

Antifungal Activity of *Polyscias scutellaria* Fosberg Leaves Against *Candida albicans*

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ABSTRACT

Polyscias scutellaria Fosberg is one of the indigenous plants from Indonesia that widely used as folk medicines for several illnesses. The leaves of *P. scutellaria* were previously studied as an antimicrobial agent. Nonetheless, this theory of antifungal activity has never been scientifically verified. This study aimed to evaluate the antifungal activity of *P. scutellaria* against *Candida albicans*. The leaves of *P. scutellaria* were macerated using an ethanol solvent. Then, the extract was evaporated and dissolved by 1% DMSO into different concentrations (100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL, 1600 µg/mL, 3200 µg/mL, and 6400 µg/mL). Fluconazole, as a common antifungal treatment, was used as the positive control. The antifungal activity of *P. scutellaria* against *Candida albicans* was tested using well dilution and diffusion disc methods. Ethanol extract of *P. scutellaria* leaves showed antifungal activities against *Candida albicans*. It showed the minimum inhibitory concentration of above 6400 µg/mL and formed the inhibitory zone against *Candida albicans*. As a conclusion, ethanol extract of *P. scutellaria* has the potential to be used as an antifungal agent against *Candida albicans*.

Keywords: *Candida albicans*; antifungal; *Polyscias scutellaria*; well dilution; disc diffusion

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INTRODUCTION

Indonesia is rich in biodiversity and is also known as a mega diversity country. Indonesian biodiversity has been utilized to support livelihoods, especially for food, health and energy that aim to meet human necessities. It provides plants that can be used to develop herbal medicine. In fact, many Indonesian people depend on nature to treat illness. In prior study, plants are rich in a wide variety of secondary metabolites that have antimicrobial properties (Dahanukar et al., 2000). One plant that has the potential to be used as a medicinal plant is the leaf of *P. scutellaria*. Its leaf is mostly used traditionally to treat breast inflammation, wound, urinary tract problems, and body odor (Rosa et al., 2019). Based on the previous study *P. scutellaria* leaves have antibacterial properties. The leaves contain alkaloid, saponin, tannin, and flavonoid. The most bioactive compound contained in *P. scutellaria* is alkaloid that is used to bother the stability of peptidoglycan and degrade the fungal cell walls. Another active compound is triterpenoid saponins, which is widely known as one of the antifungal materials from natural herb plants (Arif, Bhosale, & Kumar, 2009).

Polymorphic yeast, like *C. albicans*, is part of normal flora in the human body. In some cases, *C. albicans* can cause several infections, starting from superficial infections on the skin to systemic infections. The growth

of the yeast can be inhibited by the antifungal agent presence. Therapy for candidiasis can be carried out by using topical antifungal agents and oral antifungal agents from azole classes (Katzung, 2010). The type of azole antifungal works by inhibiting 14 α -lanosterol demethylase in the ergosterol synthesis pathway (Ghannoum & Rice, 1999). Fluconazole is one of the antifungal agents against most isolates of *Candida* (Lyon, Karatela, & Sunay, 2010). Recently, many researchers go over the case to investigate traditional medicine. Therefore, based on the benefits of *P. scutellaria* leaves, this study aims to determine the activity of ethanol extract of *P. scutellaria* leaves on the *C. albicans* as the cause of several infections. This research can be a start line for antifungal activity study in *P. scutellaria* leaves. Our aim of this study was to identify antifungal activity, which is expected from the presence of the active compound that is contained in *P. scutellaria* leaves.

METHODS

Sample Preparation

First, the leaves were cleaned and rinsed with water. Afterward, the leaves of *P. scutellaria* were dried for preparation of the plant extract in the oven within the temperature of 50 °C. The leaves of *P. scutellaria* were collected from Lenteng Agung Area, South Jakarta, Indonesia. For the extract preparation, 50 g of dry powder of the leaves were placed in a 1000 mL of Erlenmeyer

containing 500 mL of ethanol solvent. The content in the Erlenmeyer was mixed at first with stirrer stick and being macerated for 24 hours at room temperature. The extract was then filtered using Whatman® paper No.2 (Cat. Number: 28419866, Sigma-Aldrich, USA) Then, the solvent was evaporated by using a rotary evaporator and vacuum (Buchi R-205 and V-500) at temperature 60 °C. The obtained extract was weighed for 16 mg and dissolved in 10 mL 1% DMSO.

Preparation/Inoculation of *Candida albicans*

Candida albicans were obtained from Parasitology Laboratory, Faculty of Medicine Universitas Indonesia (FMUI). The yeast colony was suspended in 10 mL tube containing NaCl solution (0,85%) in a tube. Then, the turbidity was equalized to the standard of 0,50 McFarland (as many as $0,4 \times 10^6$ - 5×10^6 CFU/mL colonies) using the Densichek.

Disc Diffusion Method

The disc diffusion method is the most widely used method for determining antifungal activity. Extracts of *P. scutellaria* were prepared in the concentration of 100, 200, 400, 800 and 1600 µg/mL, respectively. The blank discs were moistened by 200 µL extract solution of each concentration. Afterward, *C. albicans* was smeared on the Sabouraud Dextrose Agar (SDA) growth medium and the discs were put on. Triplicate repetitions have been done for each concentration. Then, the Petri dishes were incubated for 24 hours at 37°C and diameter of the clear zone was measured. As a negative control, 20 µL of 1% DMSO was used, while the positive control was 20 µL of 0,1 mg/10 ml fluconazole. The result of this method was interpreted statistically by using one-way Anova.

Well Dilution Method

The experimental group was divided into five groups: positive control, negative control, media control, growth control, and treatment control. MIC determination was

carried out using a microplate. Microplate consists of 96 wells consisting of twelve columns and 8 rows. The first column until the fifth column were filled by 50 µL of yeast suspension and 50 µL of RPMI medium respectively. Then, 50 µL of fluconazole solution was pipetted into A1 well. Following that, 50 µL of fluconazole was pipetted from the first column and transferred to the second column well and so on until the fifth well. In the sixth column well was filled with 150 µL water for injection as a negative control with yeast suspension and RPMI medium (ratio 1:1:1). The seventh column well was filled by 150 µL suspension of DMSO solution with yeast suspension and RPMI medium. In the eighth column well, 50 µL of yeast suspension and 50 µL of RPMI medium were added. The ninth column well was filled with RPMI medium only. In the first to fifth columns in the second row, 50 µL of fungal suspension and 50 µL of RPMI medium were added. A total of 50 µL of the sample on the first well in the second row was inserted, then mixed. Next, 50 µL was pipetted to the second column well in the second row and so on until the fifth well in the second row. All experimental groups were carried out in triplicate. Microplate then incubated at 35°C in 48 h. Then, 50 µL mixed solution from each well was transferred onto Sabouraud Dextrose Agar medium. The result could be read after 48 hours of incubation.

Data Analysis.

We use One-way Anova statistical method by Microsoft Excel for data analysis.

RESULTS AND DISCUSSION

In this study, the leaf extract of *Polyscias scutellaria* Fosberg exhibited inhibitory activity against *C. albicans*. The yeast of *C. albicans* was obtained from the laboratory of Parasitology, Universitas Indonesia. It was assessed using two different methods. The diffusion disc method was done to exhibit the clear zone of ethanol

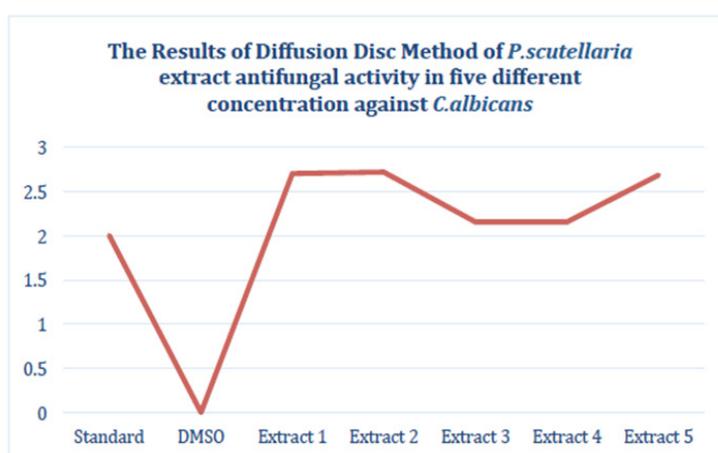
Table 1. Results of diffusion disc method

| No | Sample | Clear Zone Diameter (mm) | | | Average ± SD |
|----|--------------------|--------------------------|------|------|--------------|
| | | 1 | 2 | 3 | |
| 1 | Standard | 2 | 2 | 2 | 2 |
| 2 | 1% DMSO | 0 | 0 | 0 | 0 |
| 3 | Extract 100 µg/mL | 3 | 3.1 | 2 | 2 ± 0.37 |
| 4 | Extract 200 µg/mL | 4 | 2.8 | 1.35 | 2.72 ± 1.76 |
| 5 | Extract 400 µg/mL | 2 | 3.1 | 1.35 | 2.15 ± 0.78 |
| 6 | Extract 800 µg/mL | 2 | 3 | 1.45 | 2.15 ± 0.61 |
| 7 | Extract 1600 µg/mL | 2 | 3.35 | 2.7 | 2.68 ± 0.46 |

Table 2. Results of optical density (ELISA) readings of Well-dilution method

| | Standard | Extract 1 | Extract 2 | Extract 3 | Extract 4 | Extract 5 |
|---------------|------------------------|------------------------|------------------------|-----------------------|-----------------------|------------------------|
| Repetition 1 | 0.2994 | 0.3563 | 0.3612 | 0.3442 | 0.3485 | 0.3521 |
| Repetition 2 | 0.307 | 0.3435 | 0.3627 | 0.3351 | 0.3348 | 0.3371 |
| Repetition 3 | 0.3365 | 0.3494 | 0.3559 | 0.3569 | 0.3647 | 0.3466 |
| Mean \pm SD | 0.3143 \pm 0.0003 | 0.350 \pm 0.00004 | 0.360 \pm 0.00001 | 0.345 \pm 0.0001 | 0.349 \pm 0.0002 | 0.345 \pm 0.00005 |

Notes:

Extract 1 = 400 $\mu\text{g/mL}$ Extract 2 = 800 $\mu\text{g/mL}$ Extract 3 = 1600 $\mu\text{g/mL}$ Extract 4 = 3200 $\mu\text{g/mL}$ Extract 5 = 6400 $\mu\text{g/mL}$ **Figure 1. Results of diffusion disc method of *P. scutellaria* extract antifungal activity in five different concentrations against *C. albicans***

extract of *P. scutellaria* leaves. The concentrations of the extracts were varied into five different concentrations. Then, the experiment was repeated three times for each concentration.

As shown in Figure 1, all concentrations of the extract performed clear zone diameters representing that indicate the antifungal activity of *P. scutellaria* leaves. The development of a disk diffusion-based assay is desirable for determining the antifungal susceptibility of dermatophytes based on the advantages (Nweze, Mukherjee, & Ghannoum, 2010). Clear zone diameter of the diffusion disc method could strongly indicate the strength of antifungal activities of the extract against *C. albicans*. As the concentration lower, it exhibited smaller clear zones, which means there was no antifungal activity (Rosa et al., 2019). The clear zone diameters for different five concentrations have statistically verified to be significantly different by observing that p-value was lower than 0.05, the result showed 0.007.

The result diffusion disc was tested statistically by one-way Anova method. It can be verified by comparing the value of F with F-crit. The result showed that the value of F was higher than F-crit, it describes that the hypothesis of this experiment is accepted. This statistical result indicates that the clear zone diameters of ethanol extract of *P. scutellaria* were significantly different in each concentration.

An experiment was done in this research using fluconazole as a positive control and the clear zone was formed less than 10 mm, showing that *C. albicans* susceptibility to fluconazole. This indicated that *P. scutellaria* extracts have the possibility to become an alternative antibacterial agent against *C. albicans*. Furthermore, to measure the capability of each concentration in inhibiting the growth of *Candida albicans* by knowing the Minimum Inhibitory Concentration of the extract, a Well-Diluted method of analysis was performed. The result was depicted in Figure 2. In the dilution method analysis, The culturing

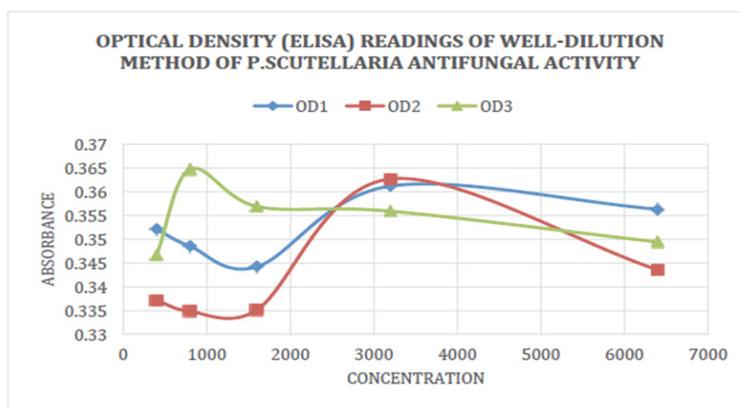


Figure 2. Results of optical density (ELISA) readings of *P. scutellaria* extract inhibiting the growth of *C. albicans* by Well-dilution method

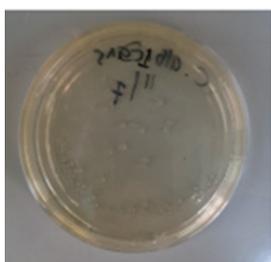


Figure 3. Visualization of fluconazole as a positive control

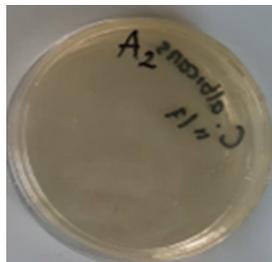


Figure 4. Visualization of *P. scutellaria* leaves extract in 400 µg/mL concentration

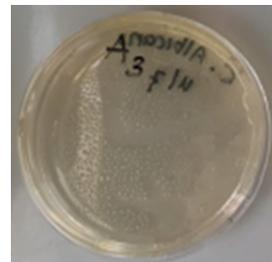


Figure 5. Visualization of *P. scutellaria* leaves extract in 800 µg/mL concentration

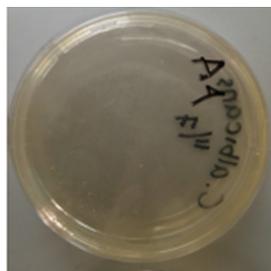


Figure 6. Visualization of *P. scutellaria* leaves extract in 1600 µg/mL concentration

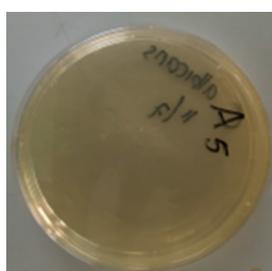


Figure 7. Visualization of *P. scutellaria* leaves extract in 3200 µg/mL concentration



Figure 8. Visualization of *P. scutellaria* leaves extract in 6400 µg/mL concentration

from wells was conducted to find concentrations that can inhibit the growth of *C. albicans* yeast. Fungal colonies that grow on fluconazole are very few. The results of cultured extracts showed that the MIC of ethanol extract of the *P. scutellaria* leaves was above 6400 µg/mL. The inhibition of *C. albicans* was caused by alkaloids that are used to interfere the stability of peptidoglycan and degrade the fungal cell walls. Another active compound is triterpenoid saponins which is known as an antifungal activity compound. Those are contained in *P. scutellaria* leaves (Arif, Bhosale, & Kumar, 2009).

The results were statistically tested using one-way anova. The turbidity of five different concentrations has also statistically verified to be different by looking

at p-value which is lower than 0.05, the result showed 0.009. It means that there is no significant difference of the turbidity in the wells of each concentration and controls.

Furthermore, the growth of *C. albicans* colonies could be seen by visual readings, as shown in figure 3 until Figure 8. The culture medium that was used for smearing the sample was Sabouraud Dextrose Agar. The result after 24 hours of incubation showed that in the highest concentration, the growth of *C. albicans* colonies could be inhibited. A reference microdilution method was approved by the Clinical and Laboratory Standards Institute (CLSI) for antifungal susceptibility testing of molds and dermatophytes.

CONCLUSION

The result showed as the concentration of *P. scutellaria* leaf extract went higher the number of *C. albicans* colonies was decreased. It showed that 6400 µg/mL was the highest concentration that significantly decreased the growth of *C. albicans* colonies. However, further studies are required to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents.

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