

Formulation of M/A-Type Ointment Dosage from Ethanol Extract of White Plumeria Leaves (*Plumeria alba L.*) Against *Candida albicans*

Dian Riana Ningsih^{1*}, Zusfahair¹, Dwi Kartika¹, Mei Lianasari¹

¹Department of Chemistry, Faculty of Mathematics and Basic Sciences,
Universitas Jenderal Soedirman

*Corresponding author: deeyanbik@gmail.com

Received 9 May 2018; Revised 23 July 2018; Accepted 26 July 2018

ABSTRACT

Candida albicans is one of the fungi that can cause various infections. The plant that can be used as antifungal is white plumeria leaves (*Plumeria alba L.*). Ethanol extract of white plumeria leaves contains alkaloid and saponin active compounds, to ease its use and to get the maximum effect, then its formulated into ointment dosage form. This research aims to formulate ointments dosage from plumeria leaves, to investigate its physical properties and its activity against *Candida albicans*. The research begins with the determination of minimum inhibitory concentration (MIC), and then formulation of ointment dosage. The physical properties of the ointment were tested including homogeneity test, pH, dispersive power, protection power, adhesion and hedonic and antifungal activity. The MIC value of extract was 5 ppm with a 1.22 mm inhibition zone. Extract has a form of semisolid, white colour, distinctive odour, homogeneous, pH of 5.07-5.59, dispersive power of 5.09-5.78 cm, adhesion of 1.00-2.33 seconds. It has antifungal activity of ointment at day 0 for concentrations of 5, 10 and 15 ppm respectively are 2.93, 5.2 and 7.87 mm and at day 15 for concentrations of 5, 10 and 15 ppm respectively are 3.68, 4.87 and 5.82 mm. This extract also able to protect skin from the outside environment.

Keywords: antifungal, ethanol extract, *Candida albicans*, white plumeria, ointment

INTRODUCTION

Indonesia is a tropical climate country with high humidity that allows the growth of various plants and microorganisms very well. One type of microorganism that can grow well in Indonesia is fungi. Skin diseases caused by some types of fungi is one of the problems in tropical countries such as Indonesia. *Candida* is the most common cause of fungal diseases/infections, specifically species *Candida albicans*.

C. albicans causes oral thrush[1], lesion[2], vaginal inflammation (vulvovaginitis) (Wilson, 2005), candida in the urine (candiduria)[3] or may even lead to cancer complications [4]. The infection caused by *C. albicans* is known as candidiasis. Candidiasis is an acute or subacute disease that attacks the mouth, vagina, skin, nails, bronchi or lungs, sometimes causing septicemia, endocarditis or meningitis. This disease can strike both men and women[5]. The most common type of diseases/infections caused by *C. albicans* is skin candidiasis.

The existed topical medications to treat skin candidiasis are nystatin, clotrimazole, ketoconazole, and miconazole. These drugs have limitations, such as severe side effects, poor

The journal homepage www.jpacr.ub.ac.id

p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

penetration of certain tissues and even the emergence of resistant fungi). The presence of antifungal drugs is relatively fewer than other antimicrobial drugs. This limitation is due to the nature of fungal cells such as human cells which are eukaryotic, making it difficult to find specific compounds against fungal cells but not toxic to its host. Fungal cells are more difficult to overcome because they easily survive even in less favorable environments compared to other microorganisms. The alternative treatment that can be done is traditional medicines derived from nutritious plants as an antifungal. Treatment with traditional medicines has the advantages such as cheap, easy to obtain, minor side effects and low toxicity levels[6].

The plants that can be used as an antifungal is white plumeria (*Plumeria alba* L.). Most parts of this plant are useful especially the leaves. Research by [7] stated that the active compounds of ethanol extract of white plumeria leaves were alkaloids and saponins. In her research, 30 ppm extract was able to inhibit the growth of *Staphylococcus aureus* bacteria. Research on antifungal substance from flower plumeria alba was also done by ⁸ which showed that essential oil of *P. alba* possesses antifungal activity, it was able to inhibit the growth of *C. albicans*.

In this research, white plumeria leaves extract is used as an antifungal compound, the extract is formulated into ointment dosage form to ease the use and to get the maximum desired effect. The ointment dosage can be prepared in two types, which are the water type in oil (A/M) and the type of oil in water (M/A). The M/A-type ointment is an ointment with oil as dispersed phase and water as dispersing phase which has advantages such as easy to clean, non-sticky, non-fatty and easy to spread on the emollient skin surface. This type is preferred in everyday use because after usage does not cause scars, it gives cool effect on the skin, not oily and has good spreading ability[9]. The selected ointment base in this research is an oil-type ointment in water. Therefore, the research is conducted on the formulation of the ethanol extract of white plumeria leaves (*Plumeria alba* L.) and its activity test against *C. albicans*.

EXPERIMENT

Chemicals and instrumentation

The tools used in this research are Heraeus incubator, hot plate, micropipette, crock borer, drugalsky, oven, analytical balance, calliper, pH meter, object glasses and Thermo Scientific Genesys 20 spectrophotometers.

This research used ethanol extract of white plumeria leaves (*Plumeria alba* L.), *Candida albicans* from Medical Microbiology Laboratory Universitas Jenderal Soedirman, Sabaroud Dextrose Agar (SDA), Sabaroud Dextrose Broth (SDB), ketoconazole tablets 200 mg (generic), stearyl alcohol, glycerol, vaseline, nipazole, nipagine, tween 80, paraffin.

Procedure reaction

Determination of minimum inhibitory concentration (MIC)

Determination of MIC aims to find the minimum concentration of the highest active extract which is able to inhibit the growth of *C. albicans*. The method used was agar diffusion method with various concentration of extract. The concentration used were 1000; 500; 250; 125; 65; 30; 15; 10; 5; and 1 ppm. One single loop of fungus culture was put into 10 mL SDB medium then incubated for 18-24 hours at 37°C. The suspension of isolates was measured by spectrophotometer at λ 600 nm to obtain a transmittance value of 25%, if yet achieved then diluted using distilled water. Then 50 μ L fungus was taken and spread evenly by spread plate streaking using a *drugalsky* over the SDA medium on a sterile petri dish. Media containing tested fungus was perforated with diameter of ± 6 mm using crock borer. Each concentration

of extract was taken 50 μ L and then inserted into the hole on SDA medium which had been inoculated with tested fungi. After that, it was incubated at 37°C for 24 hours. The clear zone around the hole is measured as the value of antifungal activity of extract.

Fabrication of M/A-type ointment[10]

The ointment is made by the fusion method. Fabrication of M/A-type ointment was conducted by mixing aqueous phase and oil phase solution. Aqueous phase was made by heating distilled water with glycerol at 70°C while stirring and then added with tween 80, nipagin and 5, 10 and 15 ppm white plumeria leaves extracts. The oil phase was made by melting stearyl alcohol with vaseline at 70°C while stirring and then added with nipazole. The aqueous phase and the oil phase were then mixed at 70°C while stirring, the stirring was conducted until an ointment mass was formed and then cooled and an M/A-type ointment was obtained. Formulation of M/A-type ointment dosage form can be seen in Table 1.

Table 1. Formulation of M/A-type ointment

Materials	F1	F2	F3	F4	F5
Distilled water	50.07 g	50.07 g	50.07 g	50.07 g	50.07 g
Glycerol	9.98 g	9.98 g	9.98 g	9.98 g	9.98 g
Tween 80	5 g	5 g	5 g	5 g	5 g
Nipagine	0.0025 g	0.0025 g	0.0025 g	0.0025 g	0.0025 g
Stearyl Alcohol	9.98 g	9.98 g	9.98 g	9.98 g	9.98 g
Nipazole	0.0025 g	0.0025 g	0.0025 g	0.0025 g	0.0025 g
Vaseline	24.96 g	24.96 g	24.96 g	24.96 g	24.96 g
Ethanol extract of white plumeria leaves	0 ppm	5 ppm	10 ppm	15 ppm	-
Ketoconazole Tablet	-	-	-	-	15 ppm

Note : F = Formula

Testing physical properties of ointment

Testing on the physical characteristics of ointment is conducted to determine the pH value and emulsion stability of ointment after addition of extract leaves at several concentrations. Testing was done on day 0; 5; 10 and 15.

1. Homogeneity

The ointment preparations at the top, middle, and bottom part were then placed on a glass plate then rubbed and touched. The homogeneity test was performed by pouring 1 g of ointment on the flat glass surface. The ointment homogeneity test passed when no solid substance left during hand-applying the ointment to the flat glass such as the normal use of ointment to the skin surface [11]. The work conducted three times.

2. Ointment pH

The methanol extract ointment as much as 0.5 g was diluted with 10 mL of distilled water, then dipped pH meter for 1 minute. pH of 4.5 - 6.5 is a skin pH [12]. The work conducted three times.

3. Dispersive power

The 0.5 g ointment was placed on petri, and another petri was put on it and left for 1 minute. The spreading diameter of the ointment was measured. Thereafter, 150 g additional load was added and left for 1 minute and then constant diameter was measured. Spreadability of topical preparations is 5-7 cm [13]. The work conducted three times.

4. Protection power

Filter paper (10x10 cm) was wetted and dried with phenolphthalein. The ointment as much as 1 gram smeared on the paper. On another filter paper was made an area (2.5x2.5 cm) and bund on the edge of the area with molten solid paraffin. The filter paper was pasted on the previous filter paper. The 0.1 N KOH solution was dropped on the area. The presence or absence of stains was observed at 15, 30, 45, 60 seconds, 3 and 5 minutes, no stains appeared means that the cream provides protection. The work conducted three times.

5. Adhesion

The 0.25 g ointment was placed on the top of two object-glasses, pressed with a 1 kg load for 5 minutes. The object glass was mounted on the test kit. The test tools were loaded by 80 g load and the ointment release time from the object glass was recorded. Adhesive power should be more than 1 second [14]. The work conducted three times.

Activity test of antifungal ointment against *C. albicans*

Determination of antifungal ointment activity is conducted with the agar diffusion method. Tests were performed on ointments stored on day 0 and day 15. The activity test procedure as follows: tested fungus was grown in SDB medium for 24 hours. Fungus cultures were measured by spectrophotometer at λ 600 nm to obtain a transmittance value of 25%, if yet achieved, then diluted with distilled water. The culture was taken as 50 μ L then spread evenly by spread plate streaking using a drugal sky over the SDA medium on a sterile petri dish. The medium containing the tested fungus was perforated with a diameter of ± 6 mm using a cork borer as many as 3 holes and each hole was incorporated with 0.1 g ointment dosage of extract at specified concentration. The culture was incubated at 37°C for 1x24 hours. The inhibitory diameter was measured on each ointment. The clear area around the hole shows that the ointment has antifungal activity.

RESULT AND DISCUSSION

Determination of Minimum Inhibitory Concentration (MIC)

Determination of MIC is done to determine the minimum level of active extract that can inhibit the growth of *Candida albicans*. The method used in this test is the well diffusion method. This method is conducted by perforating the well on solid media SDA that has been inoculated with *C. albicans*. This method is practical, easy, and fast in reading the result [15]. In addition, the hole can accommodate more test materials and diffusion can occur more easily [16]. Observations are made after 24 hours by measuring the inhibition zone formed around the wellbore. The inhibitory zone diameter is an indication of the sensitivity of the tested fungi. The larger the inhibition zone, the better antifungal activity of a compound.

Determination of minimum inhibitory concentration was done by examining the ethanol extract of white plumeria leaves at various concentrations. The concentrations used are 1, 5, 10, 15, 30, 65, 125, 250, 500 and 1000 ppm, as a comparison for positive control using ketoconazole 1000 ppm and negative control using distilled water. The determination of MIC can be seen in Figure 1.

The results as shown in Figure 1 show that extract are able to inhibit the growth of *C. albicans* at the smallest concentration of 5 ppm with the inhibition zone diameter of 1.22 mm. The concentration of 1000 ppm has the highest antifungal activity with the inhibition zone diameter of 2.81 mm. The positive control gives an inhibition zone of 10.22 mm, whereas negative control (ointment without extract) does not. The higher concentration of extract, the higher inhibition zone against *C. albicans*.

The active compounds of extract, according to ⁷ are alkaloids and saponins. These compounds are expected to have antifungal activity by inhibiting the growth of *C. albicans*. Alkaloids are compounds that have antimicrobial activity by inhibiting nucleic acid biosynthesis [17]. Alkaloids are also able to bind strongly with ergosterol forming a hole that causes cell leakage. It causes permanent damage and even death to cell of fungi [18]. Saponins are able to break down the fat layer on cell membranes that ultimately lead to the impaired permeability of cell membrane. It causes disruption of the diffusion process of materials or substances required by the fungi so that eventually the cell swells and breaks.

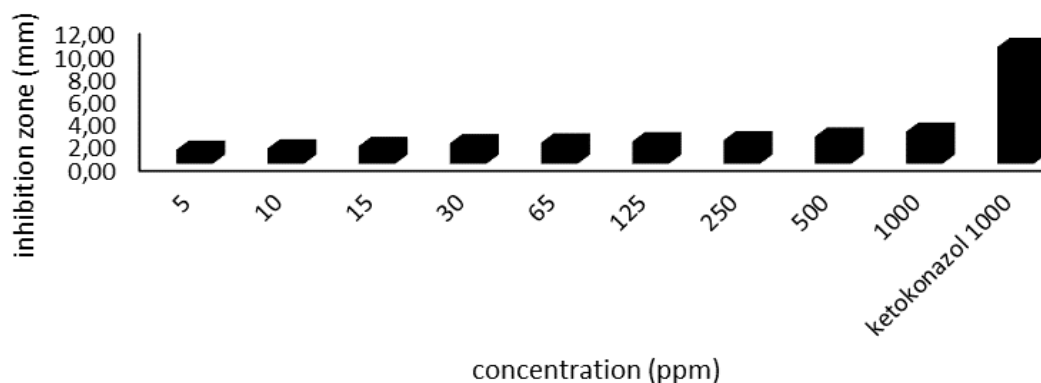


Figure 1. Chart of the relation between the concentration of extract and the clear zone.

Oil-in-water Ointment Dosage (M/A)

The extract is formulated into an oil-in-water ointment dosage (M/A). M/A-type ointment is an ointment with water as dispersing phase and oil as dispersed phase. The M/A-type ointment is preferred due to several advantages, such as easy to evenly spread, give a cool effect on the skin, easy to clean or wash and not sticky in its use ⁹. The method used in the fabrication of ointment dosage form is the fusion method by dividing the material used into two phases, namely the oil phase and the water phase. The oil phase consists of white vaseline, stearyl alcohol, and nipazole, whereas aqueous phases consists of distilled water, glycerol, tween 80, nipagine, and extract. The fusion method is conducted by adding the water phase as the dispersing phase into the oil phase as dispersed phase at 70°C with constant stirring. This method is selected because the material in the oil phase can melt perfectly during the melting process and the materials in the water phase can dissolve perfectly.

The fabrication of the ointment dosage begins by fusing stearyl alcohol in a hot mortar, then after melting added with vaseline and lastly added with nipagine. The fusion is conducted because the oil phase material is solid, so it needs to melt to lower its viscosity, therefore the material can mix homogeneously. The materials in the water phase are further heated at a temperature of $\pm 70^{\circ}\text{C}$. This heating process aims to increase the solubility of the materials. The two homogeneous phases are then mixed while constantly stirring until the ointment dosage is formed. The resulting ointment dosage is inserted into an ointment pot.

The white vaseline in this ointment is used as an oil base that acts as emollient and stearyl alcohol is added to increase emulsion stability and to form an ointment texture. Nipagine and nipazole are added as a preservative to prevent microbial growth. Glycerol is added as a humectant that will retain water evaporation on the dosage and as a moisturizer on the skin. Tween 80 is added as an emulsifier to lower surface tension, so that able to mix different polarity of two compounds[9]. The extract was added to the dosage with various

concentrations of 5, 10 and 15 ppm which acts as an active compound to inhibit the growth of *C. albicans*.

Testing Physical Properties of Ointment Dosage

This test aims to determine the existence of physical changes of the dosage during the storage for 15 days with the test time on day 0; 5; 10 and 15. The test includes homogeneity, pH, dispersive power, protection power, and adhesion. Testing antifungal activity of ointment is done on day 0 and 15. One of the requirements of good ointment is to be stable due to physical and chemical influence during the use.

Homogeneity

Homogeneity test aims to determine the homogeneity of the resulting ointment dosage, whether the medicinal materials and the basic ingredients are mixed homogeneously. The consistency of ointment dosage should be homogeneous. The ointment dosage should show a homogeneous arrangement because it is smeared on the skin. Homogeneity test results for ointment dosage with the addition of extracts showed homogeneous results. This is based on the absence of lumps or coarse grains on the ointment. In addition, the ointment also did not undergo homogeneous changes during the 15 days of storage. Homogeneity test results for the dosage without the addition of extracts showed the presence of coarse grains and water and oil separation on the day 10, indicating that the ointment dosage without the addition of the extract is not homogeneous. The addition of extracts into the ointment dosage is expected to affect the homogeneity of the dosage.

According to [19], homogeneous ointment dosage indicate that mixture of ointment materials and extracts is good so that no grains are found in the dosage. An ointment dosage should be homogeneous and even avoid irritation and evenly distributed when used. Homogeneous preparations will produce good results because the drug ingredients are dispersed in the basic material evenly so that each part of the preparation contains the same amount of drug ingredients. If the drug ingredients are not dispersed evenly in the basic materials then it will not achieve the desired therapeutic effect [20].

pH

The pH test is a part of the chemical physics examination criteria in predicting the stability of ointment. The pH profile determines the stability of active ingredients in acidic or alkaline conditions). The good pH value of the ointment according to Indonesian National Standard (SNI) 16-4946.2-1998 is 4.5-7. The pH test results can be seen in Figure 2.

The pH test results as shown in Figure 2 show that the pH values of the ointment at various concentrations tend to be stable for 15-days storage. The pH value for 0 ppm is 5.19 to 5.92; for 5 ppm is 5.17-5.53; for 10 ppm is 5.29-5.59 and for 15 ppm is 5.07-5.47. The resulting pH value has fulfilled the good pH requirement for topical dosage according to SNI, which is 4.5-7. The overly alkaline pH of the ointment may cause dry skin, whereas overly acidic pH may induce skin irritation.

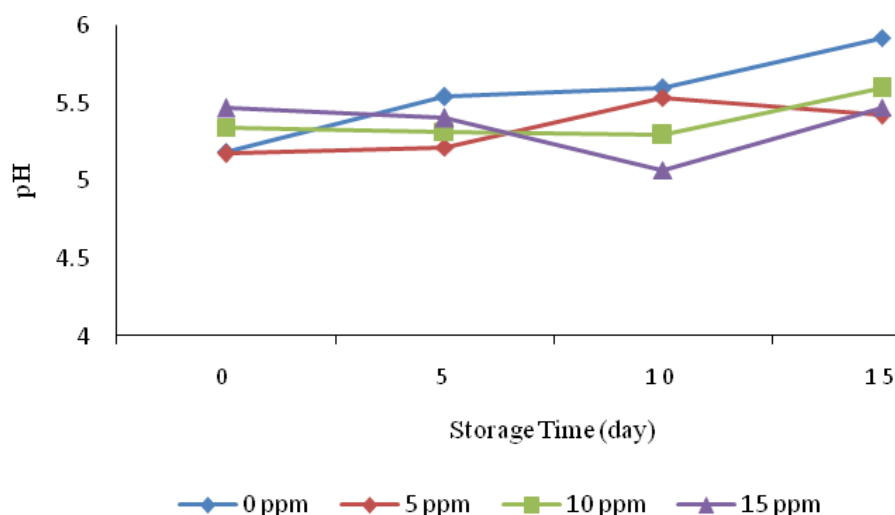


Figure 2. The effect of storage time and ethanol extract concentration to pH value of the ointment

Dispersive power

The dispersive power test of the ointment aims to see the ability of the dosage to spread on the skin. An ointment base should have good dispersion to ensure good drug delivery¹⁹. The larger value of dispersion, then the ointment is easier to smear on the skin, so that drug ingredients absorb maximally on the skin. The result of the test can be seen in Figure 3.

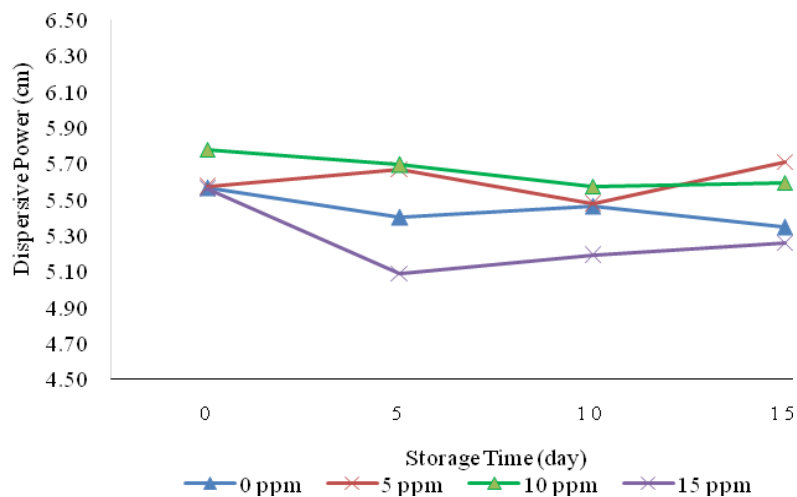


Figure 3 The effect of storage time and ethanol extract concentration to dispersive power value of the ointment

The dispersive power value of 0 ppm ointment is 5.34-5.56 cm, 5 ppm ointment is 5.48-5.71 cm, 10 ppm ointment is 5.57-5.78 cm and 15 ppm ointment is 5.09-5.56 cm. A good dispersion for semisolid preparations is 5-7 cm. Therefore the resulting value meets the standard value of good dispersion. Good dispersion causes extensive contact between the drug and the skin, resulting in rapid drug-to-skin absorption [20].

Protection power

The test aims to know the ability of the ointment to protect against external influences. The test was performed by smearing one gram of the ointment on a filter paper which is soaked with a phenolphthalein indicator, then a smaller filter paper placed on the previous filter paper by gluing it using liquid paraffin on the edge borders. After that, the area containing the ointment was dropped with a 0.1 N KOH solution and observed the formation of a purplish red stain. If it forms red purplish stain quickly, then the ointment is easily penetrated by KOH, so the protection power of the dosage is relatively low.

The results show that the ointment dosage with the addition of 5, 10 and 15 ppm extract and ketoconazole did not form red stain for more than 2 minutes in 15 days storage time, but the ointment dosage without the addition of extract formed red stain less than 2 minutes on day 10 and 15. This suggests that an ointment dosage with the addition of 5, 10 and 15 ppm extracts and ketoconazole are able to provide protection, while the ointment without the addition of the extract provides a low protection. These different results are due to the nonhomogeneity of the ointment preparations without the addition of the extracts i.e. the presence of coarse grains and phase separation which lead to changes in the density of the particles in the preparation. Good ointment requirements are able to provide protection against all external influences such as acid-base, sunlight, and dust during treatment [21].

Adhesion

The adhesion test was used to study the retention time of the ointment when applied to the skin. Good adhesion showed a long life ointment that would have optimum antimicrobial effect during application [22]. The adhesion test is indicated by the time it takes to release two glass objects with a certain surface area that has been smeared by the ointment and has been given a certain load. The result of the test is shown in Figure 4.

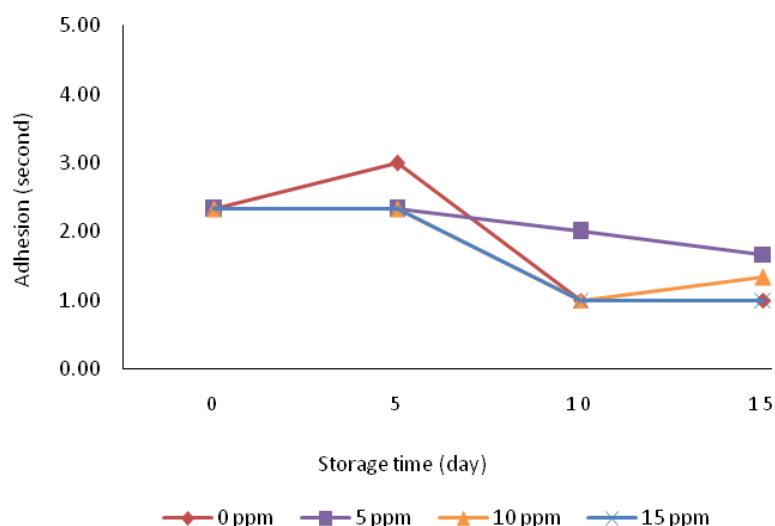


Figure 4 The effect of storage time and ethanol extract concentration to adhesion of the ointment

The adhesion value of 0 ppm concentration is, 00-1.00 seconds, 5 ppm concentration is 2.33-1.67 seconds, 10 ppm concentration is 2.33-1.00 seconds and 15 ppm concentration is 2.33-1.00 seconds. Adhesion of semisolid dosage should range more than 1 second. So, decreasing adhesion value of the ointment dosage from extract is in accordance with good adhesion criteria.

Antifungal Activity of Ointment Dosage

Tests were performed on ointment dosage at concentrations of 5, 10 and 15 ppm. The concentration of 0 ppm as negative control and ointment with the addition of 15 ppm ketoconazole as the positive control. The results of the antifungal test of the ointment can be seen in Figure 5.

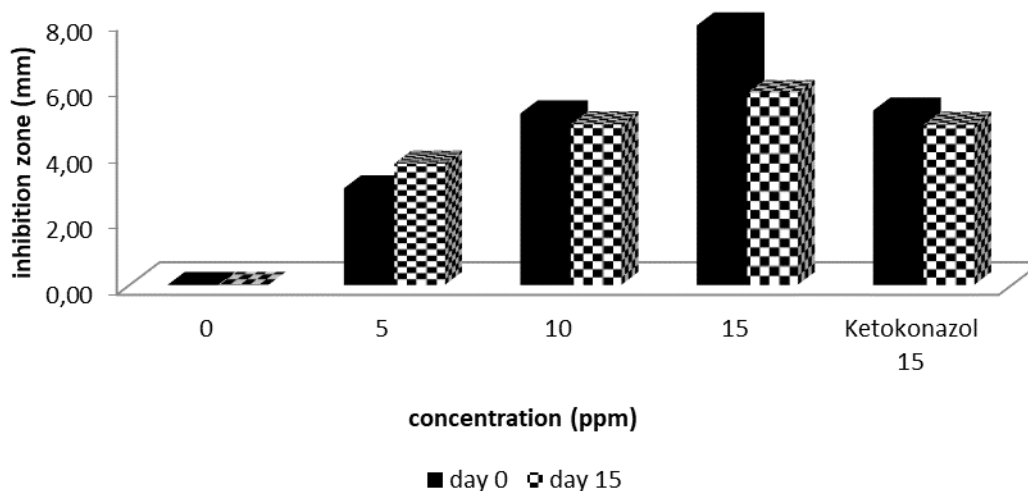


Figure 4 The effect of storage time and ethanol extract concentration to antifungal activity of ointment dosage

The 5 ppm ointment produced an inhibition zone of 2.93 mm on day 0 and 3.68 mm on day 15, the 10 ppm ointment produced an inhibition zone of 5.29 mm on day 0 and 4.87 mm on day 15, the 15 ppm ointment resulted from an inhibition zone of 7.87 mm on day 0 and 5.82 mm on day 15. The dosage without extracts did not produce inhibition zone and the dosage with ketoconazole resulted from an inhibition zone of 5.29 mm on day 0 and 4.86 mm on day 15.

Based on the test results, the extract which is formulated into ointment dosage is able to inhibit the growth of *Candida albicans*. The release of an active ingredient/drug in the ointment according to [9] depends on the chemical physics properties of the drug and also on the ointment basis properties. For example, an ointment dosage with a hydrocarbon base which is a fatty base has the fat or water-free property and a medium of its activity test tends to contain a lot of water so that the ointment is difficult to diffuse or release an active substance which eventually results in a relatively small inhibition zone[23].

CONCLUSION

Ethanol extract of white plumeria leaves has MIC against *C. albicans* at the concentration of 5 ppm with inhibition zone diameter of 1.22 mm. Ointment dosage from the ethanol extract of white plumeria leaves has fulfilled the SNI requirements in which has white color with semisolid form, the typical odor of ointment, homogenous, protective, has pH value of 5.07-5.59, the dispersive power of 5.09-5.78 cm, and adhesion of 1.00-2.33 seconds. Antifungal activity of the ointment dosage form on day 0 at concentration of 5, 10 and 15 ppm respectively are 2.93; 5.2 and 7.87 mm, and on day15 at concentration of 5, 10 and 15 ppm respectively are 3.68; 4.87 dan 5.82 mm.

ACKNOWLEDGMENT

We would like to thank the Ministry of Research, Technology and Higher Education of Indonesia and Jenderal Soedirman University through Research Grant of “Penelitian Produk Terapan”.

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