GEL POTENTIAL OF RED ONION (*Allium cepa* L.) ETHANOL EXTRACT AS ANTIFUNGAL CAUSE TINEA PEDIS

Running title: Gel Potential of Red Onion

Erza Genatrika[1*], Elza Sundhani[1], Mayang Ika Oktaviana[1]

[1]Pharmacy Faculty, Universitas Muhammadiyah Purwokerto, Purwokerto, 53182, Indonesia
*Corresponding author :
Erza Genatrika, M.Sc, Apt
Email: erzagenatrika@gmail.com
Phone: +62856-4777-2200

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ABSTRACT
Introduction: Tinea pedis is a dermatophyte infection of the foot, especially between the fingers and soles of the feet. Tinea pedis is caused by a fungal infection of Trichophyton *rubrum*. Red onion is one of the spices that has been widely known by the public and has been used as a traditional medicine in the prevention of fungus. The objective of this research was to determine the antifungal activity of gel from an extract of red onion on *Trichophyton rubrum*.
Materials and Methods: The gel was formulated with various concentration of red onion, FI with a concentration of extract (5%), F2 (7,5%) and F3 (12,5%). Each formula was tested the physical characteristics and antifungal activity toward *T.rubrum*. The antifungal activity was determined by the agar well diffusion method using SDA plates. Furthermore, the antifungal activities were assessed by the presence or absence of inhibition zones after the plates were incubated at 28^{o}C for 7 days.
Results: F3 has the greatest inhibitory power than F1 and F2 ($p<0,05$). Then, F3 has the same inhibitory power as a positive control ($p>0,05$).
Discussion: All gel understudy at various concentrations of red onion formulated in gel exhibited antifungal activity. Antifungal activity of red onion because it is containing Allicin. Therefore we can use these gel as a natural antifungal in the healing of tinea pedis caused *T.rubrum*.
Conclusion: The gel from an extract of red onion showed significant antifungal activity against *T.rubrum.*
Keywords: *Trichophyton rubrum*, red onion, gel, tinea pedis.

INTRODUCTION
Tinea pedis is a dermatophyte infection in the feet, especially between the fingers and soles of the feet. Tinea pedis is found throughout the world with a high prevalence. The incidence of tinea pedis in Indonesia is currently quite high at 74.9%, and the results of culture show that the most common cause is the fungus Trichophyton *rubrum* [1,2]. *T. rubrum* can be treated using a group of antifungal drugs. Unfortunately, these drugs can cause side effects, so the need

for alternative medicines from natural ingredients. One of the plants that can be used is an onion.

Red onion is included in the genus *Allium* which is often used as cooking spices, but besides that they also have a variety of benefits. Mercy et al (2014) stated that the ethanol extract of garlic at a concentration of 12.5% had activity against the fungus of *T. rubrum* [3]. In addition, in a study by Nurhasanah (2015), it was stated that the red onion juice had activity on the fungus of Candida albicans [4]. Antifungal activity of onion bulbs is known because of the content of the allicin [5].

In line with changes in people's lifestyles that require practicality so that the development of herbal products is still very necessary. This gel is expected to be able to penetrate better on the skin compared to the extract form [6]. So far there are no studies that reported the activity of gel products from onion extract on T. *rubrum*. Therefore, this research needs to be done to prove that the gel from the onion extract is efficacious for treating tinea pedis.

MATERIALS AND METHODS

Plant materials

Allium *cepa* L. were collected from Gebang Village, Cirebon City, West Java, Indonesia. The leaves of the plant were authenticated by Faculty of Biology, Jenderal Soedirman University, Indonesia.

Microorganisms

Trichophyton rubrum was obtained from the Parasitology Laboratory, Poltekkes Kemenkes, Bandung, West Java. *Trichophyton rubrum* was cultured in Saboround Dextrose Agar (SDA) at 28°C for 7 d.

Extraction of plant materials

The cleaned materials were cut, and the ethanolic extract was prepared using 96% ethanol by maceration method followed by steam evaporation in rotary flask evaporator. The extract was transferred into clean and dried airtight vial and stored at until ready for use. A net yield of 75,88 g (11,67% w/w) was obtained by macerating 650 g of the red onion.

Formulation of gel

Table 1: Formula of gel selected for study

Material	Formulation Code (w/w)			
	F1	F2	F3	F0 (-)
Extract	5	7,5	12,5	-
Carbopol 940	2	2	2	2
TEA	3,5	3,5	3,5	3,5
Propylene gycol	10	10	10	10
Methyl paraben	0,2	0,2	0,2	0,2
Prophyl paraben	0,02	0,02	0,02	0,02
Purified water	ad 100	ad 100	ad 100	ad 100

F1 : gel with 5% extract concentration
F2 : gel with 7,5% extract concentration
F3 : gel with 12,5% extract concentration
F0 : gel with 0% extract concentration as negative control

Gel was formulated as given in table 1, then it was evaluated with cycling test in 6 cycles (one cycle consist of temperature 4^{o}C for 24 hours and 40^{o}C for 24 hours) and observed physical characteristic change of the gel at the beginning and end of the cycle which included organoleptic, homogeneity, pH, viscosity, disperse and adhesiveness test. The pH of the gel

was determined using digital pH meter, and then the viscosity of the gel was determined by using Brookfield Viscometer (LV).

Antifungal activity determination

The antifungal activity of the gel of red onion was determined by the agar well diffusion method [7,8]. Saboround Dextrose Agar (SDA) was prepared by dissolving 65 g in 1000 ml of distilled water; by sterilizing in the autoclave for 15 minutes at 15 lb pressure (121^{o}C) and by pouring 12 ml of the prepared medium to each petri dish that has a bacterial suspension. After that, the wells were made with a diameter of 6 mm by punched aseptically with a sterile cork borer. Approximately 200 mg of the gel at different concentrations 5; 7,5, and 12,5% were loaded into the wells, and the positive control was used an x-Brand antifungal gel and an F0 (no extract of red onion) was used as a negative control.

Each test plate contained five samples of gel at various concentration of red onion placed about equidistant to each other. After that, the plates were incubated at 28°C for 7 days. The diameter of inhibition zones was measured in millimeter and the study was carried out in triplicate [7,8].

Data Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) to test the difference between inhibition zone of red onion extract gel formula, negative control, and positive control group.

RESULTS

The gel was tested for physical characteristics including organoleptic, homogeneity, viscosity, pH, disperse and adhesiveness. After that, the gel was determined antifungal activity. The results of this research can be seen in table 2.

Table 2: Physical characteristic test result and antibacterial activity result

Test	Formulation code				
	F0	**F1**	**F2**	**F3**	**F4**
1st cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	light yellow	yellow	dark yellow	transparent
Homogeneity	homogen	homogen	homogen	homogen	homogen
Viscosity	11306,67±220,08	10765,33±401,6	11260±260	11100±420	11567±380
Disperse (m)	3,78±0,31	3,53±0,26	3,42±0,21	3,36±0,27	5,81±0,17
Adhesiveness (s)	0,8±0,27	0,50±0,11	0,72±0,37	1,06±0,35	4,51±0,12
pH	8	6	6	6	6
6th cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	brown	brown	brown	transparent
Homogeneity	homogen	homogen	homogen	homogen	homogen
Viscosity	11380±75,5	11193,33±220,08	11556,67±276,47	11623,3±203,06	11386±246,67
Disperse (m)	3,34±0,08	3,05±0,13	2,86±0,14	2,76±0,22	5,32±0,13

Adhesiveness (s)	0,64±0,17	0,39±0,08	0,66±0,08	0,75±0,17	4,32±0,18
pH	8	6	6	6	6
Antifungal activity	0 ± 0	0,51±0,11	15,43±1,71	20,82±5,83	45,53±2,23

F0 : gel with 0% extract concentration as negative control
F1 : gel with 5% extract concentration
F2 : gel with 7,5% extract concentration
F3 : gel with 12,5% extract concentration
F4 : X-Brand antifungal gel

DISCUSSION

Physical characteristic

The evaluation of the gel was carried out at the beginning of the preparation and after the cycling test. The results of organoleptic testing on all three formulas were the same, the results of the characteristics are semisolid dosage form, yellow color, and smell was typical of red onion. Homogeneity test to determine the homogeneity of extracts in gel. Homogeneity of dosage form will affect the antifungal power of gel. This is because with a homogeneous gel, the distribution of the active ingredients in gel will be evenly distributed so that the release of the active compound by the base through the test media will be good and the antifungal effect will be maximized. The difference in the concentration of red onion extract does not affect the homogeneity of gel, because the process or treatment of each formula is the same.

And then, disperse test was conducted to determine the ability of gel's diffusion on the skin. The easier the gel is flattened on the skin, then the gel is in contact with the surface of the skin more widely and active substance will be well distributed [9]. The addition of the extract can decrease the disperse of gel, however the addition of the extract can increase the viscosity and adhesiveness of gel.

The results of pH test were found that the higher concentration of extract did not affect the pH value of each formula. The pH value of the three formulas still meets the SNI requirements of 4.5-6.5. pH value should not be too acidic because it can cause skin irritation and also it should not be too alkaline because it can cause scaly skin [10].

The results of the evaluation after the cycling test of 6 cycles showed that each formula did not change the smell, pH, dosage forms and homogeneity, but there has been a change in color, viscosity, disperse and adhesiveness. Nevertheless, the changes in viscosity, disperse and adhesiveness are still within the standard range of good gel parameters (adhesiveness> 1 second; disperse 5-7 cm; and viscosity 2000 - 50000 cps). According to Yati (2018) states that stable dosage form is still within acceptable limits during the storage period, in which the characteristics are the same as they had when they were made [11]. Unstable gels show irreversible changes in their rheological properties, such as the separation of the liquid phase (syneresis) and of the solid phase (sedimentation) [12].

Antifungal activity

In the study, positive control (X-brand gel) was produce inhibition zones against *T. rubrum* than the negative control (F0), so no zone of inhibition was noted for the negative control. Furthermore, the zones of inhibitions are shown by gel of red onion at different concentrations against T. *rubrum* depicted in table 2. The test results showed that the higher the concentration of red onion, so the higher the inhibitory power. So, F3 has the greatest inhibitory power than F1 and F2 ($p<0,05$). F1 has a weak inhibitory ability (<10 mm), while F2 and F3 have moderate to strong ability to inhibit *T. rubrum* (10-20 mm) [13].

The antifungal activity of gel of red onion can be attributed to the different phytochemicals present in the red onion. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemicals are nonnutritive plant

chemicals that may have protective or disease preventive antifungal activities. Because of their structural differences from those of the more studied antibacterial sources, their mode of action may too differ [14].

Allicin, flavonoid, triterpenoid, saponin, tannin dan alkaloid are found to be associated with antifungal effects in various studies. Allicin can inhibit the activity of enzyme in fungi including proteinase cysteine enzyme and alcohol dehydrogenase enzyme, cysteine proteinase enzymes that cause infections and disorders of skin metabolism while alcohol dehydrogenase enzymes that help fungi stay alive and multiply in cells [15]. Flavonoid, saponin, tannin, and triterpenoid have been found to exhibit antifungal activity through mechanisms like interfere with the permeability of fungal cell so that it causes damage to the membrane and causes the release of various important component from inside the fungal cell such as protein, nucleic acid and nucleotides [13,16,17]. And then, the mechanism of action of alkaloids is inhibiting esterase, DNA and RNA polymerase, and also inhibit cell respiration [18]. These may explain the probable mechanism of antifungal activity of the plant.

CONCLUSION

The study reveals the antifungal activity of the gel of red onion against *T.rubrum*. Gel with 12,5% extract concentration has the greatest inhibitory power than gel with 5 dan 7,5% extract concentration. The present study can pave a way for further research to develop newer antifungal dosage form in this era of resistance.

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CONFLICTS OF INTERESTS

All authors have none to declare