

Formulation and Evaluation of Natural Anti *Candida albicans* Ointment Containing Mango Leaf (*Mangifera indica* L.) Extract (Formulasi dan Penilaian Semula Jadi Salap Anti *Candida albicans* yang Mengandung Ekstrak Daun Mangga (*Mangifera indica* L.))

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ABSTRACT

Arumanis mango is one of plants potentially used as a medicine. *Arumanis mango* leaf is potentially used as antimicrobial, which are as antibacterial and antifungal. Mango leaf extracts are able to inhibit *Candida albicans* with inhibition zone of 8.12 mm. The use of extracts for various purposes has disadvantages. This research aims to formulate ointments dosage from mango leaf extracts, to know its physical properties and its activity against *Candida albicans*. Antifungal activity test is conducted using diffusion method. The fungus used is *Candida albicans*. The absorbent ointment preparations has homogeneous semisolid form, yellowish white color, and distinctive odor. The ointment preparations has dispersive power value of 4.90-6.23 cm, adhesiveness of 2.0-7.0 seconds, pH value of 6.56-6.99 and able to protect skin from the outside environment. Inhibition zone of absorbent ointment with concentrations of 30, 65, and 125 ppm, respectively, was 3.07; 5.96; and 9.51 mm.

Keywords: Antifungal; *Candida albicans*; mango leaf; ointment

ABSTRAK

Mangga *Arumanis* adalah salah satu tumbuhan yang berpotensi digunakan sebagai ubat. daun mangga *Arumanis* berpotensi sebagai antimikrob, iaitu sebagai antibakteria dan antikulat. Ekstrak daun mangga dapat merencat *Candida albicans* dengan zon perencatan 8.12 mm. Penggunaan ekstrak bagi pelbagai tujuan mempunyai kelemahan. Kajian ini bertujuan untuk memformulasi dos salap daripada ekstrak daun mangga, untuk mengetahui sifat fizikal dan aktiviti terhadap *Candida albicans*. Ujian aktiviti antikulat dijalankan menggunakan kaedah penyebaran. Kulit yang digunakan ialah *Candida albicans*. Persediaan salap penyerap yang mempunyai bentuk semipepejal seragam, warna putih kekuningan dan bau tersendiri. Persediaan salap yang mempunyai nilai kuasa serakan 4.90-6.23 cm, perekatan 2.0-7.0 saat, nilai pH 6.56-6.99 dan mampu untuk melindungi kulit daripada persekitaran luar. Zon perencatan penyerap salap dengan kepekatan 30, 65, dan 125 ppm masing-masing adalah 3.07; 5.96; dan 9.51 mm.

Kata kunci: Antikulat; *Candida albicans*; daun mangga; salap

INTRODUCTION

Indonesia is a tropical country with humid air. One of the fungi that can cause disease is *Candida albicans*. *C. albicans* can cause candidiasis (Casari et al. 2010). Topical medications that have been used to treat skin candidiasis include nystatin, clotrimazole, miconazole, ketoconazole and other azoles. Chemical drugs have negative effects (Brooks et al. 2005).

Negative effects caused by synthetic antifungal drugs can be overcome with exploration of herbal medicines. Traditional medicine derived from plants has less side effects and risk than chemical drugs (Muhlisah 1999). One of sources that can be used as an herbal antifungal drug is a plant. Mango plants (*Mangifera indica* L.) has the potential as an herbal medicine because it contains secondary metabolite compounds. Preliminary and experimental studies have shown that the ethanol extracts of *M. indica* leaf is efficacious as analgesic, anti-

inflammatory in experiments on mice, and antimicrobial (Islam et al. 2010). Methanol extract of mango leaf contains alkaloids, flavonoids, steroids, polyphenols, tannins, and saponin (Ningsih et al. 2017). Their results showed that methanol extract of mango leaf (*Mangifera indica* L.) can inhibit the growth of *C. albicans*. Ethanol extract of white plumeria can be formulated into ointment preparations with semisolid-shaped, white color, typical odor of ointment, homogeneous but not protective, pH of 4.57-6.10, dispersive power of 10-6.06 cm, adhesion of 1.67-3 s, and antibacterial activity of optimum ointment preparations at 5 ppm resulted an inhibition zone of 24.00 mm (Ningsih et al. 2017). This results showed the potency of plant extracts formulated as ointments.

Based on the explanation mentioned, methanol extracts of *Arumanis mango* leaf are expected to have activity against the growth of *C. albicans* and able to be formulated into ointment preparations.

MATERIALS AND METHODS

MATERIALS AND APPARATUS

Material used was mango leaf (*M. indica* L.) from local source (Karangwangkal Purwokerto, Indonesia), *Candida albicans* INAcc obtained from Lembaga Ilmu Pengetahuan Indonesia (LIPI), *Sabaroud Dextrose Agar* (SDA), *Sabaroud Dextrose Broth* (SDB), cera alba, paraffin, Na-borax, propyl paraben, methyl paraben, methanol, ketoconazole tablet 200 mg.

Apparatus used were oven crock borer, mortar, test tube, incubator, drugalsky, Vernier scale, watch glass, pH meter, object glass, and spectrophotometer *Thermo Scientific Genesys 20*.

FORMULATION OF ABSORBENT OINTMENT PREPARATIONS

The ointment preparations are formulated based on the composition prepared according to the previous reported (Himawati & Erawati 2003) with methanol extract of mango leaf as an antifungal agent in various concentrations incorporated into the preparations. Formulation of absorbent ointment from methanol extracts of mango leaf can be seen in Table 1.

EXAMINATION ON THE OINTMENT PHYSICAL PROPERTIES

Examination on the physical characteristics of antifungal ointment were performed to determine the pH value and emulsion stability for each ointment type, due to the addition of methanol extract at various concentrations. Examination on day 0; 5; 10; and 15.

Organoleptic Ointment was observed on the shape, odor, and color.

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 normal use of ointment to the skin surface (Bayuaji et al. 2012).

Ointment pH The methanol extract ointment as much as 0.5 g was diluted with 10 mL of aquades, then dipped pH meter for 1 min. pH of 4.5 - 6.5 is a skin pH (Wasitaatmadja 1997).

Dispersive power The 0.5 g ointment was placed on petri, and another petri was put on it and left for 1 min. The spreading diameter of the ointment was measured. Thereafter, 150 g additional load was added and left for 1 min and then constant diameter was measured. Spreadability of topical preparations is 5-7 cm (Kumaresan et al. 2012).

Adhesion The 0.25 g ointment was placed on the top of two object glasses, pressed with 1 kg load for 5 min. The object glass was mounted on the test kit. The test tools was loaded by 80 g load and the ointment release time from the object glass was recorded. Adhesive power should be more than 1 s (Lieberman et al. 1998).

Protection power Filter paper (10 × 10 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 (2.5 × 2.5 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 s, 3 and 5 min, no stains appeared means that the cream provides protection. The procedure was conducted 3 times for each ointment.

ANTIFUNGAL OINTMENT ACTIVITY TEST
AGAINST *C. albicans*

This test was conducted after ointment storage for 15 days (Septiadi et al. 2013). The *C. albicans* was grown in Sabaroud Dextrose Broth (SDB) medium for 24 h. The fungus cultures were measured by a spectrophotometer at λ 600 nm to obtain a 25% transmittance value. When the transmittance more than 25%, the culture was diluted. The fungus culture solution of 50 μ L was then spread on the *Sabaroud Dextrose Agar* (SDA).

SDA medium was then made one holes by using crock borer and added by 0, 1 g methanol extract ointment of mango leaf at 30; 65; and 125 ppm in each hole and then

TABLE 1. Formulation of absorbent ointment from methanol extracts of mango leaf

Materials	F ₁	F ₂	F ₃	F ₄	F ₅
Cera alba	10 g	10 g	10 g	10 g	10 g
Liquid Paraffin	59.91 g	59.91 g	59.91 g	59.91 g	59.91 g
Propylparaben	0.0025 g	0.0025 g	0.0025 g	0.0025 g	0.0025 g
Aquades	20.06 g	20.06 g	20.06 g	20.06 g	20.06 g
Na-borax	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g
Methylparaben	0.005 g	0.005 g	0.005 g	0.005 g	0.005 g
Methanol extract of mango leaf	0 ppm	30 ppm	65 ppm	125 ppm	-
Ketoconazole Tablet	-	-	-	-	125 ppm

incubated for 1×24 h at 37°C . Then, inhibition zone of each *C. albicans* for each ointment was measured (clear area around the hole). Negative control is ointment preparations without extracts and positive control is ointment with 125 ppm ketoconazole.

RESULTS AND DISCUSSION

STUDIES ON OINTMENT PHYSICAL PROPERTIES

Organoleptic Organoleptic test was conducted by observations on physical appearance of ointment preparations including the color, the dosage form and the smell. It aims to see the effect of storage on the ointments. This organoleptic test was performed on ointment preparations with addition of methanol extract of mango leaf (*M. indica* L.) at 0; 30; 65; and 125 ppm.

Based on the observations, the ointments at concentration of 0 and 30 ppm show yellowish white color, and at concentrations of 65 and 125 ppm show yellow color. The more extracts are added; the thicker color it shows. The odor test of ointment at concentrations of 0, 30, 65, and 125 ppm show typical ointment smell with ointment form is semi solid. Overall, the ointment preparation did not change after 15 days of storage. This indicates that the ointment has stable properties. According to Voigt and Noerono (1994), the ideal ointment physical properties must be stable.

Homogeneity Homogeneity test aims to determine whether during formulation of ointment, the active substance along with the basic materials and other necessary additives are mixed homogeneously. Homogeneity test was conducted by observing ointments on glass objects at the top, middle, and bottom part. The parameters observed were the texture of ointment (Indonesia 2000).

Homogeneous test of absorbent ointments at various concentrations has no difference after storage for 15 days. The result shows a homogeneous form and the absence of

coarse or clumped particles. Homogeneous preparations will give good results because the drug substances are dispersed in the basic material evenly, therefore, each part of the preparation contains the same amount of drug substances and is evenly distributed when used.

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 had optimum antimicrobial effect during application (Wibowo et al. 2017). The test was conducted by measuring the release time of the ointment on the plate. The graph of relations between adhesion of absorbent ointment preparations and the storage time can be seen in Figure 1.

The result showed that adhesion value during 15 days storage at concentration of 0 ppm is 7.0 - 2.0 s; concentration of 30 ppm is 3.0 - 2.1 s; concentration of 65 ppm is 3.1 - 1.3 s; and concentration of 125 ppm is 3.0 - 1.3 s. This result is in accordance with the reference which suggested that the ideal adhesion value of semisolid preparations must be more than 1 s (Lieberman et al. 1998).

The result of statistic analysis with ANOVA ($\alpha = 0.05$) on absorbent ointment with concentration of 0, 30, 65 and 125 ppm obtained significantly different result, and the result of analysis of absorbent ointment preparations towards storage time obtained the same. This shows that the addition of extracts and length of storage affect the adhesion of the absorbent ointment.

Dispersive power The dispersive power was performed to study the spread ability of the ointment. Good ointment should have complete dispersive power that facilitated the antimicrobial effect during application (Naibaho et al. 2013). Dispersive power is related to usage comfort. Good dispersion is highly expected in topical preparations. The research result shows that the dispersive power value during 15 days storage at concentration of 0 ppm is 6.03 -

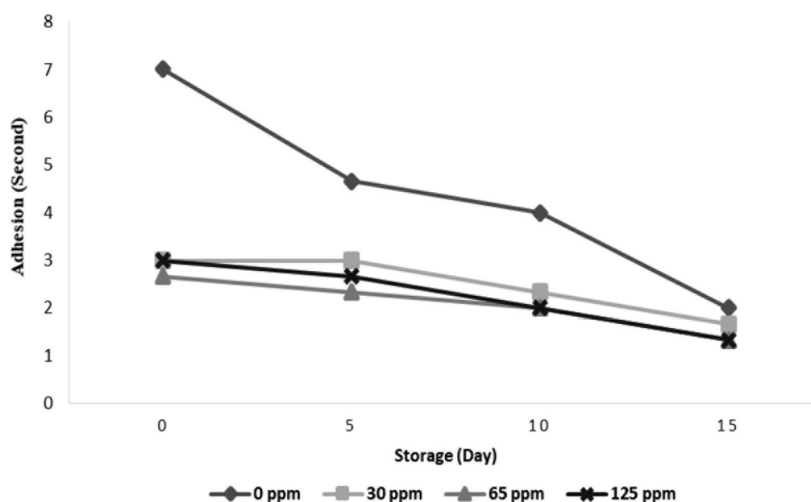


FIGURE 1. Effect of storage on the adhesion of different concentration ointment

6.23 cm; 30 ppm is 4.90 - 5.59 cm; 65 ppm is 5.05 - 5.64 cm; and 125 ppm is 5.02 - 5.72 cm. This corresponds to a good dispersion reference for topical preparations which is 5-7 cm (Kumaresan et al. 2012; Ulaen et al. 2012). The results of the test can be seen in Figure 2.

The result of statistic analysis with ANOVA ($\alpha = 0.05$) on absorbent ointment with concentration of 0, 30, 65 and 125 ppm obtained significantly different result. This suggests that the addition of the extract affects the ointment dispersion. The results of analysis for the 15 days storage time does not obtain significantly different result.

pH The pH values were observed before and after 15 days storage. The pH value is important to determine the acidity level of the preparations to avoid skin irritation, overly acidic pH cause skin irritation while overly alkaline pH causes scaly skin (Mappa et al. 2013; Swastika & Mufrod 2013). The result of pH test of ointment is shown in Figure 3.

Based on the result, during 15 days storage pH value at concentration of 0 ppm is 6.99 - 6.74; 30 ppm is 6.59

- 6.72; 65 ppm is 6.56 - 6.77; and 125 ppm is 6.72 - 6.83. The pH obtained does not correspond to Indonesian National Standard (SNI) range, which is pH of 4.5-6.5.

The result of statistical analysis with ANOVA ($\alpha = 0.05$) test on absorbent preparations with concentration of 0, 30, 65 and 125 ppm obtained no significantly different result whereas the 15 days storage obtained significantly different result. This suggests that the length of storage affects the absorbent ointment.

Protection power The protection power showed the stability of ointment during application (Stiani et al. 2016). The ointment protection test was performed with 0.1 N KOH. The ointment preparations may provide protection against 0.1 N KOH liquids when red stain does not appear on the traces of KOH 0,1N droplets on the filter paper. The appearance of red stains on filter paper is due to the interaction between phenolphthalein and alkaline compound (KOH 0.1 N). This result show that the absorbent ointment preparations provides protection from outside environment.

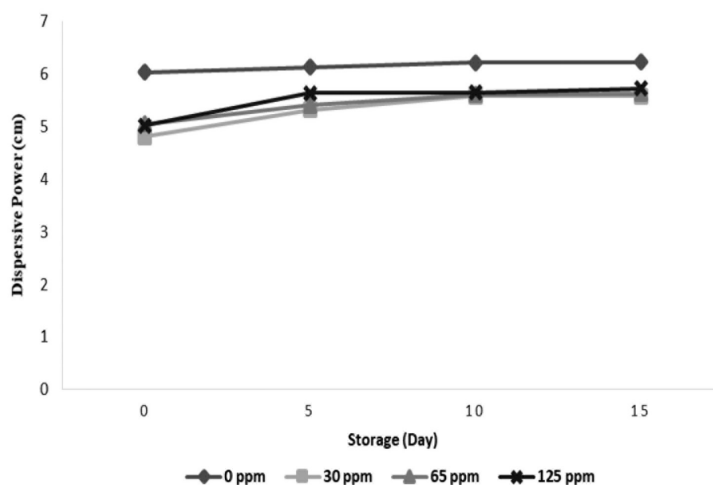


FIGURE 2. Effect of storage on the dispersive power of different concentration ointment

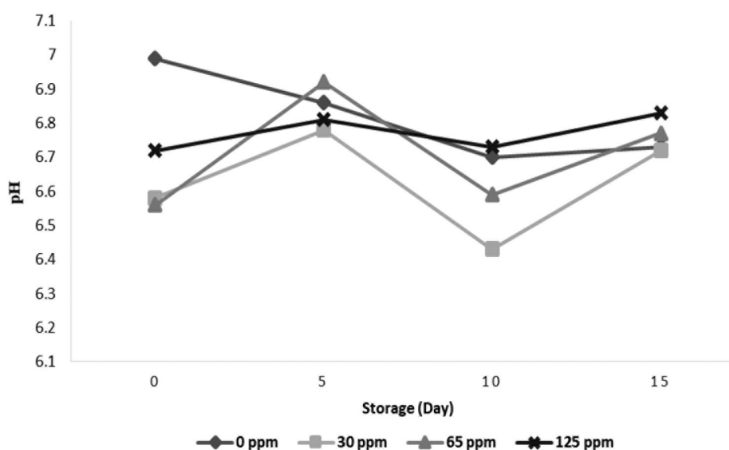


FIGURE 3. Effect of storage on the pH of different concentration ointment

ANTIFUNGAL ACTIVITY OF OINTMENT
AGAINST *Candida albicans*

The result of antifungal test of the ointment against *C. albicans* at concentrations of 30, 65, and 125 ppm shows respective inhibition zones of 3.07; 5.96; 9.51 mm. Concentration of 0 ppm or negative control does not show any inhibition zone while the positive control shows the inhibition zone with a diameter of 14.13 mm. The result can be seen in Figure 4. The more methanol extracts of mango leaf are added, the greater inhibition zone are resulted. Positive control used was ketoconazole tablet formulated into dosage at concentration of 125 ppm.

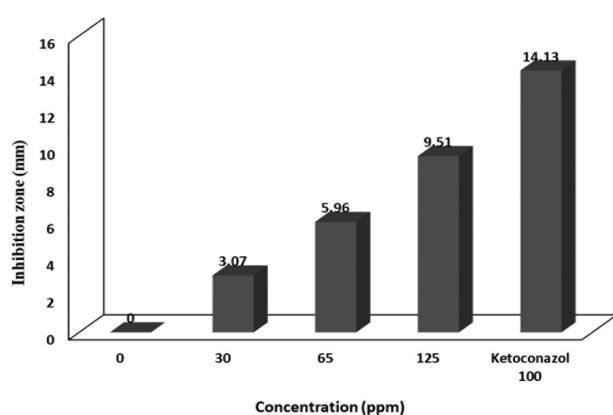


FIGURE 4. effect of ointment concentration to the inhibition of *Candida albicans*

The result of statistical analysis with ANOVA ($\alpha = 0.05$) on absorbent ointment with concentration of 0, 30, 65, 125 ppm and positive control obtained significantly different result. This shows that the addition of extracts affects the resulting inhibition zone. The analysis was continued with the Duncan test. Duncan test result showed that 125 ppm concentrations were significantly different from other concentrations and positive controls.

CONCLUSION

The absorbent ointment preparations have homogenous semisolid form, white yellowish color, and typical odor of ointment. It has dispersive power value of 4.90-6.23 cm, adhesion value of 2.0-7.0 seconds, pH value of 6.56-6.99 and absorbent ointment provides skin protection from outside environment. Activity of absorbent ointment preparations at 30, 65, and 125 ppm respectively are 3.07; 5.96; 9.51 mm.

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