2.09	2.19	2.14	2.17
10	1.98	2.38	2.20	2.41
20	2.41	2.55	2.47	2.52

*S. aureus*: *Staphylococcus aureus*

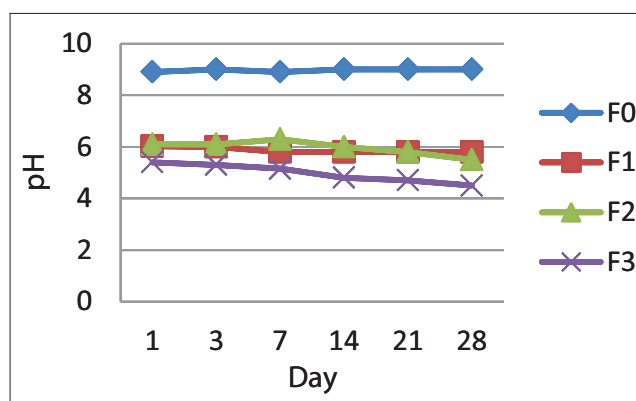
**Table 10: Effectiveness test of antiseptic gel formulation Kawang fruit extract against *E. coli* ATCC 25922**

Concentration	Average of inhibitory zone diameter (cm)			
	Beginning		End	
	Gel	Extract	Gel	Extract
F0	-	-	-	-
F1	0.95	1.10	1.02	1.32
F2	1.49	1.44	1.55	1.57

*E. coli*: *Escherichia coli*, Well diameter: 0.8 cm

**Table 11: Physical appearance antiseptic gel day-1 result**

Gel	Appearance		
	Form	Smell	Color
F0	Gel	Odorless	Transparent
F1	Gel	Typical extract	Black
F2	Gel	Typical extract	Black
F3	Gel	Typical extract	Brown

**Fig. 2: Graph of antiseptic gel pH changes during storage**

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