

FORMULATION OF LENGKUAS RHIZOME (*Alpinia galanga* L.) EXTRACT'S GEL AS ANTIFUNGAL WITH HIDROXY PROPHYL METHYL CELLULOSAE (HPMC) AND CARBOPOL BASE

FORMULASI GEL EKSTRAK LENGKUAS (*Alpinia galanga* L.) SEBAGAI ANTIJAMUR DENGAN BASIS HIDROKSI PROPIL METIL SELULOSA (HPMC) DAN CARBOPOL

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ABSTRACT

Infection of Malassezia furfur on skin can be caused by bad factor. One of the materials that can be used as an antifungal is Lengkuas rhizome extract (Alpinia galanga L.). This study had purpose to determine the antifungal activity and the effectiveness of gels Lengkuas rhizome extract on pathogenic fungi using maseration method with etanol 96%. Gel making is used HPMC and carbopol base. Testing activity of extract and testing the effectiveness of the gel by disc diffusion method (Kirby-Bauer test). Screening result showed that the extract contains triterpenoids, flavonoids and essential oil. Based on the test results against Malassezia furfur, antifungal activity of the extract in the gel increased compared to extracts without formulated into a gel. But the activity increasing was not significant based on statistical analysis with one-way ANOVA test obtained a significance of 0.234 ($p > 0.05$).

Keywords: gel, lengkuas, antifungal, HPMC, carbopol

ABSTRAK

Infeksi Malassezia furfur pada kulit bisa karena faktor buruk . Salah satu bahan yang dapat digunakan sebagai anti jamur adalah ekstrak rimpang Lengkuas (Alpinia galanga L.). Penelitian ini bertujuan untuk mengetahui aktivitas antijamur dan efektivitas gel ekstrak rimpang lengkuas pada jamur patogen menggunakan metode maserasi dengan etanol 96 %. Pembuatan gel digunakan HPMC dan basis Carbopol. Pengujian aktivitas ekstrak dan menguji efektivitas gel dengan metode difusi cakram (uji Kirby - Bauer). Hasil skrining menunjukkan bahwa ekstrak mengandung triterpenoid, flavonoid dan minyak esensial . Berdasarkan hasil uji terhadap jamur Malassezia furfur aktivitas antijamur dari ekstrak dalam gel meningkat dibandingkan ekstrak tanpa dirumuskan menjadi gel . Namun peningkatan aktivitas tidak signifikan berdasarkan analisis statistik dengan satu - way ANOVA diperoleh signifikansi sebesar 0,234 ($p > 0,05$).

Keywords: gel, lengkuas, antifungal, HPMC, karbopol

INTRODUCTION

Kalimantan Barat has high temperature. Hot conditions is one of factor on Tinea versicolor / pityriasis versicolor disease, disease of the skin caused by a fungal infection, *Malassezia furfur*. One of the plants that have the potential as an antifungal is lengkuas. The chemical compounds are essential oils which are composed of eugenol, sesquiterpenes, pinene, methyl-cinnamic, kaem-erida, galangan, and galangol (Handajani, 2008).

While research done by Setyarini and Krisnansari (2011) showed that the compounds flavonoids, phenols, triterpenoids and essential oil has inhibitory effects against fungi. Mechanism of lengkuas rhizome as antifungal is inhibition growth of fungal that broke permeability of cell membrane. Generally, gel used in many medicinal products, cosmetics, food and some industrial processes. Drug formulation in gel preparation will affect the amount and speed of active substances that can be absorbed. From the above description it will be tested formulation of lengkuas rhizome extract's gel with HPMC and

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Carbopol base. Evaluation preparations are organoleptic, viscosity, dispersive, stickiness, pH, and safety of gel.

METHODOLOGY

The research material includes extracts of lengkuas rhizome, the reagents for the phytochemical screening, gelling materials, media Sabouraud Dextrose Agar (SDA), and the fungus *Malassezia furfur*. Research test of lengkuas rhizome extract's gel on *Malassezia furfur* was done in Laboratory of Pharmacy Studies Program, Medicine Faculty, Tanjungpura University; Laboratory of Forestry, Forestry Faculty, Tanjungpura University; Laboratory of Microbiology, Health Analysis Faculty, Health Polytechnic, Pontianak, West Kalimantan.

Sampling and Sample Preparation

Sampling

The sample used in this research was lengkuas rhizome with age 3-4 months. The rhizome was taken in Rasau Jaya II, Kubu Raya District, West Kalimantan.

Sample Preparation

The stages in the preparation of botanicals includes several stages. Rhizomes that have been obtained are then sorted wet, washed, chopped, dried, sorted dried, crushed, and saved.

Making of Lengkuas Rhizome Extract

Extraction using a maceration method. Maserat collected and repeated until a clear liquid extract. Then evaporated by an evaporator to obtain extracts (Depkes RI, 1979).

Analysis using Phytochemical Screening

Phytochemical screening were include on the examination of alkaloids, triterpenoids/steroids, tannins, flavonoids, saponins, and essential oils.

Testing Activity of Lengkuas Rhizome Extract

The medium used is the medium Sabouraud Dextrose Agar (SDA). SDA instant mixed with antibiotics, olive oil, and distilled water. Manufacture of suspension fungus was fungus colony taken by ose needle and suspended in a tube containing 5 mL of sterile 0,9% NaCl solution and then compared by standard Mc.Farland 0.5 (ICMR, 2009).

Testing of antifungal activity using the disc diffusion method (Kirby-Bauer test). Ethanol as a negative control. Petri dishes were incubated at 37°C for 48 hours and observed inhibition zone formed (ICMR, 2009).

Table I. Concentrations

Material	Concentrations		
	3%	4%	5%
Stok (mL)	1,5	2	2,5
Ethanol (mL)	ad 5	ad 5	ad 5

Lengkuas Rhizome Extract's Gel-Making

Formulation reference to research Helal *et al.* (2012) with some modifications. HPMC was dissolved in cold water. Carbopol was dissolved in hot water and add TEA. Methyl paraben was dissolved in propylene glycol. Added to a mixture of methyl paraben and propylene glycol. After that add lengkuas rhizome extract which has been diluted with glycerin. Last, 250 g water was added to the gel formulation (Wathoni dkk., 2009). Table 2 is the design formulation used (Helal *et al.*, 2012).

Table II. Draft Formulation

Material	Formula (%)
Lengkuas extract	x
Glycerin	10
Methyl paraben	0,2
HPMC	2
Propylene glykol	20
Aquadest	ad 250 g

Testing Effectiveness Lengkuas Rhizome Extract's Gel

Testing the effectiveness of the gel using the disc diffusion method (Kirby-Bauer test). Positive control used was Ketomed gel containing Ketoconazole 2%. Negative controls used were formulated gel without extract.

Evaluation Preparations

Evaluation preparations made to give a general overview of gel.

RESULT AND DISCUSSION

Raw Materials Processing Results

Harvesting was done at 09.00 a.m., because on the morning, plant contains many metabolites. Harvesting was done on lengkuas age 3-4 months because of many component water (Setyarini and Krisnansari, 2011). Processing of raw materials was done to obtain a stable crude drug until the next treatment.

Extraction of Simplicia

A cold maceration extraction method was chosen so that there was no damage caused by heating, especially chemicals contents. Ethanol can extract all the active ingredients contained in

lengkuas. Antifungal component largely soluble in ethanol as galangin, eugenol, kaempferol, quercetin (Windholz, 1983). Ethanol can dissolve essential oils (Soebagio *et al.*, 2006) which is thought to be the active ingredient. Extraction was done until there was clear and constant color of maserat. Maserat was evaporated by a rotary evaporator. Extract obtained is 16,2132 g with a yield of 3.945%.

Phytochemical Screening of Extract

Results of phytochemical screening of the extracts of lengkuas rhizome can be seen in Table III.

Table III. Phytochemical Screening of Extract Results

No.	Examination	Result
1	Alkaloid	-
2	Triterpenoid/ Steroid	+/-
3	Tanin	-
4	Flavonoid	+
5	Saponin	-
6	Essential oil	+

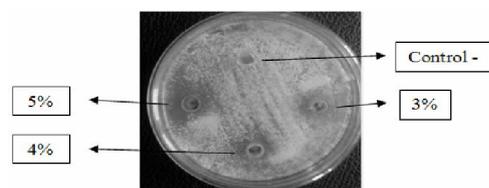
Preparation and Testing Antifungal Activity by Disc Diffusion Method (Kirby-Bauer test) Results

Media made in this research was the SDA. In the research Gholib (2011) used the media SDA to test antifungal power. SDA is instant media with the addition of antibiotics and olive oil. Antibiotics used were chloramphenicol 250 mg, so bacteria does not grow on the medium. While the purpose of the addition of olive oil was a supplemental nutrition.

Testing Antifungal Activity by Disc Diffusion Method (Kirby-Bauer test) Results

Disc diffusion method (Kirby-Bauer test) was used for the purpose in accordance with this research, to see a large sample of antifungal resistance, demonstrated by the clear zone. The results showed that ethanol did not cause inhibition zone. It meant that stock solutions and variation of concentration showed pure antifungal activity. Concentration of the extract were 3,4, and 5% and had inhibition zone showed on figure 1.

Figure 1. Result of Extract Activity Testing



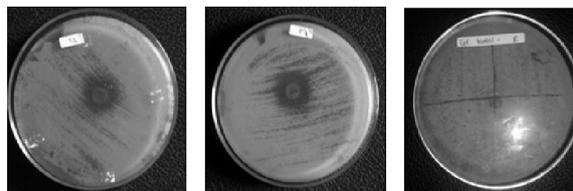
Lengkuas Rhizome Extract Gel-making

Extract formulation in gel form was intended to allow the base to hold the evaporation of the active compounds contained so that it can withstand the loss of extract on the skin due to the various activities by the user, thus resulting antifungal effect will be longer and efficient when used. Overall gelling materials have their respective. HPMC can produce a neutral, clear, colorless and tasteless, stable at pH 3-11, has a good resistance against microbial attack and provide good film strength when it dries on the skin (Suardi *et al.*, 2004). Carbopol is used as high gelling agent because in low concentration, it can make mass of gel (Carter, 1975).

Effectiveness Testing Antifungal Disc Diffusion Method (Kirby-Bauer tes)

Antifungal efficacy testing used the disc diffusion method (Kirby-Bauer test) where the concentrations chosen for making gel was 3% because of extract antifungal activity that made a zone of inhibition.

Figure 2. Inhibition zone diameter of gel 3%, positive control, negative control



The results showed that the negative control did not produce inhibition zone against the test fungi. While the positive control caused inhibition zone which was characterized by a clear zone. Results Inhibition Zone Diameter Extracts and Gel can be seen in Table IV.

Evaluation preparations

Organoleptic: Brown gel, characteristic lengkuas odor, thick and creamy texture; Coverage: The results showed that the average value of the dispersive gel is an area of 55,7689 cm²; Sticking power: The results showed that the average value of the stickiness of the gel was 6,1 minutes; Viscosity: It was unknown In this study because the value of viscometer instrument was not spinning so that can not be used to determine the value of the viscosity of the gel; pH: The results showed an average value of 6.233; Test Security: The results showed that lengkuas rhizome extract's gel was safe to use because it did not cause irritation after topical at the back of the hand.

Table IV. Results Inhibition Zone Diameter Extracts and Gel

Fungus	Concentrations (%)	Inhibition zone (mm)			Average (mm)	
		I	II	III		
<i>Malassezia furfur</i>	Extract	3	25	18	20	21
	Activity	4	20	23	26	23
	Test	5	24	24	27	25
	Control -	-	-	-	-	-
	Effectivity	3	22	24	20	22
	Gel Test	Control +	25	24	25	24,6
	Control -	-	-	-	-	-

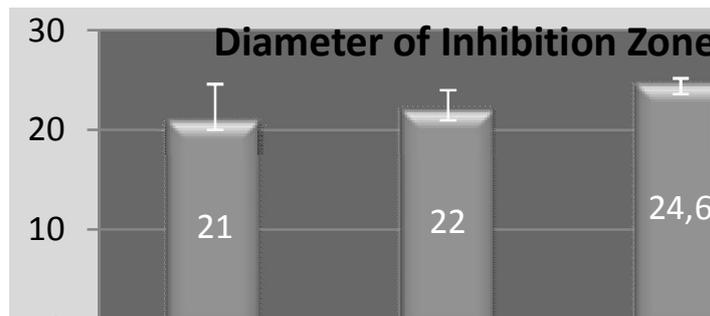


Figure 3. Diagram of Inhibition Zone

Analysis Result

Analysis of the data in this study was done by statistical tests One-way ANOVA (Analysis of Variance) to look for significant value of the ratio between the diameter of inhibition zone in extracts, gels, and a positive control. Statistical analysis was performed using the program R. ANOVA test. The first was a test of normality to determine that the data were normally distributed or not. This test can be done with the analytic method Shapiro-Wilk to know the normality. Data were normally distributed when the significance value > 0.05 and the result was obtained significance value of 0.0793 (> 0.05), which meant that the data were normally distributed. The second requirement, dependent variable should have the same variance between two or more groups of data. To find Levene's test of homogeneity of variance and the significance of the results obtained rate > 0.05 (0.122) which means between homogeneous variance. The data were analyzed using one-way ANOVA by significant numbers of 0.234 (p> 0.05), which meant there was no difference in outcome between the inhibition zone extracts, gels, and a positive control. Based on the analysis, showed that gel with extract concentration of 3% effective as an antifungal in the *Malassezia furfur* fungi.

CONCLUSION

Lengkuas rhizome extract contains metabolites such as triterpenoids, flavonoids, and essential oils. The effectiveness of antifungal gel greater antifungal activity than extracts. Lengkuas rhizome extract gel formulations had organoleptic, dispersive power, adhesion, good pH as physical properties and chemical profiles.

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