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FORMULATION OF ETHANOL EXTRACT OF *CITRUS SINENSIS* LIQUID SOAP AS AN ANTIFUNGAL AGAINST *CANDIDA ALBICANS*

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ABSTRAK

Candidiasis is an infection that affects 20-25% of the world's population. The prevalence of infections caused by *Candida albicans* is estimated to reach 25,000 cases annually. *Candida albicans* can be opportunistic, attacking internal organs such as the heart, lungs, and kidneys, and spreading through the bloodstream, leading to severe symptoms. Treatments for candidiasis often involve synthetic antifungal medications. However, some of these drugs can cause side effects such as nausea, vomiting, and skin burning sensations. This study aims to formulate and test the antifungal effectiveness of a liquid soap preparation made from ethanol extract of *Citrus sinensis* peel against the growth of *Candida albicans*, the causative agent of candidiasis, in vitro. The method used is a laboratory experimental study with a One-Way Analysis of Variance (ANOVA) test. The analysis will evaluate the inhibitory effect of the ethanol extract liquid soap formulation from *Citrus sinensis* peel on the growth of *Candida albicans* in vitro and assess the organoleptic characteristics of the liquid soap. The results of this study showed that the ethanol extract of *Citrus sinensis* peel can be formulated into a liquid soap preparation with characteristics that meet the Indonesian National Standards for liquid soap. Formulation I of the liquid soap containing *Citrus sinensis* peel extract demonstrated the greatest inhibitory effect compared to other formulations. All formulations were able to inhibit the growth of *Candida albicans* in vitro, ranging from strong to very strong categories. The conclusion of this study is that *Citrus sinensis* peel extract can be formulated into a liquid soap and possesses antifungal activity against *Candida albicans*, categorized as strong to very strong.
Keywords: Liquid soap formulation., Citrus sinensis peel., Candida albicans

INTRODUCTION

Fungal infections are increasing year by year. The World Health Organization (WHO) says that one of the biggest health threats that continues to grow to the health of the world's population is fungal infections. Candidiasis is a disease caused by infection with the fungus of the genus Candida. Candidiasis is a significant health problem worldwide with an estimated incidence of around 75% in women and 50% in men [1]. The main fungal species causing candidiasis is *Candida albicans*. The prevalence of candidiasis in Indonesia continues to increase every year, with cases rising by approximately 25-50% annually. Pathological leukorrhea is the most common type of candidiasis [2].

Pathological vaginal discharge that continues will disrupt the function of female reproductive organs, especially in the fallopian tubes, which can cause infertility. In pregnant women, it can cause miscarriage, fetal death in the womb (KJDK), congenital abnormalities, and premature birth. In addition, *Candida albicans* in the human mouth can cause thrush. This fungus can attack the oral mucosa (oral candidiasis). If not treated properly, Candidiasis can spread and affect other parts of the body such as blood cells, kidneys, heart, brain and intestines. The worst possibility of Candidiasis is death. [3] [4].Candidiasis disease treatment therapy includes using synthetic drugs or those that have been commercialized such as Fluconazole. Fluconazole can cause side effects such as digestive disorders and headaches and even symptoms of hypoglycemia. In addition, the use of antifungal drugs that are not in accordance with doctor's recommendations can increase fungal resistance. The critical need for Candidiasis disease treatment therapy other than using synthetic drugs is currently in great demand [5].

Alternative natural medicine for Candidiasis disease such as using natural ingredients can be a solution to Candidiasis problems. The potential of natural ingredients such as sweet orange peel (*Citrus sienensis*) has been proven to inhibit the growth of *Candida albicans*. According to research [6] Ethanol extract of Citrus sinensis peel can inhibit the growth of Candida albicans due to the secondary compounds it contains such as flavonoids, alkaloids, phenolics, and steroids. According to [7] Ethanol extract of *Citrus sinensis* peel can inhibit the growth of *Candida albicans* in the moderate to very strong category.Candidiasis treatment therapy using natural ingredients can be an alternative in treating this disease. By making liquid soap for example, liquid soap made from natural ingredients such as Citrus spp extract can be an alternative treatment for Candidiasis, of course through various tests first such as testing the characteristics of national standards for liquid soap preparations and testing irritation of liquid soap.Several studies related to the formulation of liquid soap preparations from natural ingredient extracts that can have antimicrobial properties include: soap preparations from essential oils of citronella (*Cymbopogon nardus* L.) and cat's whiskers leaves (*Orthosiphon aristatus* (Bl) Miq.) have been proven to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* [8]. The formulation of liquid soap preparations containing Batak onion extract can inhibit the growth

of *Candida albicans* [9]**.** Study [10] stated that the formulation of liquid soap preparations containing star fruit extract at concentrations of 5 and 10% has the potential as an antimicrobial. According to [11] The liquid soap formulation with bacang leaf and nutmeg leaf extract can inhibit the growth of Candida albicans with a strong activity category with an inhibition zone diameter of 18.8 mm.

Based on the state of the art above, it is important to conduct research on the formulation of liquid soap preparations from ethanol extract of Citrus sinensis peel on the growth of Candida albicans, considering that ethanol extract of Citrus sinensis peel contains several secondary metabolic compounds that can inhibit the growth of Candida albicans. This research is a new strategy and alternative in the discovery of drugs other than synthetic drugs to treat Candidiasis without causing side effects during use, namely in the form of liquid soap preparations based on Citrus sinensis peel extract which has been proven to inhibit the growth of Candida albicans.

This study aims to formulate and test the antifungal effectiveness of liquid soap preparations from Citrus sinensis peel extract against Candida albicans which causes Candidiasis in vitro. The results of this study are expected to be a new reference in the pharmaceutical world, namely in the discovery of therapy to treat Candidiasis, namely in the form of liquid soap from ethanol extract of Citrus sinensis peel.

***Candida albicans* infection**

Candidiasis is an infectious disease caused by *Candida* spp. Candidiasis in female genitals is called pathological vaginal discharge. Pathological vaginal discharge occurs due to vaginal secretions containing high levels of sugar. This can occur during pregnancy, uncontrolled diabetes, and when women wear damp underwear that can support the growth of *Candida albicans*. *Candida albicans* can infect other organs such as the lungs, heart, and kidneys or be carried through the bloodstream and cause severe conditions. [12].

***Citrus sinensis* peel**

Citrus sinensis peel is one of the organic wastes that contains secondary metabolite compounds such as flavonoids, terpenoids, alkaloids and saponins. The content of these metabolite compounds can inhibit the growth of *Candida albicans* [13].

Materials And Methods

The research was conducted in the microbiology laboratory of Syedza Saintika University, Padang. This research is a type of laboratory experimental research, the data were analyzed using SPSS One-Way Analysis of Variance (ANOVA) test. The sample of this research was sweet orange peel (Citrus sinensis). Samples were taken in the Pasar Raya area of Padang City. Primary data were obtained from the diameter of the inhibition zone formed after the paper disc diffusion test was carried out. The formulation of liquid soap with ethanol extract of Citrus sinensis peel was made with variations of 5 different formulas, namely: (base formula, formula 1, 2 3, and 4) with a positive control of ketoconazole.

The formulation of liquid soap preparations was made with variations in the concentration of ethanol extract of Citrus sinensis, namely 0%, 50%, 25%, 10% and 5%. The formulation of liquid soap preparations refers to [14]. Other primary data is in the form of a test of the characteristics of liquid soap preparations from ethanol extract of *Citrus sinensis* peel.

RESULTS

A. Characteristic Test of Liquid Soap Preparations

1. Organoleptic Test

Table 1. Organoleptic test results

	Organoleptic	Days			Ket
		1	7	14	
Basis (0%)	Texture	Thin	Thin	Thin	MS
	Aroma	Orange	Orange	Orange	MS
	Color	Yellow	Yellow	Yellow	MS
FI (50%)	Texture	Thick	Thick	Thick	MS
	Aroma	Orange	Orange	Orange	MS
	Color	Thick yellow	Brownish yellow	Brown	MS
FII (25%)	Texture	Thick	Thick	Thick	MS
	Aroma	Orange	Orange	Orange	MS
	Color	Thick yellow	Brownish yellow	Brown	MS
FIII (10%)	Texture	Thick	Thick	Thick	MS
	Aroma	Orange	Orange	Orange	MS
	Color	Yellow	Yellow	Brown	MS
FIV(5%)	Tekstur	Thick	Thick	Thick	MS
	Aroma	Orange	Orange	Orange	MS
	Warna	Yellow	Yellow	Yellow	MS

Description :MS : Qualify

TMS : Not Qualify 2. Homogeneity Test

Table 2. Homogeneity Test Results

	Hari ke			Ket
	1	7	14	
Basis (0%	Homogen	Homogen	Homogen	MS
F I (50%)	Homogen	Homogen	Homogen	MS
F II (25%)	Homogen	Homogen	Homogen	MS
F III (10%)	Homogen	Homogen	Homogen	MS
F IV (5%)	Homogen	Homogen	Homogen	MS

Description :MS : Qualify

TMS : Not Qualify 3. pH Value Test

Table 3. pH Value Test

	Days			Ket
	1	7	14	
Basis	12,4	12,3	12,2	MS
F I	12,3	12,4	12,5	MS
F II	12,4	12,5	12,6	MS
F III	12,2	12,5	12,5	MS
F IV	12,2	12,4	12,5	MS

Description :MS : Qualify

TMS : Not Qualify4. Foam Height Test

Table 4. Foam Height Test

	Days						Ket
	1		7		14		
	Foam height (mm) vs (minute)						
	0	5	0	5	0	5	
Basis (0%)	15	11	10	7	7	4	MS
F I	4	3	3	1	2	1	MS
F II	7	5	5	3	2	1	MS
F III	13	11	12	10	10	6	MS
F IV	11	9	9	7	6	5	MS

Description :
MS : Qualify
TMS : Not Qualify

Organoleptic test shows that the texture of the base liquid soap is thinner than the liquid soap formula I, II, III and IV. The color of the formulation added extract is more concentrated than the base soap. The overall aroma of the formulation and base has an orange aroma. The homogeneity test from table 3 above shows that all formulations of liquid soap preparations produce homogeneous results. From table 4, the pH value of the liquid soap preparation ranges from 12.2 -12.6 and is categorized as basic. The foam height of the liquid soap preparation of all formulations is stated as stable.

B. Antifungal Test of Liquid Soap Preparation from Ethanol Extract of Citrus Sinensis Peel

Table 5. Diameter of inhibition zone of *Citrus sinensis* peel extract on the growth of *Candida albicans*

No	Formulation	Inhibition zone diameter (mm)		
		1	2	3
1	Basis (0%)	17	14	11
2	F I (50%)	30	34,5	35
3	F II (25%)	27,5	24,5	15,5
4	F III (10%)	22,5	16	11,75
5	F IV (5%)	11,5	15	19
6	Kontrol +	22,5	23	26

From table 5 above, it can be seen that the formulation of liquid soap formulation I has the largest inhibition zone diameter of 35 mm. While the base formulation has the smallest inhibition zone of 11 mm. From the results of the SPSS one way ANOVA test, it was found that all treatments had different effects on the growth of Candida albicans with a p value of 0.005 <0.05.

The inhibition zone is the area around the disc paper on the SDA media, where there is no growth of Candida albicans. This indicates that all formulations of liquid soap preparations of Citrus sinensis peel extract can inhibit or kill Candida albicans. This can be seen in the image below:

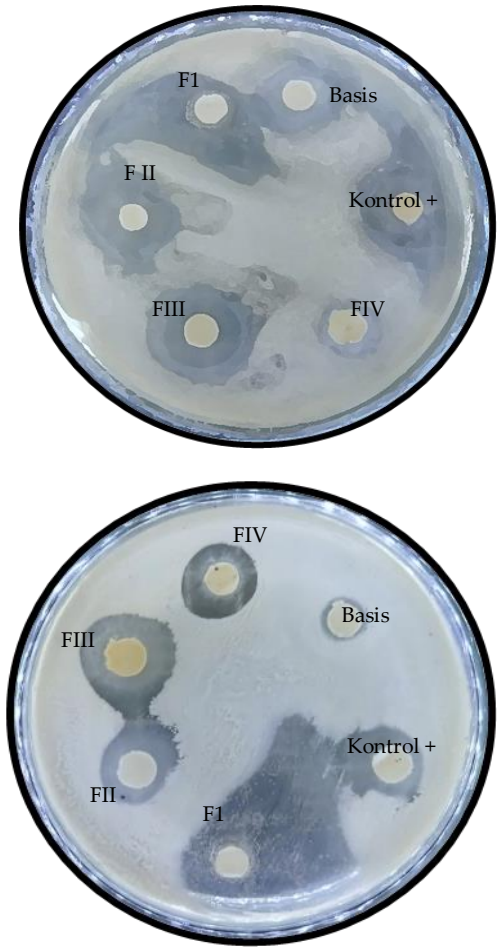

Figure 1. Inhibition zone of ethanol extract of skin

From Figure 1 above, it can be seen that the largest inhibition zone is formulation I followed by formulation II, III, IV and control. While the smallest inhibition zone is the inhibition zone without the addition of Citrus sinensis peel extract, namely the base. Liquid soap formulation without the addition of extract causes a smaller inhibition zone than the others, this is because this formulation does not add extracts containing antimicrobial compounds such as flavonoids, saponins and others.

DISCUSSION

Organoleptic tests showed that the liquid soap preparation of Citrus sinensis peel extract had a thin texture for the soap base and thick for liquid soap formulations I, II, III, and IV. The color of the formulation with the added extract was more concentrated than the base soap. The overall aroma and color of the formulation and base had an orange aroma, but the addition of extract to the formulation resulted in a more concentrated orange aroma in line with the concentration of the added extract. This is in line with research by [9], increasing color intensity of liquid soap preparations along with increasing concentration of added extract.

The homogeneity test aims to determine whether or not there are mixed particles and the distribution of color in liquid soap. From table 3 above, it can be seen that all formulations of liquid soap preparations produce homogeneous results. This is evidenced by the absence of floating and undissolved particles. [15].

The pH test of liquid soap was carried out to determine the pH value of the liquid soap preparation. From table 3, the pH value of the liquid soap preparation ranges from 12.2 -12.6 (base). This can be caused by the addition of KOH (strong base) to the liquid soap, the addition of extracts and the length of storage of the liquid soap preparation [14].

The foam height test of liquid soap preparation with Citrus sinensis peel extract was conducted to determine the ability of liquid soap preparation to form stable foam. The stability of liquid soap preparation foam can be caused by the CMC content in soap, CMC can play a role in foam stability, CMC and strengthens the foam wall and reduces water flow so that the foam can be stable [14].

From table 5 above, it can be seen that the formulation of liquid soap formulation I has the largest inhibition zone diameter, which is 35 mm. From the results of the SPSS one way ANOVA test, it was found that all treatments had different effects on the growth of Candida albicans with a p value of 0.005 <0.05. This is in line with research [16] which states that Citrus sinensis peel extract can inhibit the growth of Candida albicans. All formulations are categorized as having strong to very strong inhibition zones. The inhibition zone is categorized based on the size of the diameter of the inhibition zone formed, all formulations in this study showed a large inhibition zone, namely above 10 mm. This proves that the formulation of liquid soap preparations with ethanol extract of Citrus sinensis peel is categorized as susceptible, meaning that Candida albicans is susceptible to ethanol extract of Citrus sinensis peel [17].

From Figure 1 above, it can be seen that the largest inhibition zone is formulation I and followed by formulations II, III and IV while the smallest inhibition zone is the inhibition zone without the addition of Citrus sinensis peel extract, namely the base. Liquid soap formulation without the addition of extract causes a smaller inhibition zone than the others, this is because this formulation does not add extracts containing antimicrobial compounds such as flavonoids, saponins and others.

The formation of inhibition zones in the medium with the disc paper method is caused by the extract content in the liquid soap preparation. The higher the concentration of the liquid soap preparation extract, the greater the inhibition zone formed in the medium. This is in line with research by [18] which states that the higher the concentration of the extract, the greater the inhibitory power against the test microbes. In addition to the concentration of the extract, there are several factors that can affect the microbial inhibition zone, including the type of microorganism, media conditions and incubation time [19].

The content of metabolite compounds contained in Citrus sinenis peel extract is the main factor that influences the size of the inhibition zone formed. According to [20] The content of metabolite compounds contained in the ethanol extract of Citrus sinensis skin includes terpenoids, alkaloids, steroids, glycosides and flavonoids. And has antioxidant and antibacterial activity.

CONCLUSION AND SUGGESTIONS

Based on the results of the research that has been done, it was concluded that Citrus sinensis peel extract can be formulated into a liquid soap preparation with several characteristics that are in accordance with the Indonesian National Standard (SNI) as a liquid soap. However, further formula development is still needed to obtain a preparation that meets all the requirements. Each formulation can inhibit the growth of Candida albicans with a strong to very strong category. Each formulation (base, f I, II, II, IV and control +) has a significant difference in inhibiting the growth of Candida albicans with a sig p value of 0.005 <0.05.

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