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Development and Evaluation of a Topical Herbal Gel for the Treatment of *Tinea pedis*

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ABSTRACT

Allium cepa fractions have been shown to possess antifungal activity against *Trichophyton rubrum*. The antifungal effect of the plant is mainly attributed to the presence of Allicin. This study aims to formulate a topical antifungal gel and assess its physical stability and efficacy parameters against *T. rubrum*. The gel was formulated with various concentration of *Allium cepa* fractions FI (5%), F2 (7.5%), and F3 (12.5%). Each formulation was tested for physical stability and efficacy against *T. rubrum*. The agar well diffusion method was used to determine their antifungal activities using SDA plates. Furthermore, the antifungal activities were assessed by the presence or absence of inhibition zones after incubating the plates at 28^{o}C for 7 days. The topical gel was a fawn-coloured homogenous semi-solid preparation that was readily spreadable upon skin contact with no tackiness sensation. The optimized gel showed no phase separation after the cycling test in six cycles (one cycle consists of temperatures of 4^{o}C for 24 hours and 40^{o}C for 24 hours). The efficacy test showed that the topical gel produced better inhibition zones against *T. rubrum* than the negative control. F3 (12.5%) showed the most inhibitory potentials of all the formulations (F1 and F2). These results demonstrate that *Allium cepa* fractions can be formulated into a stable semi-solid dosage form and with antifungal activity against *T. rubrum*.

***Keywords*:** Tinea pedis, Red onion, Gel, Fraction.

Introduction

Fungal infection of the skin is one of the common dermatological problems, making the skin look scaly with rashes. A good example is tinea pedis (athletes' feet), with a relatively high (74.9%) prevalence in Indonesia. Skin culture results indicate that the most common cause is the fungus *Trichophyton rubrum.*[1,2] There are many treatment choices, from antifungal medicine to traditional medicine. One of the plants that can be used is *Allium cepa* L (Onions). Studies on *Allium cepa* L. as an antifungal agent against *Aspergillus niger*, *Aspergillus, fumigatus*, *Candida albicans*, and *Aspergillus flavus* have been developed.[3] In addition, results of a study by Nurhasanah *et al.* stated that onion juice exhibited antifungal activity against the pathogenic fungus *Candida albicans.*[4] Also, a study conducted by Mercy *et al.*. revealed that the ethanol extract of garlic (*Allium sativum*) at a concentration of 12.5% was active against *T. rubrum*.[5] Similarly, we have reported the antifungal activity of *Allium cepa* against *T. rubrum.*[6] The antifungal activity of *Allium cepa* is linked to its allicin content.[7] Allicin was first reported in 1944 as a colourless oil with low and relatively unstable solubility in water.[8] Ilic *et al.* also stated that allicin is unstable in non-polar organic solvents.[9] Hence, a semi-polar solvent such as ethyl acetate may be a solvent of choice. In this era, people like products that provide significant benefits and can be used practically in their daily lives.[10] Therefore, this study aimed to develop a topical gel containing an ethyl acetate fraction of *Allium cepa* L, with better skin penetrations and expected significant activity against *T. rubrum*, the causative agent of tinea pedis.

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Materials and Methods

Materials and equipment

Materials used include Carbopol 940, TEA, propylene glycol, methylparaben, propylparaben, purified water, ethyl acetate, n-hexane, ketoconazole, silica gel F_{254} (Merck), aluminium foil, glacial acid vanillin reagents, X-brand gel.

Sample collection and preparation

Allium cepa L. was collected from Gebang Village, West Java, Indonesia, in April 2018. The bulbs were authenticated at the Faculty of Biology, University of Jenderal Soedirman, Indonesia, with certificate number 1329/UN23/02/8/PP/08/00/2018. Moreover, *Trichophyton rubrum* was obtained from the Parasitology Laboratory, Health Polytechnic, Ministry of Health, West Java. *Trichophyton rubrum* was cultured in Saboround Dextrose Agar (SDA) at 28°C for 7 days. The equipment used includes a rotary evaporator (IKA), Laminar Air Flow (Mascotte, LV-S Model), Viscometer Brookfield (DV2T), pH indicator strips (MQuant), petri dish (Steriplan), autoclave, oven (Memmert, USA), and glassware (Iwaki Pyrex).

Fractionation of plant materials

Allium cepa was peeled and washed, then squeezed to obtain red onion juice. The red onion juice obtained was partitioned using ethyl acetate solvent (1:2.5) in a separating funnel. The ethyl acetate phase (fraction) was collected, while the aqueous (water phase) was re-extracted with ethyl acetate. The combined ethyl acetate fractions collected were then evaporated using a rotary evaporator at 25ºC.[11]

Preparation of topical gel

Carbopol 940 was dissolved slowly by stirring in hot distilled water until fluffy, and TEA was added. Then, the ethyl acetate fraction dissolved in propylene glycol containing propyl paraben and methyl paraben was added. Finally, a sufficient amount of distilled water was added to make a 100 mL gel. Carbopol 940 is used as a gelling agent in formulations because it is biodegradable, bio-adhesive, biocompatible, free of irritation, and is not absorbed into the body.[12] Furthermore, since DMSO was reported to cause skin erosion, propylene glycol was used as a permeation enhancer in this formulation.

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Table 1: Formula of gel selected for study[6]

Material	Formulation Code (% w/w)			
	F0	F1	F2	F3
Fraction	0	5	7.5	12.5
Carbopol 940	2	2	2	2
Triethanolamine (TEA)	3.5	3.5	3.5	3.5
Propylene glycol	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 (qs)	100	100	100	100

F0: gel with 0% fraction concentration as a negative control
F1: gel with 5% fraction concentration
F2: gel with 7.5% fraction concentration
F3: gel with 12.5% fraction concentration
qs: quantity sufficient

Previous researchers stated propylene glycol as the best permeation enhancer. [13]TEA was used in the formulations to regulate the gel's pH. In addition, preservatives such as methyl paraben and propyl paraben are known to be easily compatible with other components.[14]

Physicochemical stability
The gel was evaluated with a cycling test in six cycles (one cycle was set at a temperature of 4^{o}C and lasted for 24 hours and then 40^{o}C for 24 hours). The gel's physicochemical parameters (organoleptic, homogeneity, pH measurement, viscosity, spreadability and adhesiveness test) were observed at the beginning and the end of the cycle.[15]

Antifungal activity
The *Allium cepa* L. fraction formulated into a gel was tested for antifungal activity using the agar well diffusion method. *T. rubrum* was smeared on a petri dish containing Saboround Dextrose Agar (SDA) media. Next, the SDA media was perforated aseptically by 6 mm. Each petri dish test contained five gel samples (F0-F4), i.e., about 100 mg was placed into wells equidistant from each other. After that, the petri dish was incubated at 28°C for 7 days. The diameter of the inhibition zone was measured in millimetres, and the study was carried out in triplicate.[16,17]

Data analysis
One-way analysis of variance (ANOVA) was used to determine whether or not there were differences in the zone of inhibition between each test group, positive and negative controls. Data collection for each group was carried out three times. The level of significance was 5% (0.05). *A p*-value less than 0.05 indicates a difference in the zone of inhibition between each group.

Results and Discussion

Physicochemical stability
Five different gel formulations (F0-F4) were prepared, and their physicochemical parameters were evaluated. These formulations were carried out at the beginning of the preparation and after the cycling test. Physicochemical parameters include organoleptic, homogeneity, viscosity, pH, spreadability, and adhesiveness. The result of the physicochemical parameters is shown in table 2.
The results showed prepared gels to be homogeneous and in good appearance and consistency. The homogeneity of dosage form will affect the antifungal power of the gel. This is because with a homogeneous gel, the distribution of the active ingredients in the gel will be good, and the antifungal effect will be maximized.[6]
The pH values of all formulations were in the range of SNI requirement (4.5-6.5); hence, they may not cause skin irritation. pH values should not be too acidic due to skin irritation, and also, they should not be too alkaline because they can cause scaly skin.[15]
The consistency of the substance is one of the most critical aspects that need to be considered in the formulation of topical antifungal gels because it is meant to be applied to a thin layer of skin. Therefore, the value of gel viscosity plays a vital role in controlling drug permeation. Polymers such as Carbopol 940 are used in these topical formulations to control drug release and maintain drug concentrations within the effective therapeutic range. The viscosity value of this topical gel is in the range of SNI requirements, namely 2000 and 50000 cps, according to SNI requirements.[18] And then, the spreadability of topical herbal gel was considered quite good. The therapeutic efficacy of gels depends on their spread. The gel spreading helps in the uniform application of the gel to the skin, so the prepared gels must have good spreadability and meet the ideal qualities in topical application.[19]
In addition to spreadability, attention should be given to its adhesiveness value. The decrease in the viscosity value can increase the spreadability.[20] This is inversely proportional to adhesiveness. If there is a decrease in the viscosity value, the adhesiveness of the preparation to the skin will be faster. Immediate contact with the skin will undoubtedly cause non-optimal drug absorption.[21]
Furthermore, the cycling test of six cycles was carried out for the five gel formulas (F0-F4). Results showed that the characteristic of the gel formulations remained the same before and after the cycling tests were done. The gels did not experience crystallization, syneresis, and other changes (colour, pH values, viscosity values, dispersion, and adhesion). These results indicate that this topical gel formulation can be said to be stable. Previous studies stated that a dosage form is stable if it is still within acceptable limits during the storage period when the characteristics are the same as when it was created.[21] Unstable gels exhibit irreversible changes, such as separating the solid phase (sedimentation) and liquid phase (syneresis).[22]

Antifungal activity
The presentation of the inhibition zone diameter of each topical herbal gel can be seen in Figure 1. The results describe that F3 has the most significant antifungal activity compared to F1 and F2. However, the topical herbal gel activity was not as significant as that of F4 (positive control). The results reveal that the bulb of the red onion has potential antifungal properties against *T. rubrum*. The topical gel with a 12.5% fraction concentration (F3) showed the highest zone of inhibition compared to a 7.5% fraction concentration (F2) and 5% fraction concentration (F1). Therefore, F3 showed better inhibitory power than F1 and F2 but not significant (p>0.05). Furthermore, all formulations were significantly different from negative controls/F0 (p<0.05), but their activities were not the same as positive control/F4 (p>0.05). However, all formulations of the topical gel containing red onion fraction have moderate to strong potential to inhibit *T. rubrum* (10-20 mm).[23] This study is experimental, so our results are limited to the reported findings. The findings of this study are consistent with the reports by Skerget et al. that the ethanol and acetone extracts of red onion's skin and edible part possess antifungal activity against *Aspergillus niger, Trichoderma viride* and *Penicillium cyclopyum*.[24]
The antifungal activity of the topical gel can be attributed to the different phytochemicals present in the red onion. Phytochemicals are non-nutritive plant chemicals that may have protective or disease preventive antifungal activities.[25,26] Allicin, thiosulfonates, and other phytochemical compounds from onions showed fungistatic activity against *Aspergillus niger, Rhodotorula nigricans, Penicillium italicum, Penicillium cyclopium, Aspergillus flavus, Cladosporium macrocarpus, Aspergillus fumigatus, Aspergillus alutaceus,* and *Aspergillus terrygenum*.[24] Allicin can inhibit the activity of enzymes in fungi, such as alcohol dehydrogenase enzymes and cysteine proteinase enzymes. Cysteine proteinase enzymes cause infections and skin metabolic disorders, while alcohol dehydrogenase enzymes help fungi stay alive and reproduce in cells.[27] In addition, secondary metabolites such as flavonoids, saponins, tannins, and triterpenoids have been found to exhibit antifungal activity through mechanisms such as interfering with the permeability of fungal cells, causing membrane damage and the release of various vital components from inside fungal cells such as proteins, nucleic acids, and nucleotides.[23,28,29]

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Table 2: Physicochemical stability test result

Test	Formulation code				
	F0	**F1**	**F2**	**F3**	**F4**
Before Cycling Test					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	11306.67±220.08	11306.7±281.1	11226.7±261.0	11193.3±89.6	11567±380
Spreadability (cm)	3.78±0.31	3.19±0.05	3.54±0.29	3.21±0.06	5.81±0.17
Adhesiveness (s)	0.80±0.27	0.70±0.13	0.85±0.23	0.88±0.42	4.51±0.12
1st cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	10468±336.7	11333.3±294.8	11076.7±465.0	11037.3±104.3	11487±127
Spreadability (cm)	3.50±0.18	3.47±0.08	3.40±0.07	3.62±0.11	5.90±0.11
Adhesiveness (s)	0.81±0.26	0.70±0.15	0.72±0.05	0.94±0.64	4.62±0.13
2nd cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	10374.7±404.6	11121.3±538.2	10647.3±181.8	10858±122	11348±142
Spreadability (cm)	3.52±0.11	3.23±0.08	3.14±0.05	3.17±0.15	5.83±0.13
Adhesiveness (s)	0.88±0.13	1.00±0.06	1.05±0.50	1.15±0.81	4.61±0.07
3rd cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	11160±465.2	11420±202.2	11526.7±251.5	11496.7±310.9	11439±235
Spreadability (cm)	3.36±0.04	3.32±0.30	3.30±0.21	3.49±0.30	5.76±0.17
Adhesiveness (s)	0.70±0.24	1.07±0.43	0.82±0.42	1.16±0.36	4.52±0.12
4th cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific

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Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	11493.3±312.1	11536.7±372.9	11510±96.44	11160±260	11368±180
Spreadability (cm)	3.54±0.01	3.09±0.01	3.30±0.11	3.16±0.13	5.68±0.14
Adhesiveness (s)	1.08±0.33	0.96±0.28	0.84±0.10	0.68±0.14	4.37±0.29
5th cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	11827.3±54.1	11456.7±309.9	11226.7±168.0	11142.7±480.4	11323±260
Spreadability (cm)	3.54±0.01	3.09±0.01	3.30±0.11	3.16±0.13	5.69±0.56
Adhesiveness (s)	1.08±0.33	0.96±0.28	0.84±0.10	0.68±0.14	4.31±0.12
6th cycle					
Organoleptic :					
Form	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Smell	none	specific	specific	specific	specific
Colour	transparent	fawn-coloured	fawn-coloured	fawn-coloured	transparent
Homogeneity	homogenous	homogenous	homogenous	homogenous	homogenous
pH	8	6	6	6	6
Viscosity (Cps)	11380±75.5	11750±36.1	11706.7±106.9	11843.3±15.3	11386±246.67
Spreadability (cm)	3.34±0.08	3.31±0.00	3.39±0.15	3.11±0.88	5.32±0.13
Adhesiveness (s)	0.64±0.17	0.91±0.21	1.44±0.42	0.67±0.09	4.32±0.18

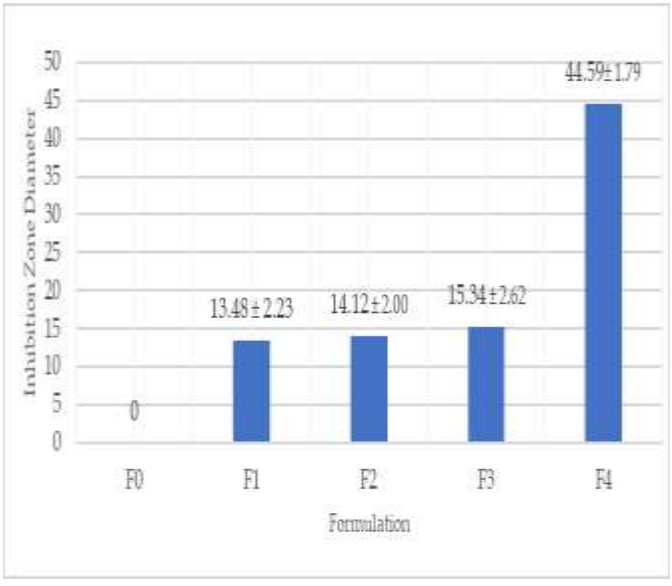

Figure 1: Diameter of inhibition zone of each topical herbal gel; F0 (gel with 0% fraction concentration as a negative control); F1 (gel with 5% fraction concentration); F2 (gel with 7.5% fraction concentration); F3 (gel with 12.5% fraction concentration); F4 (X-Brand antifungal gel as a positive control).

Alkaloids are reported to inhibit esterase, DNA and RNA polymerase, and cell respiration.[30] This might explain the possible mechanism of the plant's antifungal activity.

Conclusion

The study's results indicated that this herbal gel had suitable pH, viscosity, spreadability, and adhesiveness after the 12 days of the cycling test. Therefore, it is concluded that this herbal gel formulation can be an alternative for the topical treatment of tinea pedis due to *T. rubrum*. However, further preclinical, clinical and long-term stability studies are required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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