

ANTIFUNGAL ACTIVITY OF LIQUID SOAP CURLY RED CHILI FRUIT(*CAPSICUM ANNUUM* L. VAR. *LONGUM*) EXTRACT AGAINST *CANDIDA ALBICANS*

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Abstract

Abnormal vaginal discharge can be caused by a *Candida albicans* fungal infection. Vaginal discharge that is not treated properly can cause infertility. Curly red chili plants have the potential to have antifungal activity because they contain chemical compounds in the form of alkaloids, flavonoids, tannins, saponins and triterpenoids. The aim of this study was to determine the antifungal activity of liquid soap curly red chili fruit (*Capsicum annum* L. var. *longum*) extract against *Candida albicans*. Curly red chili fruit was extracted using the UAE method with 70% ethanol solvent. The extract was formulated into liquid soap with formula 0 (0%), formula 1 (5%) and formula 2 (15%) which was tested against the *Candida albicans* fungus using the well diffusion method with control (+), namely Resik V Godokan Sirih. The research results showed that liquid soap with extract of curly red chili fruit could inhibit *Candida albicans* with the diameter of the inhibition zone of formula 0, formula 1 and formula 2 respectively 9.11 ± 0.31 mm (medium); 16.43 ± 0.11 mm (strong) and 20.19 ± 0.34 mm (strong). Control (+) had an inhibition zone diameter of 32.40 ± 0.59 (very strong). The optimal liquid soap formula for curly red chili fruit extract (*Capsicum annum* L. var. *longum*) in inhibiting *Candida albicans* is formula 2.

Key words: [Antifungal, Curly red chilies, *Candida albicans*, Liquid soap.]

INTRODUCTION

Vaginal discharge is the discharge of thick mucus, not blood, from the vaginal canal (Shalma & Puspitasari, 2020). Based on the cause, vaginal discharge is divided into two, namely normal and abnormal vaginal discharge. Normal vaginal discharge is influenced by hormones, while abnormal vaginal discharge is caused by bacterial, fungal or parasitic infections (Abid et al., 2016; Marhaeni, 2016). One of the fungi that most often causes vaginal discharge is *Candida albicans* (Tivani & Amananti, 2020). Women in Indonesia have the potential to experience vaginal discharge because Indonesia is a country with a tropical climate, so fungi easily grow which results in many cases of vaginal discharge. Vaginal discharge that is not treated properly can cause infertility (Emilia & Fadilah, 2019).

Vaginal discharge caused by *Candida albicans* infection can be treated using butoconazole, miconazole, nystatin, ketoconazole, itraconazole, and fluconazole (Haliyah & Yelda, 2015). These drugs have undesirable side effects such as nausea, stomach discomfort, diarrhea, flatulence, headaches and rashes (BNF, 2014). Improper use of drugs can also cause drug resistance (Candrasari, 2014).

In research by Lubis et al., (2022) stated that green chili extract has great potential as an antifungal against *Candida albicans* with a strong inhibitory zone diameter of 11.2 mm at a concentration of 6%. Curly red chilies contain alkaloids, flavonoids, tannins, saponins and steroids/triterpenoids which have the potential to have antifungal activity against *Candida albicans* (Sapitri et al., 2021). One of the preparations that can be used as an antifungal against *Candida albicans* is in the form of liquid soap. Therefore, research will be carried out on the antifungal activity of liquid soap with extract of curly red chili fruit (*Capsicum annum* L. var. *longum*) against *Candida albicans*. It is hoped that the results of this research will increase public knowledge about liquid soap with extract of curly red chili fruit (*Capsicum annum* L. var. *Longum*) as an alternative to herbal-based antifungal drugs.

METHODS

Types of Research

This type of research is experimental research with the aim of determining the antifungal activity of curly red chili fruit extract liquid soap (*Capsicum annum* L. var. *longum*) against *Candida albicans*. The research was carried out using the completely randomized design with concentration variations of 5% and 15%. Tools and Materials The tools and materials needed for this research are UAE (Ultrasonic Assisted Extraction, Laminar

Air Flow (LAF), ose needle, drigalski spatula, perforator, pH meter, caliper, curly red chilies, 70% ethanol, H₂SO₄, CH₃COOH, HCl 2N, bouchardat's reagent, dragendorff's reagent, mayer's reagent, NaOH 10%, distilled water, FeCl₃ 3%, chloroform, fungal culture *Candida albicans* ATCC 10231, Resik V Godokan Sirih, KOH, olive oil, rosae aroma, SLS, PDL (Potato Dextrose Liquid) and PDA (Potato Dextrose Agar).

Extraction of Curly Red Chili Fruit

Curly red chili fruit powder was extracted using UAE with a frequency of 40 KHz and a temperature of 40°C for 15 minutes with a break every 1 minute. The solvent used is 70% ethanol with a powder to solvent ratio of 1:10.

Phytochemical Screening

1. Alkaloids: the alkaloid test uses 3 reagents, namely Bouchardat, Mayer and Dragendorff.
2. Flavonoids: the flavonoid test uses 3 reagents, namely Wilstater, Bate Smith-Metcalf and 10% NaOH.
3. Saponin: the saponin test uses distilled water reagent enhanced with 2 N HCl.
4. Tannin: tannin test using 3% FeCl₃ reagent.
5. Steroids/ Triterpenoids: steroid/ triterpenoid tests using chloroform reagents, anhydrous CH₃OOH and concentrated H₂SO₄.

Determination of MIC (Minimum Inhibitory Concentration)

A total of 5 test tubes contained 3.5 mL of PDL and 0.5 mL of *Candida albicans* suspension. Four tubes were filled with curly red chili fruit extract with a concentration of 2.5%; 5%; 10% and 15% in 1 mL and labeled 1-4. In tube 5 it is labeled Control- (C-) and 1 mL of 5% DMSO is added. The tube was incubated for 24 hours at 28°C, then the MIC was observed by visually observing its turbidity (Munira & Nasir, 2023).

Curly Red Chili Fruit Extract Liquid Soap Formula

Table 1. Liquid Soap Formula for Curly Red Chili Fruit Extract

Material	Function	Unit	Formula 0 (F0)	Formula 1 (F1)	Formula 2 (F2)
Curly red chili fruit extract	Active substance	g	-	5%	15%
Olive oil	Fatty acid	mL	15	15	15
KOH 40%	Alkali	mL	8	8	8
CMC	Fillers and thickeners	g	0,5	0,5	0,5
Citric acid	pH neutralizer	g	3	3	3
SLS	Surfactant	g	0,5	0,5	0,5
Oleum rosae	Fragrance	mL	1	1	1
Aquadest	Solvent	mL	ad 60mL	ad 60mL	ad 60mL

Making Liquid Soap

Olive oil is put into a beaker, then 40% KOH is added until a soap paste is formed. The next step is to add CMC to the soap paste, then add citric acid and SLS, then stir until homogeneous. Oleum rosae is added and stirred until homogeneous. The aroma and dissolved curly red chili fruit extract are added to the mixture and stirred until homogeneous.

Uji Fisik Sabun Cair

1. Organoleptic test: macroscopic observation including shape, smell and color.
2. Homogeneity test: liquid soap is smeared on the glass object.
3. Specific gravity test: weighing with a pycnometer.
4. Foam height test: liquid soap and distilled water are put in a test tube, then shaken and the height of the foam formed is measured.
5. pH test: the pH test is carried out using a digital pH meter.

Liquid Soap Antifungal Test

A total of 100 µL of *Candida albicans* suspension was poured into a petri dish. Add 15 mL of PDA to the petri dish, homogenize by shaking it to form a figure 8 and allow it to solidify, then make a well with a diameter of 6 mm. Put 50 µL of liquid soap F0, F1, F2 and control+ (Resik V Godokan Sirih)

into each hole and label it. Incubated for 48 hours at 28°C, then observed the inhibition zone formed using a caliper.

Data Analysis

Data obtained from the diameter of the inhibition zone was then processed using one way ANOVA, post hoc LSD and linear regression analysis.

RESULTS AND DISCUSSION

200 grams of curly red chili fruit powder was extracted with 2000 mL of 70% ethanol, resulting in a thick extract of 59 grams, resulting in a yield value of 29.5%. The thick extract of curly red chili fruit is blackish brown in color with a distinctive chili odor. The results of the phytochemical screening showed that curly red chili fruit extract was positive for containing alkaloids, flavonoids, saponins, tannins and triterpenoids.

Table 2. Phytochemical Screening

No	Secondary Metabolites	Reagents	Interpretation	Result
1	Alkaloids	Bouchardat	An brown/black precipitate is formed	+
		Mayer	A white/yellow precipitate is formed	+
		Dragendorff	An orange precipitate is formed	+
2	Flavonoid	Wilstater	An orange color is formed	+
		Bate Smite-Metcalfe	An red color is formed	+
		NaOH 10%	An orange color is formed	+
3	Saponin	Aquadest + HCl 2N	Foam 1-10 cm high is formed	+
4	Tanin	FeCl ₃ 3%	Forms violet green color	+
5	Steroid/ Triterpenoid	Kloroform +	Steroid: A greenish blue ring is formed	-
		CH ₃ COOH + H ₂ SO ₄	Triterpenoid: Brown or violet rings form	+

The results of determining the MIC of curly red chili fruit extract are presented in table 3.

Table 3. MIC (Minimum Inhibitory Concentration)

Observation Results	Observation Results
C-	Turbid
2,5%	Turbid
5%	Slightly clear → MIC
10%	Clear
15%	Clear

MIC observed turbidity visually with the eye. If the solution in the tube looks clearer than the K(-) tube, this means that fungal growth is starting to be inhibited. The minimum inhibitory concentration is determined by the smallest extract concentration in the treatment tube that has begun to inhibit fungal growth. Based on table 2, it was found that the smallest concentration of curly red chili fruit extract was clearer than the negative control, namely a concentration of 5%, which shows that a concentration of 5% is the minimum inhibitory concentration that can inhibit the *Candida albicans* fungus.

The physical test results of curly red chili fruit extract liquid soap are presented in table 4.

Table 4. Physical test

Formula	Organoleptic test	Homogeneity Test	Specific Gravity Test	Foam Height Test	pH Test
Formula 0	- Form: liquid - Color: white - Odor: rosae	Homogeneous	1,0074	16mm	6,2
Formula 1	- Form: liquid - Color: light brown - Odor: rosae	Homogeneous	1,0233	25mm	5,9
Formula 2	- Form: liquid - Color: dark brown - Odor: rosae	Homogeneous	1,0235	26mm	5,8
Kontrol + Standar	- - Form: liquid	-	-	-	3,5
		Homogeneous	1,01-1,10	-	EPO: 5,5-8,5

mutu • Color: -
 • Odor: -

Vaginal pH: 3,5-4,5

Organoleptic tests were carried out to determine the physical form of liquid soap preparations. Based on table 4, it shows that the three formulas have a liquid form and a distinctive rose odor. Formula 0 has a white color, formula 1 has a light brown color and formula 2 has a dark brown color. The darker color is proportional to the increase in extract contained in liquid soap.

The homogeneity test aims to see the homogeneity of the liquid soap preparation (Ering et al., 2020). Homogeneous liquid soap indicates that the active substances are evenly distributed in the soap base (Muna et al., 2021). The homogeneity of a preparation is influenced by stirring during manufacture. Based on table 4, it shows that the three formulas are homogeneous.

The specific gravity test aims to determine the weight of a substance in air at a temperature of 25°C divided by the weight of water at the same volume and temperature (General Director, 2020). The specific gravity test results in table 4 F0 are 1.0074; F1 is 1.0233 and F2 is 1.0235. The specific gravity values for F1 and F2 meet INS (Indonesian National Standards) requirements, namely in the range 1.01-1.10; However, formula 0 does not comply with INS (National Standardization, 1996). Based on the test results, the more curly red chili fruit extract, the greater the specific gravity of the liquid soap.

The foam height test was carried out to see the foaming power of liquid soap (Ering et al., 2020). The foam height test results in table 4 F0 are 16 mm, F1 is 25 mm and F2 is 26 mm. The foam in liquid soap can affect the skin's pH. Liquid soap that has too much foam can cause dry skin, while too little foam can cause it

The pH test is carried out to determine the degree of acidity of liquid soap. The pH must be appropriate so as not to cause irritation because the soap comes into direct contact with the skin (Kasenda et al., 2016). The pH test results in table 4 show that F0 has a pH of 6.2; F1 has a pH of 5; F2 has a pH of 5.8 and control+ has a pH of 3.5. The decrease in pH in formulas 1 and 2 occurred due to the addition of curly red chili fruit extract. Curly red chili fruit contains vitamin C or ascorbic acid, so this fruit is acidic (Badriyah & Manggara, 2015). The three formulas are in accordance with the pH standard for feminine liquid soap according to the European Patent Office, which is in the range 5.5-8.5 (Bombart, 2002). Resik V has a pH that corresponds to vaginal pH, namely between 3.5-4.5 (Bartlik et al., 2018).

The results of the antifungal test for liquid soap with curly red chili fruit extract are presented in table 5.

Table 5. Liquid Soap Antifungal Activity Test

Treatment	Inhibition Zone Diameter (mm)	Category
Formula 0	9,11 ± 0,31	Medium
Formula 1	16,43 ± 0,11	Strong
Formula 2	20,19 ± 0,34	Strong
Control +	32,40 ± 0,59	Very strong

The inhibition zone of curly red chili fruit extract liquid soap that is formed is proportional to the concentration of the extract (Lingga et al., 2016). F0 does not contain extracts, but has inhibitory power because the soap base formula contains substances that can function as antimicrobials, namely olive oil and citric acid. Olive oil contains polyphenolic compounds which have antimicrobial activity (Bilal et al., 2021). Citric acid has antimicrobial activity by lowering the pH (Sabahannur, 2020).

The antifungal activity of curly red chili fruit extract liquid soap against the growth of *Candida albicans* is produced by secondary metabolites contained in curly red chili fruit extract including alkaloids, flavonoids, saponins, tannins and triterpenoids. The mechanism of alkaloids as antifungals is by inhibiting DNA esterase and RNA polymerase, inhibiting the process of cellular respiration and causing DNA, RNA and protein synthesis to be hampered (Aniszewski, 2007). Flavonoids also have antifungal activity by inhibiting cell growth and increasing membrane permeability (Zearah, 2014). The antifungal mechanism of saponins is by reducing membrane sterols which can cause increased permeability which ultimately results in cells becoming swollen and ruptured. (Yulia et al., 2023). Another compound contained in curly red chili extract is tannin which has two antifungal mechanisms, namely by deactivating enzymes that work to catalyze the reverse transcription reaction of single RNA into double DNA, and by interfering with protein synthesis. (Agustina et al., 2021). Curly red chili fruit extract also contains triterpenoid compounds. The antifungal mechanism of triterpenoids is by inhibiting the enzyme β -1,3-D-glucan synthase as a key enzyme in the biosynthesis of (1,3)-D-glucan which is the main component of fungal cell walls (Ghannoum et al., 2020).

The positive control contains herbal ingredients, betel leaf extract and fatimah grass extract. Betel

leaf extract contains steroid compounds, alkaloids, flavonoids and essential oils (Rukmini et al., 2020). Fatimah grass leaf extract contains phenolic and flavonoid compounds (Karimi et al., 2015). Apart from herbal ingredients, it also contains chemicals that have antifungal activity such as phenoxyethanol, chlorphenesin, sodium benzoate, piroctone olamine, potassium sorbate, o-cymen-5-ol, and hexamidine.

Statistically, the results of analysis using one way ANOVA and post hoc LSD showed that all sig values were <0.05 , indicating that F0, F1, F2 and control (+) had significant differences in response to the diameter of the inhibition zone.

The results of the linear regression test obtained a value of $R^2 = 0.9667$ which shows that the diameter of the *Candida albicans* inhibition zone is influenced by curly red chili fruit extract by 96.67%. The remaining 3.33% is influenced by other factors.

CONCLUSION

The optimal liquid soap formula for curly red chili fruit extract (*Capsicum annum* L. var. longum) in inhibiting *Candida albicans* is formula 2 which has an inhibition zone diameter of 20.19 mm in the strong category.

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