Synthesis of Anti-Acne Ointment of Ethanol Extract of White Plumeria Leaves (Plumeria Alba L.)

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Abstract. Acne is a chronic inflammatory skin disease pilosebaceous follicle, where the oil-producing glands are clogged and contaminated by bacteria. Propionibacterium acne is one of the bacteria that contributes to the pathogenesis of acne. Acne treatment was done by reducing the population of bacteria using an antibacterial. One of the plants that have antibacterial activity is white plumeria. The ethanol extract of white plumeria leaves contains antibacterial secondary metabolites, which are alkaloids and saponins. The aim of this study is to formulate white plumeria leaves extract into the water leached ointment base. Characteristics of the ointment were determined by evaluating the stability of the ointment including organoleptic, adhesion test, dispersive power test, determination of pH, and the antibacterial activity test. The results showed that the ointment of ethanol extract of white plumeria leaves has some characteristics, semisolid form, white, has distinctive smell of ointment, homogeneous but not protective, has a pH of 4.57 - 6.10, dispersive power of 5.10 - 6.06 cm, the adhesiveness of 1.67 - 3 seconds, and optimum antibacterial activity at concentrations of 5 ppm providing inhibition zone of 24.00 mm.

1. Introduction
Acne is a chronic inflammatory skin disease pilosebaceous follicle, where the oil-producing glands are clogged and contaminated by bacteria. Propionibacterium acne is one of the bacteria that contribute to the pathogenesis of acne. P. acne uses glycerol in sebum as a source of nutrients. P. acne will form free fatty of the sebum, causing a response of neutrophil cells to secrete enzymes that can damage the hair follicle wall. Thus it can cause inflammation [1]. Acne treatment was done by reducing the population of bacteria using an antibacterial. Acne treatment generally uses antibiotics, one of which is erythromycin. The use of Erythromycin antibiotic in some cases can give side effects on the body. This situation prompted scientists to develop new antibacterial compounds derived from natural resources [2].

The plant which is potential to be developed as an antibacterial is white plumeria. Ethanol extract of white plumeria leaves showed antimicrobial activity against Staphylococcus aureus and E. coli [3]. Antibacterial of white plumeria leaves is due to the existence of secondary metabolites. The ethanol extract of white plumeria leaves is known to contain alkaloids and saponins [3]. Alkaloid works as an antibacterial by disrupting components of the peptidoglycan in the bacterial cell, so the cell wall layers
are not fully formed and caused the death of these cells [4]. Saponins are substances that can interact with bacterial cell wall causing a bacterial cell lysis.

Increasing the effectivity of the use of white plumeria leaves to extract as a natural remedy was done using formulations in dosage forms. Anti-acne dosage forms have been widely circulated in the market, either in the form of gels, ointments, creams and lotions, but form is more suitable for acne. Ointment dosage is a dosage form that has suitable consistency for the treatment of skin diseases caused by bacteria. Based on its composition, ointment base was classified into hydrocarbon, absorption, water-soluble, and that can be leached by water [5]. Water-leached ointment base is considered to have advantages as a cosmetic, as it is easy to wash by water, the good release of the medicine because when used on the skin, there will be evaporation and increasing concentration of a drug that dissolves in water so as to encourage absorption into the skin tissue [6].

Accordingly, this research is aimed to formulate the ointment dosage of ethanol extract of white plumeria leaves and to test the activity against bacteria that cause acne which is *P. acne*. The effectivity of the release of active substances of ointment dosage was determined by evaluating the stability of the ointment dosage including organoleptic, adhesion test, dispersive power test, determination of pH, and the antibacterial activity test.

2. Methods
2.1 Tools and Materials
Tools used in this research are test tubes, petri dish, autoclave, analytical balance, oven, measuring cylinder, Erlenmeyer, beaker glass, micropipette, stirrer glass, aluminum foil, wrapping, volumetric flask, incubators, needle ose, stirrer, paper label, spirit burner, spectrophotometers, pipette volume, filler, cork drill, drugal sky, a pot of ointment.

Materials used in this research are ethanol extract of white plumeria leaves, aquades, *P. acne* bacteria, nutrient broth medium (NB), nutrient agar medium (NA), stearyl alcohol, white vaseline, nipasol, nipagin, polysorbate 80, glycerol, phenolphthalein indicator, KOH, alcohol 70%, solid paraffin.

2.2 Experimental Procedure
2.2.1 Water-leached basis of ointment dosage
Ointment dosage was based on compositions made by [7] with the addition of white plumeria leaves extracts as antimicrobial agents against *P. acne* bacteria in various concentrations. An ointment was made by fusion method. Ointment base was made with two preparations, namely preparation A and preparation B. Preparation A was made by fusing stearyl alcohol as much as 9.98% w/w and vaseline as much as 24.96% w/w at 70 °C while stirring and adding nipasol as much as 0.0025% w/w. Preparation B was made by heating distilled water as much as 50.035% w/w with glycerol as much as 9.98% w/w at 70 °C while stirring and adding polysorbate 80 as much as 5% w/w, nipagin as much as 0.005% w/w and extracts plumeria leave with concentrations of 0; 1; 5; and 10 ppm, as well as the positive control in the form of erythromycin ointment 10 ppm. Preparation A and B were mixed at a temperature of 70 °C while stirring in the mortar until cold and oil in water ointments were obtained. Ointment formulations of white plumeria leave extract was assembled as shown in Table 1.

The Ointment were evaluated in physical and chemical properties as well as the testing stability including the organoleptic, homogeneity, pH, dispersive power, and adhesion at room temperature on days 0, 5, 10 and 15. Antibacterial activity test of ointment was performed on the 15th day.
Table 1. Ointment formulation of white plumeria leaves extract.

| Materials     | Formula Salep | F1     | F2     | F3     | F4     | F5     |
|---------------|---------------|--------|--------|--------|--------|--------|
| Stearyl alcohol | 9.98 g        | 9.98 g | 9.98 g | 9.98 g | 9.98 g |
| White Vaseline | 24.96 g       | 24.96 g| 24.96 g| 24.96 g| 24.96 g|
| Nipasol       | 0.0025 g      | 0.0025 g| 0.0025 g| 0.0025 g| 0.0025 g|
| Nipagin       | 0.005 g       | 0.005 g| 0.005 g| 0.005 g| 0.005 g|
| Glycerol      | 9.98 g        | 9.98 g | 9.98 g | 9.98 g | 9.98 g |
| Polysorbate 80 | 5 g           | 5 g    | 5 g    | 5 g    | 5 g    |
| Aquades       | 50.035 g      | 50.035 g| 50.035 g| 50.035 g| 50.035 g|
| Plumeria Extract | 0 ppm    | 1 ppm  | 5 ppm  | 10 ppm | -      |
| Erythromycin  | -             | -      | -      | -      | 10 ppm |

2.2.2 Organoleptic test
The observations made in this test the form of dosage, smell and color of ointment dosage during storage to see the physical stability of ointment..

2.2.3 Homogeneity test
A total of 0.5 grams of ointment dosage was placed on the glass and then leveled and observed based on color uniformity and the absence of lumps and granules [8].

2.2.4 Protection power test
Filter paper (10x10 cm) was wetted with phenolphthalein and dried. The Ointment was weighed as much as one gram, then smeared on that paper. On another filter paper made an area (2.5x2.5 cm), then made a barrier at the edge of the area with the melted solid paraffin. This filter paper was attached on the filter paper before. A solution of KOH 0.1 N was dripped on these areas. The presence or absence of stains was observed at the time of 15; 30; 45; 60 seconds, 3 and 5 minutes, if no stains mean that ointments provide protection. Each type of ointment was repeated for three times [9].

2.2.5 pH test
Measurement of pH used a pH meter kit that dipped into 0.5 grams of ointment that has been diluted with ten mL of distilled water. The good pH value is 4.5-6.5 for ointment or appropriate with the pH value of human skin [10].

2.2.6 Dispersive power test
Dispersive power test was conducted by placing 0.5 grams of ointment between two transparent glass plates by a load of 100 g. Measurement of dispersive power was conducted after the ointment does not spread again or approximately 1 minute after loading. Ointment dosage which is good to use has a dispersive power of 5-7 cm [11].

2.2.7 Adhesion test
A total of 0.5 grams of ointment was weighed, then smeared on glass plates with an area of 2.5 cm². Both plates was affixed and pressed with a load of 1 kg for 5 minutes then the load was removed and given a load of 80 g for testing. Recorded the time until the two plates disjoint [12].

2.2.8 Antibacterial activity tests of ointment dosage
Antibacterial activity test of ointment dosage was conducted by pitting method. A total of one ose of P. acne bacteria was taken from the stock culture and incubated in 10 ml of liquid medium (Nutrient Broth) for 18-24 hours at a temperature of 37 °C. Some bacteria in a medium NB was taken and dispersed in the medium NA based on the results of absorbance at a wavelength of 600 nm. Based on the value of absorbance, if an absorbance value is less than or equal to 0.5 then taking 100 μL of bacterial culture, whereas if it is 0.6 to 1.0 then taking 50 μL of bacterial culture. The bacterial suspension on NA medium was streaked with spread plate using drugalsky, then dried for 15 minutes
at room temperature. Agar media was drilled using a crockbor with the diameter of ±7 mm. Ointment dosage of white plumeria leaves extracts was then put into the hole as much as 0.05 g. Comparators used for the negative control is ointment base without the addition of extracts and for positive control is erythromycin ointment. Then incubated at 37 °C for 24 hours. The clear zone which is visible around the hole indicates the presence of antibacterial activity. Clear zone formed was measured using a caliper.

3. Results
3.1 Testing of Physical Properties of Ointment
3.1.1 Organoleptic test
Organoleptic testing is conducted to determine the shape, odor and color of ointment and to observe any changes that may occur during storage that last for 15 days. Organoleptic testing showed ointment dosage of ethanol extract of white plumeria leaves has a semisolid form and soft texture such as cream at concentrations of 0; 1; 5; and 10 ppm. [13] stated that water-leached ointment base has a soft creamy consistency, could be diluted and dissolved in the aqueous solution. The color test of dosage resulted the same color at concentrations of 0; 1; 5; and 10 ppm. The smell of ointment dosage by organoleptic test resulted that ointment dosage has a distinctive smell and no changes either in the concentrations of 0; 1; 5; and 10 ppm.

Testing of organoleptic stability of ointment resulted that no changes in color, smell, and shape of the four formulations during 15 days of storage indicating that the ointment is stable. Based on these results it can be said that the antiacne ointment dosage of white plumeria leaves extract has good physical stability based on its organoleptic properties. According to [14], one of the requirements for good quality of ointment was that the ointment should be stable by physical or chemical effect as long as the ointment was used to treat. Therefore, the ointment should be free from the occurrence of incompatibility and should be stable at room temperature.

3.1.2 Homogeneity test
Homogeneity test is conducted to analyze if during the synthesis process of the cream with active ingredient with its basic materials and other necessary additives are homogeneously mixed. Homogeneity test was conducted by applying 0.5 grams of ointment dosage on glass objects, and the ointment dosage is visually observed based on color uniformity and the absence of lumps and granules. The results of the homogeneity test, all creams of ethanol extract of white plumeria leaves, positive control in the form of erythromycin ointment, and negative controls in the form of ointment base without the addition of the extract, are the homogeneous ointment. This is indicated by the absence of clumps in certain material of the ointment. [15] stated that homogeneity of a dosage depends on the properties similarity of the material used, which were the base and the active substance. If there are differences in the properties of the base and the active substance, it will occur coagulation process resulting a larger particles of the dosage.

The results of this study, the ethanol extract of white plumeria leaves can be distributed evenly in a concentration of 0; 1; 5; and 10 ppm, and no effect of storage time for 15 days against homogeneity of dosage. Based on these results, it can be seen that the ointment dosage of ethanol extract of white plumeria leaves have the good physical properties based on its homogeneity. Homogeneous dosage will give good results because the drug substances are dispersed in the basic materials evenly so that in every part of dosage contain the same amount of drug. If the ingredients are not evenly dispersed in the basic materials, the drug will not achieve the expected therapeutic effect [9].

3.1.3 Adhesion test
An Adhesion test is one of the important parameters in the evaluation of the ointment because this test can analyze how far the ointment can be attached to the skin so that the expected therapeutic effect is achieved. According to [16] ointment should not impede the physiological functions of the skin, ointment base which has powerful adhesion will impede skin respiration, if it is too weak, then the therapeutic effect will not be achieved. The Adhesion test was conducted by applying 0.5 grams of
ointment on a pair of glass plates. Both plates were taped and given a load of 1 kg for 5 minutes, the next load was released by the release load of 80 grams. The time required to detach both plates was recorded as the adhesion value of ointment dosage.

The test resulted that the concentration of 0 ppm has adhesion of 1.33 seconds, 1 ppm has adhesion of 2.33 second, and 5 ppm has adhesion from 3.07 to 2 seconds and 10 ppm has adhesion of 3.07 seconds. Figure 4.4 adhesion test results of ointment dosage, shows adhesion value of ointment dosage does not change at concentrations of 0; 1; and 10 ppm, but at a concentration of 5 ppm decreases on the 5th day and does not change until the 15th day. The decrease of adhesion does not affect on the stability because the value of adhesion is still suitable for the standards. According to [17], the adhesion of semisolid dosage should be more than 1 second.

![Figure 1 The relations between adhesion value of ointment dosage and storage time](image)

3.1.4 Dispersive power test

Dispersive power test aims to analyze the dispersion of ointment dosage on treated skin. The greater dispersion of ointment, the greater medicine to be absorbed. Dispersive power test is done by placing 0.5 g of ointment between two plates of transparent objects by an additional load of 100 g. Measuring diameter of ointment that dispersal was conducted by taking the length of the average diameter of some sides. Picture of the dispersive power test with storage time can be seen in Figure 2.
Test results showed that the dispersive power value with the addition of the ethanol extract, 1, 5, and 10 ppm, increased from day 0 to day 5, but decreased on day 10 and to 15, in contrast with the ointment without the addition of extracts continues increasing from day 0 to day 15. Despite the dispersion decreased on days 10 and 15, dispersion of ointment is still appropriate with the standard, which is good ointment dosage to use has dispersive power value of 5 s/d 7 cm [11]. The changes of dispersive power can be caused by the consistent changes of an ointment because storage containers are made of plastic. Different ways of stirring could also affect the particle size of dosage. Different particle sizes would change the value of dispersive power [18].

3.1.5 pH test

pH testing is a part of the physicochemical inspection criteria in predicting the stability of the ointment where the pH profile determines the stability of the active ingredient under acidic or alkaline condition [15]. [9] also stated that the suitability of the skin's pH with a pH of the topical dosage would also affect the skin response toward the ointment. The ideal topical dosage is not irritating to the skin. The possibility of skin irritation will be enormous if the ointment is too acidic or too alkaline. pH testing was conducted by dissolving 0.5 grams of ointment perfomed in 10 mL of aquades and then the pH was measured by using a pH meter. The resulting graph of pH test of dosage towards storage time can be seen in Figure 3.

![Figure 2 Relations between dispersion of ointment and storage time.](image)
Based on the test results is known that the pH of the ointment with the addition of white plumeria leaves extract at a concentration of 1; 5; and 10 ppm from day 0 to day 15 increased to more alkaline, it is thought to be caused by the active substances contained in the white plumeria leaves extract is alkaloid that has an alkaline pH. Alkaloids are nitrogen-containing compounds that are alkaline and have pharmacological activity [19]. Although the test results of pH ointment with the addition of white plumeria leaves extract on day 0 to day 15 increased but still achieve the Indonesia National Standard (SNI) for the topical dosage is 4.5 -6.5. Ointment of extract ethanol of white plumeria leaves the concentration of 1 ppm has a pH of 4.57 to 4.76, a concentration of 5 ppm has a pH of 4.90 to 5.77, and the concentration of 10 ppm has a pH of 5.12 to 6.10. The ointment without adding the extract has a pH of 4.35 to 4.68 which shows the lowest pH value, the base does not achieve the standard of SNI. [8] stated that too acidic pH could irritate the skin; however, too alkaline pH could make a scaly skin. 

3.1.6 Protection test
Protection test is conducted to analyze the ability of protection against foreign influences from outside which reduces the effectivity of the ointment. Protection test was performed by applying 0.5 grams of ointment dosage on filter paper that has been smeared by phenolphthalein indicator, then on another filter paper smeared by melted solid paraffin. Both papers are taped and the areas which subsequently spilled ointment smeared with 0.1 N KOH. A good ointment base can protect the skin from external influences such as acid-base dust and sunlight at the time of treatment, marked by the formation of red stains after addition of KOH [9]. Based on test results, the ointment of ethanol extract of Plumeria leaves did not have any protection. It is possibly caused by the ointment base used is the water-leached base that contains very little oil and easily removed by water, so it did not provide a protective effect. [20] suggested that the water-leached ointment base should be soluble and easily removed from the skin by washing with water.

3.2 The Ointment Antibacteril Activity Test to Bacteria
The test was conducted to analyze the effect of antibacterial activity after formulated into ointment dosage forms. The test was conducted by diffusion agar. Based on the test, the value of inhibition zone at the concentration of 1 ppm was 10.4 mm. At the concentration of 5 ppm was 24.00 mm and at the concentration of 10 ppm was 24.90 mm. The value of inhibition zone white plumeria leaves extract of ointment dosage forms has a greater value than in the form of the extract solution. The increasing of inhibition zone diameter of bacterial growth in dosage forms is possibly caused by the increasing penetration of the antibacterial compound that diffuses into the test object, resulting in larger
inhibition zone diameter of bacterial growth. This is caused by the water-soluble cell wall of gram-positive bacteria that are more polar. The high water content of the water leached ointment base may increase the polarity so that it is easy to penetrate the polar peptidoglycan layer than the nonpolar lipid layer. [21] stated that the structure of the bacterial cell could determine the penetration, bond and antibacterial activity of the compounds. *P. acne* is a gram-positive bacteria whose cell wall structure has more peptidoglycan, a little lipid and cell wall contains polysaccharides (teichoic acid). Teichoic acid is a water-soluble polymer, which serves as a positive ion transport to leave or enter. The hydration effect of the preparation that is also used can lead to increased penetration of the drug into the skin [22].

The negative control of ointment base without adding the extract did not have inhibition zone. Positive controls such as erythromycin of ointment dosage have inhibition zone which is smaller than the ointment dosage of white plumeria leaves extract. This is caused by acidic ointment base affecting erythromycin work which is optimal under alkaline conditions. [23] stated that the antibacterial activity of erythromycin is bactericidal and increased at alkaline pH.

![Inhibition zone obtained by the activity of ointment dosage of ethanol extract of white plumeria leaves](image)

**Figure 5**  Inhibition zone obtained by the activity of ointment dosage of ethanol extract of white plumeria leaves

4. **Conclusion**

The evaluation of ointment dosage of ethanol extract of white plumeria leaves has some characteristics, semisolid form, white, distinctive smell, homogeneous but not providing power protection, has a pH of 4.57-6.10, dispersive power from 5.10 to 6.06 cm, adhesion of 1.67 to 3 seconds. The ethanol extract of white plumeria leaves in dosage forms can inhibit the activity of *P. acne* on the optimum concentration of 5 ppm, with the inhibition of 24.00 mm.

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