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The antifungal effect of ethanolic extract of *Aloe vera* leaves on the growth of *Trichophyton rubrum* in vitro

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Abstract. *Salim FS, Djumarga S, Sari Y. 2019. The antifungal effect of ethanolic extract of Aloe vera leaves on the growth of* Trichophyton rubrum *in vitro. Biofarmasi J Nat Prod Biochem 17: 81-85.* This study aims to determine if there is an antifungal effect of the ethanolic extract of *Aloe vera* L. leaves on the growth of *Trichophyton rubrum* in vitro. This study is a quasi-experimental study with a posttest-only-control-group design. Pure *T. rubrum* cultures were obtained from the Microbiology Laboratory, Universitas Setia Budi, Surakarta, Central Java, Indonesia. Sampling was inoculated by simple random sampling with the criteria of a 2-day-old mushroom. Colonies of *T. rubrum* on Sabouraud Dextrose Agar were taken and then diluted with 0.9% NaCl until it reached a turbidity equivalent to 0.5 Mc Farland standard, then smeared evenly on a petri dish containing Sabouraud agar. Samples were divided into 6 groups: negative control in distilled water, extracts with concentrations of 10%, 20%, 40%, 80%, and 100%. Each group consisted of 5 wells. All petri dishes were incubated for 4 days at room temperature. Data in the form of the diameter of the inhibition zone were analyzed using the Kruskal-Wallis test and then continued with post hoc Mann-Whitney analysis. The results showed at least an antifungal effect on the ethanolic extract of *A. vera* leaves on the growth of *T. rubrum* in vitro between the two groups. There were significant differences between the treatment groups except in the concentration group of 10% with 20% and the concentration group of 20% with 40%. There is an antifungal effect on the ethanolic extract of *A. vera* leaves on the growth of *T. rubrum* in vitro, with a concentration of 100% having sensitive results, 80% concentration having intermediate results, and concentrations of 10%, 20%, and 40% having positive results.

Keywords: Antifungal, *Aloe vera, Trichophyton rubrum*

INTRODUCTION

Fungal infection of the skin is a skin disease generally found in Indonesia, a tropical country with hot and humid climates. This infection can affect all levels of society regarding age, economy, and others. Various predispositions supporting this fungus's growth include the lack of public awareness about hygiene and using antibiotics for too long (Harahap 2000; Adiguna 2004).

One of the fungal infections is dermatophytosis, caused by dermatophytes. Dermatophytes are a group of fungi that can digest keratin in the epidermis. Trichophyton is a fungus that causes dermatophytosis in addition to *Microsporum* and *Epidermophyton* (Gandahusada 2003). *Trichophyton* has many species, including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, and *Trichophyton violaceum* (Patterson 2007; Sari et al. 2012; Jacqueline et al. 2018). The *T. rubrum* is the most common fungus that causes chronic dermatophytosis (Chandra 2006). In one study, Trichophyton fungi were the most common fungi found in samples of skin, hair, finger skin, and nails (Sayuti et al. 2006). The incidence of dermatophytosis of the feet in children in Barcelona, Spain, which is 31.4%, is caused by *T. rubrum* (Gonzalez et al. 2009). Bramono's research (2004) in West Cirebon stated that the most common fungal species causing dermatophytosis was *T. rubrum*, with about 75%.

The use of herbs in alternative medicine is increasing in Indonesia and abroad because it is easy to use and accessible to the public. In addition, the side effects caused by herbal medicines are smaller than chemical drugs. Therefore, herbal medicines are relatively cheaper than chemical drugs (Subroto 2006).

One of the herbal plants is *Aloe vera* L., traditional medicine and cosmetic ingredient. The benefits of *A. vera* can be used to reduce fever, cure hemorrhoids (hemorrhoids) and whooping cough, and accelerate wound healing. In addition, *A. vera* can also be used as an anti-inflammatory, antifungal, cell regeneration, stimulating immunity against cancer, and as nutritional support for PLWHA (People With HIV AIDS) (Widodo and Budiharti 2006).

The *A. vera* contains anthraquinones, especially aloe-emodin and aloin, which can be used as antifungals (Nidiry et al. 2011). Plants other than *A. vera* that can be used as antifungals are *Rheum emodi* Wall. root, which contains anthraquinone derivatives such as rhein, physcion, aloe-emodin, and chrysophanol (Agarwal et al. 2000). Another plant that has a similar effect is the leaves of *Senna Alata* Linn. containing anthraquinone derivatives in the form of rhein and aloe-emodin except for anthraquinone glycoside extract (Wuthi-udomlert et al. 2010).

Various kinds of antifungal tests of *A. vera* extract in vitro have been carried out on several species, such as *Microsporum gypseum*, *Aspergillus flavus*, and *Aspergillus niger* (Sundari and Winarno 2001). Based on the above, the

authors are interested in researching the antifungal effect of *A. vera* leaves on the growth of *T. rubrum* in vitro.

This study aims to determine whether there is an antifungal effect of the ethanolic extract of *A. vera* leaves on the growth of *T. rubrum* in vitro.

MATERIALS AND METHODS

Research sites

Research and measurement of inhibition zones were carried out at the Parasitology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, and the Microbiology Laboratory, Universitas Setia Budi, Surakarta, Central Java, Indonesia.

Research subject

Pure *T. rubrum* culture was obtained from the Microbiology Laboratory, Universitas Setia Budi, Surakarta, Central Java, Indonesia.

Sampling technique

The inoculated samples can be taken by simple random sampling with the criteria of a-2-day old mushroom. Colonies of *T. rubrum* on Sabouraud Dextrose Agar were taken and then diluted with 0.9% NaCl until it reached turbidity equivalent to 0.5 Mc Farland standards. The physiological solution of 0.9% NaCl acts as a pH buffer so that the cells in the fungus are not damaged due to the decrease in environmental pH. Meanwhile, 0.5 Mc Farland standards standardize the inoculation process (PML Microbiologicals 2001). The *T. rubrum* suspension was taken with a sterile ose and then spread evenly on a petri dish containing Sabouraud agar. The samples were divided into 6 groups, each consisting of 5 wells. The determination of the sample size is based on the Federer formula, namely (Hanafiah 2004):

$$(k - 1)(n - 1) \geq 15$$

Where:
k = number of treatments
n = number of wells per treatment

The number of treatments in this study was 6, consisting of 1 control group and 5 treatment groups. Thus, the minimum value of n is 4. However, in this study, the number of wells was determined to be more than the minimum, namely 5 wells in each group, where the number was still by Federer's formula.

Research tools and materials

Tools: petri dish with a base diameter of 95 mm, a lid diameter of 110 mm, and a height of 18 mm, an ose, a well-making tool with a diameter of 6 mm, an incubator, autoclave, denatured-alcohol lamp, and ruler.

Ingredients: Sabouraud Dextrose Agar, *T. rubrum* culture obtained from the Microbiology Laboratory of Universitas Setia Budi, Surakarta, Central Java, Indonesia ethanol extract of *A. vera* leaf from LPPT (Integrated Research and Testing Laboratory) Universitas Gajah Mada. Yogyakarta, Indonesia, antibiotic Kalmicetine capsule containing 250 mg chloramphenicol produced by Kalbe Pharma.

Antibiotic preparation of chloramphenicol (Bridson 2006)

The antibiotic dose of chloramphenicol in SDA solution was 0.4 g/L, and the dose of 0.9% NaCl solvent in 250 mg chloramphenicol was 10 mL. One capsule of 250 mg chloramphenicol is dissolved in 10 mL of 0.9% NaCl. A 1.6 mL mixture of chloramphenicol and 0.9% NaCl was taken as the antibiotic dose in the sabouraud agar solution.

Preparation of agar plates (Bridson 2006)

The fungus *T. rubrum* was reared on a slanted Sabouraud Dextrose Agar (SDA). First, 100 mL of distilled water was added to 6.5 grams of SDA powder. It is then heated and stirred until it becomes homogeneous. A total of 1.6 mL of a mixture of chloramphenicol and 0.9% NaCl that had been prepared was dissolved in a solution of sabouraud agar and then stirred until evenly dissolved. SDA tools and media were sterilized in an autoclave at 121ºC for 15 minutes. SDA liquid medium was poured into a petri dish with a thickness of 20 mL. Let it solidify.

Preparation of mushroom suspension

The *T. rubrum* culture was taken using a sterile ose and then put into a 0.9% NaCl solution, shaken, and stirred until it reached a turbidity equivalent to 0.5 Mc Farland standard. The suspension of *T. rubrum*, shaken and stirred, was immediately inoculated on the plate to solidify. The suspension was shaken and stirred just before the inoculation of each petri dish to prevent precipitation of the suspension. Petri dishes that the suspension has inoculated are then incubated at room temperature for 4 days.

Preparation of *A. vera* ethanol extract

A total of 5,926 grams of *A. vera* leaves are split to take the gel. Then, the gel was dried in a drying cabinet at 45ºC for 48 hours. The dried form of *A. vera* leaves was obtained, weighing 59 grams. The dried form of *A. vera* leaves was put into a pollinator machine to obtain a powder form of *A. vera* leaf weighing 58.410 grams. The *A. vera* leaf dry powder was macerated with 70% ethanol solvent using a homogenizer. Then shaken for 30 minutes and precipitated for 24 hours. The results were then filtered using a Buchner funnel to obtain the first pulp and filtrate. The first dregs were dissolved with 70% ethanol solvent, shaken for 30 minutes, and allowed to stand for 24 hours. The results were then filtered using a Buchner funnel to obtain the second dregs and filtrate. The second dregs were redissolved with 70% ethanol, shaken again for 30 minutes, and allowed to stand for 24 hours. The results were then filtered using a Buchner funnel to obtain the third dregs and filtrate. The three filtrates were combined and evaporated using a vacuum rotary evaporator with a

water bath heating at 70ºC to obtain a thick extract weighing 30.950 grams. The thick extract was poured into a porcelain cup and then heated in a water bath at 70ºC. The extract was diluted with distilled water to reach 100% (no dilution), 80%, 40%, 20%, and 10%, with a volume of 3 mL for each concentration.

Treatment

Six wells with a diameter of 6 mm were made in 5 inoculated petri dishes. First, the well was added 0.5 mL of the ethanolic extract of *A. vera* and distilled water. Then it was incubated at room temperature for 4 days. The clear area around the well was measured with a ruler. Measurements were made at the bottom of the petri dish by calculating the average diameter of the smallest and largest inhibition zones. The interpretation of the diameter of the sensitivity zone is as follows (Hoffman and Michael 2001): (i) 19 mm or more: sensitive, (ii) 13-18 mm: intermediate, and (iii) 12 mm: resistant. Data were tabulated, averaged, and evaluated.

Data analysis

The analysis was carried out statistically using the one-way ANOVA test with a significance of p = 0.05 and continued with the Tukey Honestly Significant Difference (HSD) test. The ANOVA test was carried out to determine if there were differences in the inhibition zones between the 5 groups by comparing the average inhibition zones of the groups. The Tukey HSD test was used to determine if there was any significance between groups in inhibiting the growth of *T. rubrum*.

The requirements for the ANOVA test are the sample must consist of more than two groups, the data distribution must be normal, and the data variance must be homogeneous. The second condition is if the significance of the p-value is <0.05, then the ANOVA test is continued with post hoc analysis. Finally, suppose one of the ANOVA test conditions is not met. In that case, it can be continued with nonparametric tests such as the Kruskal-Wallis test and then with post hoc analysis of the Mann-Whitney test (Priyatno 2009).

RESULT AND DISCUSSION

After researching the antifungal effect of ethanolic extract of *A. vera* on the growth of *T. rubrum* in vitro, data was collected on the 4th day of incubation.

The diameter of the inhibition zone for each group can be seen in Table 1. In the distilled water group, 0 mm was obtained in the five replicates, indicating that the distilled water used as a diluent extract did not affect the growth of fungi. The average diameter of the inhibition zones in the ethanol extract of *A. vera* at concentrations of 10%, 20%, 40%, 80%, and 100%, respectively, was 8.8 mm, 10.9 mm, 11.6 mm, 18.6 mm, and 22.8 mm.

Based on Table 1, Figure 1 can depict the average of various administration treatments of a certain concentration of ethanol extract with the diameter of the *T. rubrum* inhibition zone in each treatment group.

Figure 1 shows the difference in the average diameter of the inhibition zone for each extract concentration with distilled water as a negative control. It can be seen in Figure 1 that the diameter of the inhibition zone increased with the increase in the concentration of *A. vera* ethanol extract.

Data analysis

The research data regarding the antifungal activity test of *A. vera* ethanol extract on the growth of *T. rubrum* in vitro were analyzed by a one-way ANOVA test, which was then followed by a post hoc Tukey HSD test if the ANOVA test had normal, homogeneous data distribution, and had p<0.05. The data was processed using Statistical Product and Service Solution (SPSS) 17.0 for Windows software. If one of the ANOVA test conditions is not met, proceed with the Kruskal-Wallis nonparametric test followed by the Mann-Whitney post hoc test.

The test of normality using the Shapiro-Wilk analytical method showed that the inhibition zone data had an abnormal data distribution. Thus, the ANOVA test could not be performed because the data were not normally distributed. Then it is continued by conducting nonparametric statistical tests such as Kruskal-Wallis.

In the Kruskal-Wallis test, p = 0.00 (p <0.05). Thus, it can be concluded that there is at least a difference in the antifungal effect between the two groups of extract concentrations. Furthermore, post hoc analysis was conducted to determine the significance between treatment groups. The test was the post hoc Mann-Whitney test.

Table 1. The measurement results in the diameter of the inhibition zone of *Trichophyton rubrum*

Repetition	Aquadest (Distilled Water)	Diameter of inhibition zone *A. vera* ethanol extract				
		10%	20%	40%	80%	100%
I	0	9	12	11	18	22
II	0	7.5	9.5	9.5	18.5	23
III	0	8	9	12	17	24.5
IV	0	10	12	11.5	19	22.5
V	0	9.5	12	14	20.5	22
Average	0	8.8	10.9	11.6	18.6	22.8

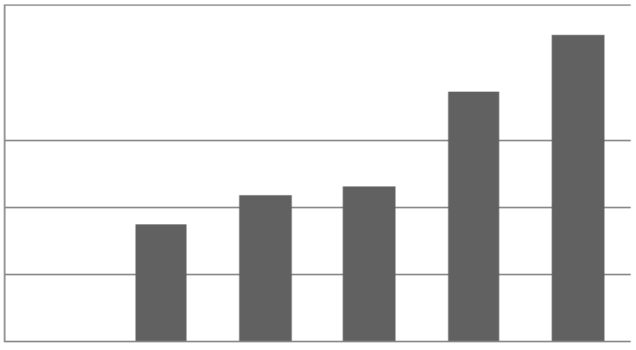

Figure 1. The average diameter of the inhibition zone (mm) in each treatment group

A post hoc Mann-Whitney analysis test on the antifungal effect of *A. vera* ethanol extract on the growth of *T. rubrum* in vitro was used to determine the significance between treatment groups. The results of the Mann-Whitney test showed that the negative control group in the form of distilled water had significant differences from other treatment groups, namely extracts with concentrations of 10%, 20%, 40%, 80%, and 100%. It can also be seen in the results of the Mann-Whitney test that the differences between the treatment groups had significant results ($p<0.05$) except between the 10% extract group with the 20% extract group and the 20% extract group with the 40% extract group which did not have significant results ($p>0.05$).

Discussion

The antifungal sensitivity test is a test to determine the effectiveness of antifungal agents in inhibiting the growth of fungi that infect humans. Fungal samples in patients were cultured in culture and then tested for sensitivity using various antifungal agents. Interpretation of the results can be sensitive, intermediate, or resistant. Thus, the doctor can determine the sensitive antifungal agent suitable for the patient.

The *A. vera* has antifungal content in anthraquinone with the main substances aloe-emodin and aloin (Nidiry et al. 2011). In an in vitro study, *T. rubrum* fungus, given with ethanol extract of *A. vera* leaves in different concentrations, had different inhibitory powers. The Mann-Whitney test analysis can be carried out to find out the average diameter of the inhibition zone between groups. Antifungal measurements can be made by measuring the diameter of the clear zone around the well.

Extract dilution into various concentrations using distilled water. It is expected that the distilled water in the ethanolic extract of *A. vera* leaves will not affect the inhibition of the fungus *T. rubrum* because there is no inhibition zone around the wells given with aquadest. Thus, distilled water did not interfere with extracts with various concentrations and had no antifungal effect.

Analysis of the Mann-Whitney test results yielded data that the comparison between groups had significant differences except between extracts with a concentration of 10% and 20% and extracts with a concentration of 20% and 40%. From the results of the study, it was proven that the ethanolic extract of *A. vera* had an antifungal effect. The *A. vera*, which has anthraquinones, especially aloe-emodin and aloin, has been shown to have antifungal properties, as stated by Nidiry et al. (2011). However, according to Phongpaichit et al. (2004), the mechanism of action of anthraquinones is still unclear. Possibly, there is a leak in the cell wall or a change in cell permeability, which eventually causes the fungal cell to lose its cytoplasm.

According to Hoffman and Michael (2001), the interpretation of the diameter of the sensitivity zone having sensitive results is the inhibition zone with a diameter of 19 mm or more. The results showed that only the concentration of 100% had an average of more than 19 mm, which was 22.8 mm. Thus, it can be said that the ethanol extract of *A. vera* leaves with a concentration of 100% effectively inhibited the growth of *T. rubrum* as a cause of dermatophytosis.

The 80% concentration has an average of 18.6 mm. It means that it is an intermediate category based on the opinion of Hoffman and Michael (2001). It indicates that the ethanol extract of *A. vera* leaves with a concentration of 80% is effective in inhibiting the growth of *T. rubrum* as a cause of dermatophytosis on the condition that the dose is given higher or the dose is given more often.

Ethanol extract with concentrations of 10%, 20%, and 40% had an average diameter of inhibition zone of less than 12 mm, which means that the three concentrations were resistant to *T. rubrum*. Thus, the ethanol extracts at concentrations of 10%, 20%, and 40%, although having antifungal effects, were not effective in inhibiting the growth of *T. rubrum*. The results of this study support the research conducted by Arunkumar and Muthuselvam (2009), which stated that the ethanolic extract of *A. vera* has an antifungal effect against *A. niger* and *A. flavus*. Therefore, in this study, it can also be concluded that the ethanol extract of *A. vera* with a concentration of 100% has an antifungal effect on the growth of *T. rubrum*.

Based on the research that has been done, it can be seen that there is an antifungal effect on the ethanolic extract of *A. vera* leaves on the growth of *T. rubrum* in vitro with a concentration of 100% having sensitive results, 80% concentration having intermediate results. On the other hand, a concentration of 10%, 20%, and 40% had resistant results.

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