An Experiment for Extracted Citrus Hystrix Leaf Effectiveness on Pityrosporum Ovale Fungi Growth

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Keywords: Effectiveness, Citrus Hystrix, Dandruff, Pityrosporum Ovale Mushroom.

Abstract: The growth of Pityrosporum ovale causes an inflammatory reaction both due to metabolite products that enter the epidermis and fungal cells itself through the activity of T lymphocytes and Langerhans cells. This causes discomfort and insecurity. Treatment of dandruff usually uses shampoos but some people often experience allergic contact dermatitis due to the chemical side effects of these shampoos. *Jeruk purut* (Citrus Hystrix) leaves are easily found in Indonesia and are natural ingredients that contain anti-fungal compounds, namely flavonoids and tannins. This type of research was a laboratory experiment with the sensitivity test method of the diffusion disk method. The sample used was 5 kg of citrus hystrix leaves and incubated for 48-72 hours. This study used a concentration of 25%, 50%, 75%, the negative control contained aquadest, while the positive control used 2% ketoconazloe. Experiments were carried out three times. The results showed that the highest inhibition zone diameter was at a concentration of 75%, namely 16.20 mm.

1 INTRODUCTION

Jeruk purut (citrus hystrix) is a plant that is known to the public as a food source and is thought to contain active compounds which are believed to be herbal medicine with very high antioxidant activity so that it is widely used in daily needs, both in medical, industrial, and household. The use of kaffir lime fruit and leaves has been known by the public for a long time as herbal medicine. Leaf and fruit parts are usually used to overcome fatigue and improve body fitness and as a flavoring for food (Adrianto, H., Yotopranoto, S. and Hamidah, H. 2014).

Citrus hystrix leaves are strongly suspected to contain triterpenoid steroids, tannins (1.8%), and essential oils (1-1.5%) so that they can be used as an anti-fungal. The scalp contains saponins, tannins (1%), and essential oils that contain citrate (2-2.5%) (Santoso, 2015).

The fungus that causes dandruff is Malassezia sp. and one of its species is Pityrosporum ovale. This fungus is a normal flora that is in the hair, but various conditions such as temperature, humidity, high oil content, and decreased immunity can trigger the growth of this fungus. (Alawiyah, Khotimah, and Mulyadi, 2016).

Dandruff is a scalp problem that makes sufferers feel disturbed both physically and psychologically. In people with dandruff, the number of Pityrosporum ovale will increase by more than 47%. Dandruff occurs due to malfunctioning caused by changes in the keratinization process which is constantly pushed to the surface of the skin and becomes layered, dry, brittle, and easily loose scales. (Karta and Burhannuddin, 2017)

Malassezia sp is reported to be resistant to the use of azole drugs. One way to cope with and prevent excessive dandruff is to use anti-dandruff shampoo. The levels of active substances in anti-dandruff shampoos may also cause allergic skin reactions. Active substances such as sulfur compounds, selenium sulfide which are accumulated and absorbed by hair follicles can also cause hair loss (Idris, 2013).

Citrus Hystrix leaf extract contains alkaloids, flavonoids, terpenoids, and phenols which have antioxidant activity. The most potent antioxidant effects of Citrus Hystrix leaf extract are flavonoids, alkaloids and phenols (Lawrence BM, 2018).

Based on the results of previous research, it was found that the test results of the IC50 antioxidant value of Citrus Hystrix leaf extract were 25.907 ± 0.187; ethosome of Citrus Hystrix leaf extract

Ramadhani Nasution, S., Nasution, A. and Nasution, S.
An Experiment for Extracted Citrus Hystrix Leaf Effectiveness on Pityrosporum Ovale Fungi Growth.
DOI: 10.5220/0010352902910295
In *Proceedings of the International Conference on Health Informatics, Medical, Biological Engineering, and Pharmaceutical (HIMBEP 2020)*, pages 291-295
ISBN: 978-989-758-500-5

formula 1 of 28.814 ± 0.431; formula 2 is 32.299 ± 1.893 and formula 3 is 30.234 ± 0.531 which shows that the three formulas have very strong antioxidant activity (Kasuan N,et all , 2009).

2 METHOD

To determine the inhibition of citrus hystrix leaf extract against the fungus Pityrosporum ovale, the sensitivity test method (disc diffusion) was used. In the experiment, 5 treatment groups included negative control using aquadest, positive control using ketoconazole ointment 2%, concentrations of 25%, 50%, and 75%. The medium used was Potato dextrose agar. The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Prima Indonesia University.

The tools and materials used in the experiment were petri dishes (7 pieces), test tubes, filter paper, measuring cups, autoclaves, incubators, disc paper, cotton swabs, tweezers, sterile cotton, calipers, drop pipettes, erlenmeyer flasks, analytical scales. , test tube racks, 5 kg kaffir lime leaves, 70% ethanol, 96% ethanol, Pityrosporum ovale mushrooms, 2% ketoconazole ointment, alcohol, aquabidest, physiological NaCl, spiritus, potato dextrose agar (PDA) media, plastic wrap, and aluminum foil.

Preparation stages, namely: The tools and materials used are sterilized in an oven at a temperature of 40-70º C for ± 2 hours, At this stage of making citrus hystrix leaf water, first clean the citrus hystrix leaves with water until clean then the citrus hystrix leaf water is sliced small and blended (Figures 1 and 2.

The citrus hystrix leaf extract which has been evaporated with ethanol was divided into 5 treatment groups with a concentration of 25%, 50%, 75%, positive control, and negative control by dissolving with 96% ethanol technique. Dissolving with ethanol aims to remove the essential oil content in the extract. The extract was made by macerating the citrus hystrix leaves which had been cleaned with running water before drying. The dried citrus hystrix leaves were mashed using a blender to form simplicia powder. From each simplicia, 1 kg of sample was taken and then dissolved or soaked using 10 liters of absolute ethanol (96% ethanol) solvent per each simplicia (Figure 1). Then stirring per day for 1 hour. This maceration process was carried out 3 times.

The citrus hystrix leaves are washed with running water then drained until dry and put in a drying cabinet for 3 days before mashed using a blender. The powder was sifted to a fine powder of the citrus hystrix leaves.

For the experiment, several tools such as petri dishes (7 pieces), test tubes, measuring cups, are sterilized first using an autocalved at a temperature of 121ºC for about 15 minutes. Meanwhile, the tools made of metal are sterilized on an incandescent fire for about 1 minute.

Preparation of Potato Dextrose Agar (PDA) as much as 65 grams of medium is suspended in 1 liter of distilled water into an erlenmayer flask and then stirred using a hotplate strirer for 1 minute or until dissolved. The sterilization was then carried out by autoclave for 15 minutes at 121 ° C and allowed to cool until the temperature has decreased to 40-45 ° C. The results obtained were in the form of jelly poured into a petri cloud and cooled until frozen.

The preparation of the Pityrosporum Ovale Mushroom suspension used in this study was obtained from the USU Pharmacy Laboratory. The Pityrosporum Ovale used was made by taking a loop of germs from the culture so that it is tilted and then put into a tube containing physiological NaCl then stirring until all fungal colonies dissolve in NaCl.

The diffusion test of pityrosporum ovale was carried out by: PDA media that had hardened, evenly scratched the entire surface of the media using a cotton swab containing the suspension of Pityrosporum Ovale fungus; a sterile blank disk is taken; dipped in 50% concentration, and finally placed on the surface of the media that has been scratched by the fungus Pityrosporum Ovale. The treatment is carried out for all concentrations then spaced apart to prevent the inhibition zones from forming. The same experiment was repeated 3 times for data collection needs. All test isolates were incubated for 36-48 hours at 37ºC in an incubator. After 36-48 hours, the zone of inhibition is measured using a caliper. The classification of the response to fungal growth inhibition is given in Table 1 (Maryanti, Marta, Della, and Hamidy, 2017).

Table 1 Classification of fungal growth inhibition responses.

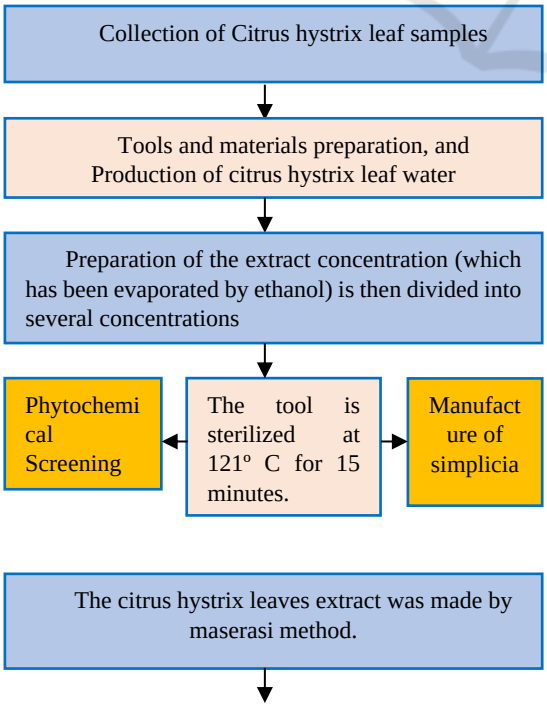
Clear zone diameter	Inhibition Growth Rresponse
>2cm	Very strong
1,6-2 cm	Strong
1-1,5 cm	Moderate
<1 cm	Weak

From Table 1, it is obtained that the larger the diameter of the clear inhibition zone was > 2 cm, then the response to fungal growth will be stronger and on

the contrary, the response to growth resistance will be weaker. In summary, the experimental process can be described as in Figure 3.

Figure 1. Drying process of Citrus Hystrix and mashed leaves

Figure 2. The process of filtering and making Citrus Hystrix leaf water



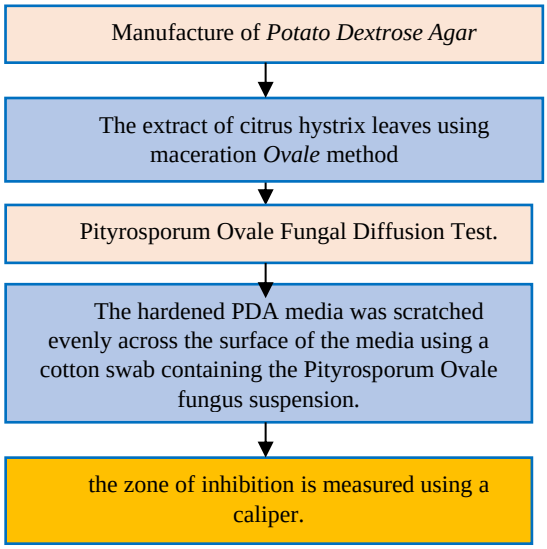


Figure 3. Scheme of Research Process

3 RESULTS AND DISCUSSIONS

3.1 Phytochemical Screening

The results of the phytochemical screening of Citrus hystrix leaf extract have been presented in the following table.

Table 2. Phytochemical Screening Results of Citrus hystrix leaf extract

No	Metabolit Sekunder	Pereaksi	Results
1	Alkaloid	DragendroffBouchardat Meyer	- - -
2	Flavonoid	Serbuk Mg+ Amil Alkohol + HCl_p	+
3	Glikosida	Molish+H_2SO_4	+
4	Saponin	Air panas/dikocok	-
5	Tanin	$FeCl_3$	+
6	Triterpen/ Steroid	Lieberman-Bourchat	+

Based on the results of the phytochemical screening of kaffir lime leaf extract (Citrus hystrix) to the six secondary metabolites as seen in Table 2, it was found that the Kaffir lime leaf extract has a chemical content consisting of flavonoids with Mg + Amyl Alcohol + HClp powder reagent, glycosides with Molish + reagent. H2SO4, Tannins with FeCl3 reagent, and triterpenoid / steroid with Lieberman-Bourchat reagent, while secondary metabolites

Alkaloid with Dragendroff Bouchardat Meyer reagent, Saponin secondary metabolites with hot water / shaken reagent were not found in the phytochemical screening results of Citrus hystrix leaf extract.

3.2 Inhibition Zone Diameter of Citrus Hystrix Leaf Extract on Pityrosporum Ovale Fungus Growth

The effectiveness of citrus hystrix leaf extract to inhibit the growth of Pityrosporum ovale fungus using disc diffusion was shown by the presence of an inhibitory or clear zone around the disc paper. Inhibition zone was measured using a caliper. The results of the study using a concentration of 25%, 50%, 75%, positive control and negative control can be seen in the Figure 4:

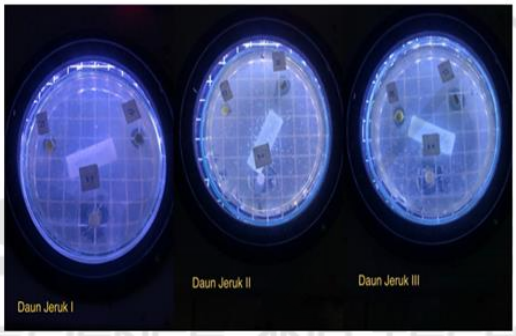


Figure 4: Inhibition zone diameter of citrus hystrix leaf extract on the growth of pityrosporum ovale fungus.

Table 3: Diameter of the Inhibition Zone of Pityrosporum ovale

Concentration	Inhibition zone diameter (mm)			
	Petri 1	Petri 2	Petri 3	Mean
25%	13,9	13,5	15,2	14,20
50%	15,3	14,8	16,1	15,40
75%	16	15,2	17,4	16,20
Mean	15,07	14,50	16,23	

From Table 3, it is found that the citrus hystrix leaf extract with the three concentrations, 25% concentration, 50% concentration and 75% concentration with 3 repetitions of petri 1, petri 2 and petri 3. found that the concentration of 75% had an increase in zone diameter. inhibition, the highest diameter of the inhibition zone was at a concentration of 75%, namely 16.20 mm, while the diameter of the lowest inhibition zone was at a concentration of 25%, namely 14.20 mm. The results of the inhibition zone diameter are displayed in graphical form in the Figure 5.

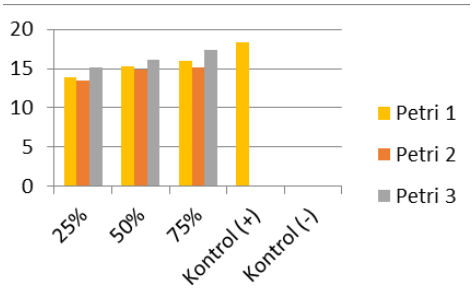


Figure 5: Graph of inhibition zone diameter of citrus hystrix leaf extract on the growth of pityrosporum ovale fungus.

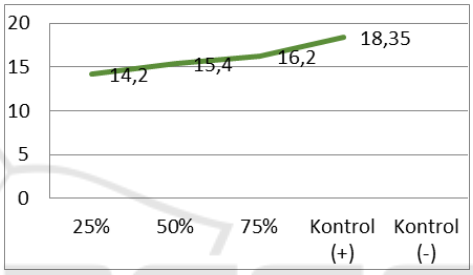


Figure 6: Average curve of inhibition zone of Citrus hystrix leaf extract on the growth of the fungus Pityrosporum ovale

Based on the curve in Figure 6, it can be seen that at a concentration of 25% the average diameter of the inhibition zone is 14.20 mm, then there is an increase in the average diameter of the inhibition zone at a concentration of 50%, namely 15.40 mm, and at a concentration of 75% an increase of 16, 20 mm. The positive control inhibition zone diameter was 18.35 mm and the negative control inhibition zone diameter was 0 mm.

4 DISCUSSIONS

Based on the results of the study, the leaf extract of citrus hystrix (Citrus Hystrix) has antifungal effectiveness against the growth of pityrosporum ovale as indicated by the formation of an inhibitory zone or clear zone around the disc paper. Then the diameter of the zone of inhibition is measured using a caliper to determine the amount of antifungal power. The concentrations used were 25%, 50% and 75%. The results of the study based on the Greenwood inhibition zone classification showed that the leaf extract of citrus hystrix (citrus hysrix) with a

concentration of 25%, 50% and 75% had antifungal effectiveness against the growth of pityrosporum ovale. With an average diameter of the inhibition zone of 14.20 mm, 15.40 mm and 16.20 mm. whereas in the positive control using ketoconazole 2% had a stronger effectiveness in inhibiting the fungus pityropsporum ovale with an average inhibition zone diameter of 18.35 mm. for negative control using aquadest did not have an inhibition zone in pityrosporum ovale.

From the results of phytochemical screening, the leaf extract of citrus hystrix (Citrus Hystrix) contains flavonoids, tannins, glycosides and triterpenes / steroids .. If tannins in low concentrations can inhibit germ growth, while at high concentrations, tannins work as an antimicrobial by coagulating or clumps of bacterial protoplasm, so that it forms a stable bond with the bacterial protein and in the digestive tract, tannins are known to be able to eliminate toxins. Whereas flavonoids have antifungal, antiviral and antibacterial activity. Several studies have examined the relationship between flavonoid structure and antibacterial activity. Flavonoids can inhibit the function of the cytoplasmic membrane and inhibit energy metabolism. (Romawati, C et.al. 2017)

The ethanol extract of citrus hystrix leaves has a larvicidal effect on Aedes aegypti larvae. The concentration of ethanol extract of citrus hystrix leaves is needed to kill 50% of the larvae population of Aedes aegypti. (Santoso, L.M, 2015).

5 CONCLUSIONS

Based on the research results, it can be concluded that the leaf extract of the citrus hystrix shows a clear zone at all given concentrations. The 50% and 75% treatments were the best treatments with an inhibitory power of about 15.40 mm and 16.20 mm. The results of phytochemical screening on the leaves of the citrus hystrix obtained secondary metabolites such as flavonoids, glycosides, tannins and triterpan.

ACKNOWLEDGMENT

The author would like to thank profusely to the Rector of the Universitas Prima Indonesia, "Dr. Chrismis Novalinda Ginting., M.Kes" who has supported the research of novice lecturers with funding from the Ministry of Research and Technology / the National Research and Innovation Agency.

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