Gel Formulation Of Ethanol Extracts Of Durian Fruit Skin (Durio Zibethinus) As An Antifungal To Candida Albicans And Trichophyton Mentagrophytes

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Abstract: Durian is a fruit that Indonesian people demand, but environmental problems arise when the durian season comes due to unutilized durian skin wastes. This research harnesses the waste of durian skin which is then processed into a formulation of gel as an antifungal. The aim of this study is to prove that this gel formulation can inhibit the growth activity of Candida albicans and Trichophyton mentagrophytes. The maceration method was used for extraction processes with 70% of ethanol solvents. Extraction results were then made of gel formulations and separated into several concentrations, formulations 1 (15%), formulation 2 (20%), formulation 3 (25%), as well as base gel as a negative control and gel containing ketoconazole 2% as a positive control. Results show that the gel of durian skin extracts provided a weak inhibition zone at the highest concentration of 25% with an average 5.70 mm ± 0.27 for Candida albicans and did not give any inhibition zone on all gel formulations for Trichophyton mentagrophytes. Preparations of gel formulations were evaluated physically including organoleptic observation resulting F1 in brown, F2 in dark brown and F3 in blackish brown. Homogeneity tests show that all formulations were homogeneous, marked by no appearance of coarse grains. The pH value of all formulations in accordance with the cosmetic standard was about 4.5–6.5. The standard viscosity value of all formulations was between 2000–4000 cps. The stability test of gel preparations shows no changes in the form of low temperature storage (4º C), room temperature and high temperature (40 º C). Thereby, it can be inferred that the durian skin extract can be made in a gel preparation. This Gel has a weak antibacterial activity against Candida albicans and has no antibacterial activity against Trichophyton mentagrophytes.

Index Terms: Antifungal,Candida albicans,Durian Skin, Ethanol Fraction, Trichopyhton mentagrophytes

1 INTRODUCTION

Durian (Durio zibethinus) is a type of fruit that originated in Indonesia and its production is very abundant if the durian season comes. Durian fruits are in demand by the community, especially in Lampung. According to the research, statistics of durian production in Lampung from 2011–2018 was up to 50,298 tons. During the durian season, environmental problems arise from the skin waste that has not been utilized to the fullest. The more fans of durian fruit grows, the more durian skin which are produced, even often the skin of durian scattered on the roadside and its smell becomes pollution. Based on the screening of phytochemicals and the identification of the main components of the durian skin extract [3], the skin of durian positively contains alkaloids, flavonoids, saponins, steroids, triterpenoids and tannins. Thus, the skin of durian can be an alternative to treatment as an antifungal. Antifungal is a compound that can kill or inhibit the growth of fungal or infectious fungi [6]. Infectious diseases caused by fungi are difficult problems. Fungi are more able to withstand the unfavorable environment compared to other microorganisms.

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One of the causes of infectious microorganisms caused by fungi is Candida albicans which can cause a candidiasis infection of the skin (Setyowati et al., 2015). In addition to Candida albicans infections, the other type of fungi causing infection is Trichophyton mentagrophytes. The infections caused by these two fungi are ringworm (a skin disease characterized by a red circular form, [5]. To date, the community overcame infectious diseases with synthetic antibiotics. Continuous and improper use of synthetic antibiotics can lead to resistance. One of the efforts to overcome this is by replacing synthetic antibiotics with antibiotics from natural substances using the durian skin. The use of durian fruit skin to cure infections can be made easy by making its gel preparations. Gel preparations have several advantages of which are not sticky, easily to apply, to wash and do not leave oily coating on the skin. It also reduces the risk of further inflammation due to accumulation of oil in the skin pores [1]. Based on the facts related to durian skin that has been a waste, and can not be utilized maximally, the researchers are interested in utilizing it by making the preparations of the ethanol extracts of the durian fruit skin to Candida albicans and Trichophyton mentagrophytes.

2 Materials and Methods

2.1 Phases of Research

This study was designed for 1 year, including 7 research phases as follows: manufacture of simplicia (simplicia characterization and standardization), manufacture of durian fruit extract (characterization and standardization extract), manufacture of gel preparations, phytochemical screening with thin layer chromatography, test of the physical properties of the gel preparations, the stability test of the gel preparations and antifungal tests.

2.2 Research Samples

Samples used were the skin of durian fruits obtained from the two line road at Way Halim, Bandar Lampung (Trade Center of Durian in Bandar Lampung). The skin was taken with a selective random sampling technique, with a color criteria (mature yellow but not yet soft).

2.3 Manufacture of Ethanol Exstract of Durian Skin

Durian skin was dried using an oven for 4 hours with a temperature of 50°–60°C. Dry skin was disortated, then made into powder and sifted with mesh no. 40, weighed as much as 500 grams. The skin powder of the durian was dissolved in ethanol 95% as much as 500 ml and then closed, left for 5 days and stirred every day. Further filtered, the dreg was macerated for 1 day for a more perfect extraction withdrawal. The extracts obtained were collected and concentrated on the evaporator and evaporated in the water bath to obtain a condensed extract [10].

2.4 Formulations of Gel Preparations of Durian Skin

A gel preparation should have both components as below: 1. Gelling Agent. A number of polymers was used in forming tissue-shaped structures that are an important part of the gel system. Included in this group are natural gom, cellulose derivatives, and carbomers. Most of these systems function in water media, other than those that form gel in non-polar liquids. Some colloidal solid particles can behave as a gel-forming due to the occurrence of particle flocculation. High concentrations of some non-ionic surfactants can be used to produce clear gel in the system containing up to 15% mineral oil. 2. Additional Materials:

- **a.** Preservatives, although some gel bases are resistant to microbial attacks, but all the gel contain a lot of water so it requires preservatives as antimicrobials. The selection of preservatives should notice its compatibility
- **b.** with gelling agents.
- **c.** Addition of hygroscopic material aims to prevent water loss. E.g. glycerol, propylen glycol and sorbitol with a concentration of 10–20%.
- **d.** Chelating agents aim to prevent bases and substances that are sensitive to heavy metals. The components of the gel preparations are presented in table 1.

Table 1. *Formulation of gel preparations*

Materials	Formula (gram)				
	F1	F2	F3	F4	F5
Durian skin extracts	0%	15%	20%	25%	0%
Carbopol	2	2	2	2	2
Triethanol amine	0.5	0.5	0.5	0.5	0
Propylen glycol	10	10	10	10	0
Methyl paraben	0.1	0.1	0.1	0.1	0
Aquadest ad	10	100	100	100	0
Ketoconazole gel	0	0	0	0	2

Explanation: In Formula 1 (FI), it is used as a negative control (not given extract), Formula 2 (F2) with centration extracts 15%, Formula 3 (F3) 20%, Formula 4 (F4) 25% and Formula 5 (F5) is used as a positive control (gel medicinal preparations that serve as an antifungal).

Thin Layer Chromatography (TLC) Separation

Compound content tests with TLC. The idle phase used was the $G_{60}F_{254}$ with a size of silica gel plate 7 cm×2.5 cm, activated earlier by heating in the oven at a temperature of 100 ºC for 30 minutes. The motion phase used was chloroform : methanol : water with a ratio (2:5:3) (V/V/V). The silica plate was then boiled with samples 5 times using the capillary pipe. The silica plate was left for several minutes to dry and inserted into the chamber that had been saturated with the phase of the motion used. The stain that appeared on the chromatogram was observed in UV with a wavelength of 254 nm and 366 nm, then calculated its Rf value. Spots detected with Bouchardat spray reagent for alkaloids would show a color in brown, ammonia for flavonoids in yellow, green, brown or pink, FeCl3 for tannins produced colors of green, red, purple, blue or strong black, and Liebermann-Burchard for saponins [7]. Non-Specific Simplicia Characteristic Test A test of the simplicia characteristics carried out included a test of non-specific parameters. The minimum limit of standard tests performed were three tests such as moisture content, ash content and ash content that is not soluble in acids [4].

1. Moisture determination

Enter 10 grams of simplicia and weigh carefully in the container that has been tested. Then the cup and sample was dried at 105 ºC for 5 hours, cooled and weighed, continued drying and weighing at a distance of 1 hour until the difference between 2 consecutive weighing of no more than 0.25%, and moisture content not more than 10%. Moisture content can be calculated using the formula:

$$\frac{Initial\ weight\ of\ simplisia - average\ weight}{weight\ of\ simplisia}$$

2. Determination of ash content

As much as 2–3 grams of simplicia was crushed and weighed thoroughly, put into the silicate block, then flatten. Samples were rolled slowly until the charcoal ran out, chilled and weighed. If in this case, charcoal cannot be eliminated, filter it by using ash-free filter paper. Rub until the weight was fixed, then weighed. Calculated the ash content that is not soluble in acid against the material that is dried in air, ash contents not more than 8.6%. Ash contents can be calculated using the formula:

$$Ash\ content = \frac{Ash\ weight}{Simplisia\ weight} 100\%$$

3. Determination of insoluble ash content in acid

Ash obtained at the determination of its content, were boiled with 25 mL aqueous HCl for 5 minutes. The insoluble part in the acid was collected, strained with a filter paper, washed with hot water, and rubbed. Calculated the insoluble ash content in acid against the material that has been dried in air, the insoluble ash content is not more than 2.9%. The insoluble ash levels of acids can be calculated using the formula:

$$Acid\ insoluble\ ash = \frac{Ash\ weight}{Simplisia\ weight} 100\%$$

Antifungal Test

The manufacture of mushroom suspension was carried out by taking 1–2 cc mushrooms (using an inoculum loop) to be dissolved in NaCl 0.9% and equated with McFarland 0.5 Standard (1.5 x 108 CFU/mL). Testing of activity of durian fruit skin gel against the growth of fungus Candida albicans and Trichophyton mentagrophytes was carried out using the disk diffusion method. A total of 200 µL of mushroom suspension with a turbidity of 1.5 x 108 CFU/mL was dripped on the SDA media and applied evenly to the media. Then each section was made of a hole diameter of 6 mm with a depth of 4 mm, then inserted the formulation of the gel in each hole. It would be incubated for 2 days in the temperature of 37^{o}C. After it was observed, the barrier zone formed around the eastern hole was measured using the caliper [6].

Data analysis

Data obtained was analyzed using analysis of variance (ANOVA). If there was a noticeable difference then a further test was done to see the difference in each concentration.

3 Results and Discussion

Simplicia Characteristic Test

Testing characteristic of simplicia that has been performed was a test of non-specific parameters with a minimum test limit that was three tests included the moisture content, ash content and an insoluble ash content. The test results of non-specific characteristics of simplicia can be seen in table 2.

***Table 2**. Test Results of Simplicia Characteristics*

Parameter	Result	Term
Moisture Conten t	7.8%	<10%
Ash Content	4.6%	<8.6%
Acid Insoluble Ash Content	2.0%	<2.9%

Determining moisture contents of simplicia aims to provide minimal or range limitation on the amount of the water content in the ingredients. The water content test used the gravimetric method and the water content of durian skin fruit obtained 7.8% of the water content that has been fulfilled the quality of the prescribed simplicia standard (less than 10%). Determination of the ash content and acid insoluble ash content aimed to provide an overview of the content of internal and external minerals originating from the beginning until the formation of extracts. The ash content obtained at 4.6%, the results obtained has fulfilled the quality standard of the simplicia set (8.6%). The acid insoluble ash content of durian skin has fulfilled the standard obtained by the result of 2% of the prescribed standard of no more than 2.6%. Simplicia that has passed the characteristic test has been stabilized and proved that the simplicia of durian fruit is simplicia which has a moisture content and good internal and external mineral content [4].

The Evaluation Result of Gel Preparations

On the observation of organoleptic, the durian skin extract was done to assess shape, smell and color. The preparation forms for all four formulations were a gel, in accordance with its definition that is a clear semi-solid preparation (no bubbles), translucent and contains active substances. The resulting smell of the three formulations was the distinctive smell of durian fruit skin extracts. The colors produced were distinctive brown as a typical extract. This can be seen in table 3.

***Table 3.** The Evaluation Result of Gel Preparations*

Sampl e	Form	Smell	Color	Imag e
K (-)	Gel	Typical gel base	Inhibit	
F1 (15%)	Gel	Typical extract	Brown	
F2 (20%)	Gel	Typical extract	Dark brown	
F3 (25%)	Gel	Typical extract	Blacki sh brown	
K (+)	Gel	Chemical antibiotics	Inhibit	

This test aims to observe the alteration of shapes, colors and smells. After testing all formulations with the appropriate form of gel, the resulting color for the gel base is without clear colored extracts and for all three colored formulations as well as the resulting odor of the three formulations smelled like typical extracts.

Homogeneity Test

A homogeneity test is performed to see if the preparation has been made homogeneous or not. The way to figure it out is by applying it to the glass of objects that are closed with a glass cover and see whether it appears coarse bubbles or not [10].

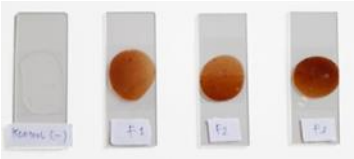

***Image 1.** The Homogeneity Test*

In this test, the four formulations showed good or homogeneous results as they did not look at the visible coarse grain so that they fulfilled good gel requirements.

pH Measurement Results

The measurement value of the gel preparations will affect the quality of the gel preparations. pH of topical preparations must comply with the requirements of intervals 4.5–6.5, and should not be too acidic because it will cause irritation and also should not be too alkaline because it will cause dry and scaly

skin [10]. The pH value can be seen in table 4.

***Table 4**. The Result of Measurement of Durian Skin Gel*

Sample	pH sample	Term
K (-)	6.9	4.5–8.0
F1	6.3	
F2	5.8	
F3	5.2	

The gel preparation formula is made up of alkaline material so that the gel base pH tends to be high (close to neutral) (K-) while the extract-containing gel preparations (F1, F2, F3) have a lower pH (approaching acid) compared to the preparation that does not contain extracts. It can be influenced by the addition of extracts. The higher the centration of the extract then the pH will approach the acid [2].

Viscosity Measurement Results

The measurement of viscosity values affects the quality of the gel. The viscosity value is presented in table 5.

***Table 5**. The Viscosity Measurement Results*

Sample	Viscosity (cps)	Term
K (-)	3500	2000–4000 cps
F1	2880	
F2	2690	
F3	2320	

Based on table 5, the results of the viscosity measurements of each formulation experience a difference in this case because of the centration of the addition of different extracts. The higher the centralised extract added, the smaller the viscosity value. The viscosity of durian extract gel is still within the range of good gel viscosity requirements [13].

Stability Test Accelerated by Cycling Test Method

This stability test was performed by storing gel at a low temperature of 4 º C for 24 hours. And later the gel was moved at a high temperature of 40 º C for 24 hours after it was stored at room temperature (27–28 º C). Storage was done by 6 cycles then the physical evaluation test was tested

***Table 6.** Results of Organoleptic Cycling Test; Measuring gel stability*

Sample	Organoleptic			Note
	Temp. (40ºC, 27-28ºC, 4ºC)			
	Form	Color	Smell	
K(-)	Gel	Inhibit	Typical base	Stable
F1	Gel	Brown	Typical extract	Stable
F2	Gel	Dark brown	Typical extract	Stable
F3	Gel	Blackish brown	Typical extract	Stable

All formulations, after stored at high temperature, room temperature and high temperature stabilized, had no changes in shape, color and smell. It was characterized by the preparation of fixed-form gel, a typical color that was brown to blackish brown, and smelled like typical durian skin extracts.

PHYTOCHEMICAL SCREENING RESULTS WITH THIN LAYER CHROMATOGRAPHY (TLC)

Thin layer chromatographic spot results showed positive results containing alkaloids after being sprayed with bouchardat reagent, characterized by a brown color formed on the TLC plate. The making of the reagent Bouchardat iodin reacts with ion I^- from potassium iodide to produce an ion-colored I^{3-} in brown color. A bouchardat test of K^+ ions would form a covalent bond of coordinates with nitrogen in the alkaloids, forming a complex potassium-alkaloids [14].

Thin layer chromatographic spot results in the reagent ammonia show positive flavonoids results due to the formation of orange color after the reagent was sprayed on the TLC plate. The result of the color tannins reagent formed on the TLC plate shows the black color. The color change on the plate shows the positive result of the tannins compound inside the durian skin extract gel. The change of color is due to the formation of compound tannins with metal Fe. Complex compounds are formed due to the coordination of covalent bonds between ions or metal atoms with nonmetallic atoms [8].

Test result with Liberman-bouchardat reagent for the saponins test shows positive result and purple color in TLC plate. The Rf calculation was obtained at 0.76, while in the related research to raw saponins as a comparison using pure saponins had Rf value of 0.8. The Rf value obtained in this study approached the Rf value [11].

4. Results of Antifungal Tests

An antifungal test of durian skin extract gel againsts two different fungi, namely T. Mentagrophytes and C. albicans. Test results can be seen at the image below

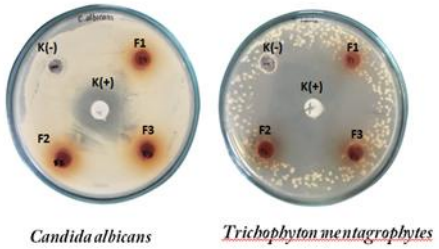

Image 2. Tests of C. albicans and T. mentagrophytes

Results of antifungal preparation tests of durian skin extracts against C. albicans and T. Mentagrophytes may inhibit the growth of fungi on Candida. It can be seen from the formation of the disk. But the inhibition zones were small in F1, F2 and F3. In F3, the inhibition zone formed amounted to 5.70 mm with a moderate response of inhibition. Then, it did not form an inhibition zone in K- and had 18.17 mm an inhibition zone on K+. This is in line with the previous research [12] at the centration of 40% of durian skin fractionate forming an inhibition zone of 5.70 mm with medium-inhibitory response category. At T. Mentagrophytes for all test formulas were not formed inhibition zones, but there was a wide inhibition zone in K+ namely 33.12 mm. Further, the centration of gel extracts or active substances is too small so it can not inhibit the growth of the fungi. The previous research on the centration of 40% of fractionate inhibited growth of fungi of 3.47 mm with the

category of weak inhibitory response [12]. The inhibition zone measurement data can be seen in table 7.

Table 7. *Data of The Inhibition Zone Diameter*

Fungi	Formulation (F)	Average diameter of the inhibition zone (mm)	Inhibitory Response [5]
C. albicans	K (-)	0±0.00[a]	None
	F1	3.30±0.23[b]	Weak
	F2	4.12±0.34[b]	Weak
	F3	5.70±0.27[c]	Average
	K (+)	18.17±0.42[d]	Strong
T. mentagrophytes	K (-)	0±0.00[a]	None
	F1	0±0.00[a]	None
	F2	0±0.00[a]	None
	F3	0±0.00[a]	None
	K (+)	33.12±0.35[b]	Very Strong

Explanation: The numbers in the same column followed by the same upper case letter (at the end of the standard deviation) are no different from the actual 5% Tukey Test level. After the data obtained, the zone diameter of the barrier was conducted by a statistical test. The results of the homogeneity test obtained homogenous data and the significant value was greater than 0.05, which amounted to 0.120 for T. Mentagrophytes and 0.279 for C. Albicans. Homogeneous data has qualified the Anova test and advanced test using Tukey's advanced test. The result of Anova on C. albicans in K- was significantly different from F1, but F1 and F2 were not significantly different, but had a significant difference with F3 and K+. In T. Mentagrophytes for all formulas (F1, F2, F3 and K-) did not have a significant difference but did with K+. The secondary metabolite compounds in the preparation of the durian skin extracts are flavonoids, tannins, alkaloids and saponins. Flavonoids play an important role in the intercelation or bonding of hydrogen with complex compounds of extracellular and dissolved proteins that destroy fungi cell membranes and are followed by the discharge of intracellular compounds [11]. The alkaloid antifungal mechanism is by working inhibition enzymes that play a role in DNA replication. DNA replication inhibition causes fungi to not be able to prevent cleavage, preventing growth of fungi. Interactions between saponins compounds with cell walls resulted in damaged cell walls and cell membranes until finally the walls and membranes of lysis fungi (Juariah and Wahyuni, 2017). Tannin compounds have antifungal activity by inhibiting the synthesis of chitin used for the formation of cell walls in fungi and damaging the cell membranes so that the growth of fungi is hindered [3]. Based on the results of the phytochemical screening of durian skin extract gel preparations, there are secondary metabolite compounds that should inhibit the growth of these fungi. However, due to the small concentration used in the formulation of gel-making, results are less potent in inhibiting the growth of these two fungi.

5. ACKNOWLEDGMENTS

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