

## Cream Formulation Of Extract Of Maja Leaves (*Crescentia cujete*) As An Antimicrobial Against *Staphylococcus aureus*

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### ABSTRACT

*Maja (Crescentia cujete L)* is a plant that has medicinal properties for various diseases. Leaves of *Maja* contain some compounds such which are thought to have the potential to be antibacterial. The purpose of this study was to determine the potential of *maja* leaf extract in cream dosage form and to prove the presence of Antibacterial activity against *Staphylococcus aureus*. The cream of *Maja* leaf extract was made into several formulations; (5, 10, 15 %) The results was the cream of *Maja* leaf extract was able to inhibit the growth of *S. aureus* with the largest inhibition zone at a concentration of 15% with an average of  $11.52 \pm 0.95$ mm. Cream formulations were evaluated physically; organoleptic was resulting F1 in brownish-green, F2 in brown, and F3 in dark brown. Homogeneity tests show that all formulations were homogeneous. The pH value of all formulations following the cosmetic standard was about 4.5 – 6.5. The standard viscosity value was between 2,000 – 4,000 cps. The stability test of cream preparations shows no changes in the form of low-temperature storage (4°C), room temperature, and high temperature (40°C), so that *maja* leaf extracts can be formulated in cream preparations and cream formulations.

### Introduction

*Maja (Crescentia cujete L)* is one of the plants that is often used as traditional medicine. *Maja* leaves are used for diuretics and treating tumors, but they are also used to antibacterial for several infection of bacteria (Hartati et al., 2017). Based on the screening of phytochemicals and the identification of the main components of *maja* leaves extract. Leaves of *maja* positively contains alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins (Dewi et al., 2014).

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Infection is caused by the entry of microbes or parasites or even metabolites from a microbe into the host cell and causes physiological disturbances in the cells. Examples of microbes that cause infection include *Staphylococcus aureus*. *S. aureus* is often found as normal bacterial flora on the skin and mucous membranes of humans. Can be a cause of infection both in humans and in animals. Some of these bacteria can make enterotoxins that can cause food poisoning. Any tissue can be infected and cause disease

with characteristic signs, namely inflammation, necrosis, and abscess formation (Ardianti & Kusnadi, 2014).

Research results on the antimicrobial activity of *Crescentia cujete* leaf extract can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* with an inhibition zone of  $7.07 \pm 0.44$  mm in *S.aureus*,  $6.70 \pm 0.46$  mm in *E. coli* and  $6.77 \pm 0.72$  mm in *C. albicans* (Amrulloh et al., 2021; Hartati et al., 2017). Another study also states that maja leaf extract has antibacterial activity against the growth of *Ralstonia solanacearum* with optimal concentrations of 85% and 95% with inhibition zones of  $11.4 \pm 0.50$  mm and  $12.4 \pm 1.32$  mm.

The use of maja leaves to cure microbial infections on the skin can be facilitated by making a cream dosage form. Cream preparations have several advantages including; simple in making, easy to apply, able to stick to the surface where it is used for a long time to reduce the risk of further inflammation and provide a cool feeling to the skin. The purpose of this study was to determine the potential of maja leaf extract in cream dosage form and to prove the presence of Antibacterial activity.

## Materials and Methods

### Phases of Research

This study was designed for 1 year, including 7 research phases as follows: preparation of simplicia (simplicia characterization and standardization), preparation of maja leaf extract (characterization and standardization extract), preparation of cream, test of the physical properties of the cream preparations, the stability test of the cream preparations and antibacterial tests.

### Research Samples

The samples of this study were mature Maja leaves (dark green in color), and healthy (no yellow and brown spots on the leaves) were taken in Sukaratu village,

Pagelaran sub-district, Pringsewu district. Samples were taken using the random sampling technique.

### Preparation of Ethanol Extracts of Maja Leaves

Maja leaves was dried using an oven for 4 hours with a temperature of  $50^{\circ}$ – $60^{\circ}$ C. Dry skin was disortated, then made into powder and sifted with mesh no. 40, weighed as much as 300 grams. The leaf powder of the maja was dissolved in ethanol 70% as much as 500 ml and then closed, left for 5 days and stirred every day. Further filtered, the dreg was macerated for 1 day for a more perfect extraction withdrawal. The extracts obtained were collected and concentrated on the evaporator and evaporated in the water bath to obtain a condensed extract (Departemen Kesehatan RI, 2000).

### Formulations of Cream Preparations of Maja Leaf Extracts

The oil phase ingredients (Vaseline album and propylparaben) and the water phase (Maja leaf extract, glycerin, methylparaben, and distilled water) were separated. The oil phase and water phase are heated to a temperature of  $70$ – $80^{\circ}$ C. After everything melts, the water phase is put a little bit into the oil phase, stirring slowly until a cream base forms, stirring until it is homogeneous, and put into the container. The next step was to test the cream preparation's physical properties, which included organoleptic tests, homogeneity, pH, dispersibility, adhesion, and consistency. In addition, the stability test of cream preparations was also carried out with the Cycling test, storage at low temperatures, storage at room temperature, and storage at high temperatures, which can be seen in Table 1.

### Non-Specific Simplicia Characteristic Test

A test of the simplicia characteristics carried out included a test of non-specific parameters. The minimum limit of standard tests performed were three tests such as moisture content, ash content and ash content that is not soluble in acids (Depkes RI, 1995).

**Table 1. Formulation of cream preparations**

Materials	Formula (gram)			
	F0	F1	F2	F3
Maja Leaf Extracts	-	10	15	20
Cetyl Alcohol	3	3	3	3
Stearic acid	10	10	10	10
Glycerin	6	6	6	6
Vaseline album	10	10	10	10
Methyl Paraben	0.02	0.02	0.02	0.02
Propyl Paraben	0.18	0.18	0.18	0.18
Propylen glycol	8	8	8	8
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

### 1. *Moisture determination*

Enter 10 grams of simplicia and weigh carefully in the container that has been tested. Then the cup and sample was dried at 105°C for 5 hours, cooled and weighed, continued drying and weighing at a distance of 1 hour until the difference between 2 consecutive weighing of no more than 0.25%, and moisture content not more than 10%. Moisture content can be calculated using the formula (Depkes RI, 1995).

$$\frac{\text{Initial weight of simplisia} - \text{average weight}}{\text{weight of simplisia}}$$

### 2. *Determination of ash content*

As much as 2–3 grams of simplicia was crushed and weighed thoroughly, put into the silicate block, then flatten. Samples were rolled slowly until the charcoal ran out, chilled and weighed. If in this case, charcoal cannot be eliminated, filter it by using ash-free filter paper. Rub until the weight was fixed, then weighed. Calculated the ash content that is not soluble in acid against the material that is dried in air, ash contents not more than 8.6%. Ash contents can be calculated using the formula (Depkes RI, 1995).

$$\text{Ash content} = \frac{\text{Ash weight}}{\text{Simplisia weight}} 100\%$$

### 3. *Determination of insoluble ash content in acid*

Ash obtained at the determination of its content, were boiled with 25 mL aqueous HCl for 5 minutes. The insoluble part in the acid was collected, strained with a filter paper, washed with hot water, and rubbed. Calculated the insoluble ash content in acid against the material that has been dried in air, the insoluble ash content is not more than

2.9%. The insoluble ash levels of acids can be calculated using the formula (Depkes RI, 1995).

$$\text{Acid insoluble ash} = \frac{\text{Ash weight}}{\text{Simplisia weight}} 100\%$$

### **Antibacterial Test**

The test for antibacterial activity used the diffusion method. A 100 µl bacterial suspension was poured into a petri dish then add NA media, homogenize it by moving the petri dishes to form figure eight, then let it solidify. Several holes were made in the media using a blue tip. Then input the three formulations, the antibiotic gentamycin as a positive control and cream base as a negative control into the holes. Then it was incubated for 24 hours at 37°C and the resistance zone formed was measured.

### **Data analysis**

Analysis of test data for physical properties of cream preparations which included organoleptic tests, homogeneity, pH, dispersibility, adhesion and consistency were carried out descriptively based on Indonesian Pharmacopoeia. Meanwhile, the results of the stability test of cream preparations with the Cycling test were also analyzed based on the resulting descriptions and presented in tables and graphs.

## **Results and Discussion**

### **Simplisia Characteristic Test**

Testing characteristic of simplicia that has been performed was a test of non-specific parameters with a minimum test limit that was three tests included the moisture content, ash content and an insoluble ash content. The test results of non-specific characteristics of simplicia can be seen in Table 2.

**Table 2. Test Results of Simplicia Characteristics**

Parameter	Result	Term
Moisture Content	8.4%	≤10%
Ash Content	6.6%	≤8.6%
Acid Insoluble Ash Content	2.3%	≤2.9%

Determining moisture contents of simplicia aims to provide minimal or range limitation on the amount of the water content in the ingredients. The water content test used the gravimetric method and the water content of maja leaf obtained 8.4% of the water content that has been fulfilled the quality of the prescribed simplicia standard (less than 10%). Determination of the ash content and acid insoluble ash content aimed to provide an overview of the content of internal and external minerals originating from the beginning until the formation of extracts. The ash content obtained at 6.6%, the results obtained has fulfilled the quality standard of the simplicia set (8.6%). The acid insoluble ash content of maja leaf has fulfilled the standard obtained by the result of 2% of the prescribed standard of no more than 2.3%. Simplicia that has passed the characteristic test has been stabilized and proved that the simplicia of maja leaf is simplicia which has a moisture content and good internal and external mineral content (Departemen Kesehatan RI, 2000).

**The Evaluation Result of Cream Preparations**

On the observation of organoleptic, the maja leaf extract was done to assess shape, smell and color. The preparation forms for all four formulations were a cream, in accordance with its definition that is a clear semi-solid preparation (no bubbles), translucent and contains active substances. The resulting smell of the three formulations was the distinctive smell of maja leaf extracts. The colors produced were distinctive brown as a typical extract (Figure 1). This can be seen in Table 3.

This test aims to observe the alteration of shapes, colors and smells. After testing all formulations with the appropriate form of cream, the resulting color for the cream base is without clear colored extracts and for all three colored formulations as well as the

resulting odor of the three formulations smelled like typical extracts. From Figure 1, it can be seen that organoleptically, the more the concentration of the extract, the darker the cream color. The color that appears on the cream matches the color of the Maja leaf extract.



**Figure 1. Organoleptic Result of Maja Cream**

**Homogeneity Test**

A homogeneity test is performed to see if the preparation has been made homogeneous or not. The way to figure it out is by applying it to the glass of objects that are closed with a glass cover and see whether it appears coarse bubbles or not (Chen et al., 2016).

In this test, the four formulations showed good or homogeneous results as they did not look at the visible coarse grain so that they fulfilled good cream requirements.

**pH Measurement Results**

The measurement value of the cream preparations will affect the quality of the cream preparations. pH of topical preparations must comply with the requirements of intervals 4.5–6.5, and should not be too acidic because it will cause irritation and also should not be too alkaline because it will cause dry and scaly skin (Chen et al., 2016). The pH value can be seen in Table 4.

The cream preparation formula is made up of alkaline material so that the cream base pH tends to be high (close to neutral) (K) while the extract-containing cream preparations (F1, F2, F3) have a lower pH (approaching acid) compared to the preparation that does not contain extracts. It can be influenced by the addition of extracts. The higher the concentration of the extract then the pH will approach the acid (Chen et al., 2016).

**Table 3. The Evaluation Result of Cream Preparations**

Sample	Form	Smell	Color
K (-)	Krim	Typical cream base	White
F1(5%)	Krim	Typical extract	Brownish-green
F2(10%)	Krim	Typical extract	Brown
F3(15%)	Krim	Typical extract	Dark brown
K (+)	Krim	Chemical antibiotics	White

### Viscosity Measurement Results

The measurement of viscosity values affects the quality of the cream. The viscosity value is presented in Table 4.

**Table 4. The pH and Viscosity Measurement Results**

Sample	Viscosity (cps)	pH sample
K (-)	3,200	6.7
F1	2,980	6.0
F2	2,870	5.9
F3	2,540	5.4

Based on Table 4, the results of the viscosity measurements of each formulation experience a difference in this case because of the concentration of the addition of different extracts. The higher the concentrated extract added, the smaller the viscosity value. The viscosity of maja extract cream is still within the range of good cream viscosity requirements (Chen et al., 2016).

### Stability Test Accelerated by Cycling Test Method

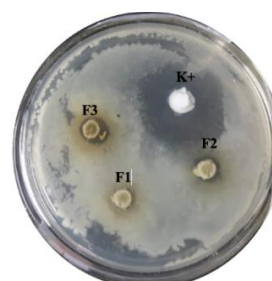
This stability test was performed by storing cream at a low temperature of 4 °C for 24 hours. And later the cream was moved at a high temperature of 40 °C for 24 hours after it was stored at room temperature (27–28 °C). Storage was done by 6 cycles then the physical evaluation test was tested (Table 5). From the table it can be seen that at the three different temperature conditions, the cream is in a stable condition in terms of shape, color and smell.

All formulations, after stored at high temperature, room temperature and high

temperature stabilized, had no changes in shape, color and smell. It was characterized by the preparation of fixed-form cream, a typical color that was brown to blackish brown, and smelled like typical bereuk leaf extracts.

### Results of Antibacterial Tests

An antibacterial test of maja leaf extract cream against *S.aureus* can be seen at the Figure 2.

**Figure 2. Antibacterial test against *S. aureus***

Results of antibacterial tests of maja extracts cream against *S. may* inhibit the growth of the bacteria. It can be seen from the formation of the clear zone from Figure 2. But the inhibition zones were small in F1, F2 and F3. In F3, the inhibition zone formed amounted to 11.53 mm with a weak response of inhibition. Then, it had 29.88 mm an inhibition zone on positive control (K+) (Table 6). This is in line with the previous research at 100% concentration of ethanol extract of maja leaves of forming an inhibition zone of 4.00 mm with weak-inhibitory response category (Hasanah et al., 2017).

**Table 5. Results of Organoleptic Cycling Test; Measuring cream stability (temp. 40°C, 27-28°C,4°C)**

Sample	Organoleptic			Note
	Form	Color	Smell	
K(-)	Cream	Inhibit	Typical base	Stable
F1	Cream	Brownish-green	Typical extract	Stable
F2	Cream	Brown	Typical extract	Stable
F3	Cream	Dark brown	Typical extract	Stable

**Table 6. Data of The Inhibition Zone Diameter**

Formulation	Diameter inhibition zone (mm) $\pm$ SD	Inhibitory Response
F0	0.00 $\pm$ 0.00	None
F1	9.048 $\pm$ 0.91	weak
F2	9.86 $\pm$ 0.97	weak
F3	11.52 $\pm$ 0.95	weak
K+	29.88 $\pm$ 3.2	strong

The results showed that the maja extract cream had antimicrobial inhibition against *S.aureus*. This is because the cream of maja leaves extract contain metabolic compounds such as phenolic, flavonoids, saponins and alkaloid which are antimicrobial (More et al., 2013). The mechanism of action of phenol is by increasing the permeability of the cytoplasmic membrane so that it causes intracellular leakage and cytoplasmic coagulation resulting in cell lysis. Flavonoids play an important role in the intercelation or bonding of hydrogen with complex compounds of extracellular and dissolved proteins that destroy bacteria cell membranes and are followed by the discharge of intracellular compounds (Ejelonu et al., 2011).

Saponins are antibacterial by denaturing proteins. Because the surface active substances of saponins are similar to detergents, saponins can be used as antibacterial where the surface tension of the bacterial cell walls will be lowered and the permeability of the bacterial membrane is damaged, the survival of the bacteria will be disrupted due to damage to the cell members (Gustomi et al., 2018; Pandey et al., 2010). The alkaloid antibacterial mechanism is by working inhibition enzymes that play a role in DNA replication. DNA replication inhibition causes bacteria to not be able to prevent cleavage, preventing growth of bacteria (Das et al., 2014).

However, previous studies showed that the ethanol fraction of maja leaves produced a larger diameter of the inhibition zone than the cream form. This is because the active ingredients contained in the cream preparations are less than the fraction. Cream preparations generally contain more cream

base ingredients (Azubuike et al., 2015; Sonia et al., 2017).

### Conclusion

This research can be concluded that maja leaf extracts can be formulated in cream preparations and cream formulations. The cream preparations of maja leaf extracts provide the zone of inhibition in *Staphylococcus aureus* the widest the inhibition zone at a formulation of 3 to 11.53 mm with strong an inhibitory response.

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