Phytochemical analysis and antifungi activity of methanol extract of Acalypha hispida Burm. F. flower against to Candida albicans (Y116)

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Abstract. Candidiasis is a fungal infection caused by the fungus Candida albicans. One of the plants that has potential as an antifungal activity is Acalypha hispida. The objectives of this research determined the phytochemical analysis and antifungal activity of A. hispida flower methanolic extract on the growth of C. albicans using the disc diffusion method. Identification of compounds using thin layer chromatography (TLC). This study consisted of six treatments, namely control using ketoconazole and concentrations of A. hispida methanol extract of 0, 0.4, 0.6, 0.8, and 1.0 g / mL. The results showed that all concentrations of A. hispida methanolic extract had antifungal properties against the growth of C. albicans. The concentration of 1.0 g / mL showed the best inhibition against C. albicans. The methanol extract of A. hispida flowers contains alkaloids, flavonoids, terpenoids and phenols.

1. Introduction
Fungi are microorganisms that cause infectious diseases in humans. One of the disease-causing fungi is a member of the genus Candida. Febriani et al. [9] stated that the fungus members of the genus Candida are commonly found in the mucous membranes of the respiratory tract, digestive tract and female genitalia. Infections that occur in the oral cavity are usually caused by this fungus. Fungi that are members of the Candida albicans species are opportunistic pathogens, meaning that they are not pathogenic in healthy individuals but will become pathogens in individuals with decreased immune system conditions or so-called immunocompromise [10]. The disease is known as candidiasis. Candidiasis is an infection by a fungal member of the C. albicans species that can attack the skin folds (intertriginosa), the vagina (vulvovaginitis), the inside of the oral cavity (thrush), and nails (paronychia) [3, 13].

A way to prevent candidiasis infection due to fungi is by giving antifungals. Antifungal is a material that can interfere with the growth and metabolism of fungi [19]. Currently, efforts to prevent disease are mostly carried out with natural based remedies. Materials that can be used are in the form of secondary metabolites derived from plant extracts. One of the plants that can be used is the Acalypha hispida.

A. hispida is an ornamental plant that usually grows in the yard and can be used as medicine. According to Dalimartha [6], A. hispida has been known by the public for the treatment of white patches on the skin (vitiligo), coughs, mouth sores, dysentery, and nosebleeds. Parts of plants that can be used for treatment are the leaves and flowers. This plant contains several secondary metabolite...
compounds such as tannins, flavonoids, saponins, essential oils, and acalyphin. The content of flavonoid and saponin compounds is known to inhibit microbial growth. Previous research conducted by Moningka et al. on the antibacterial activity of A. hispida leaf extract tested against Staphylococcus aureus and Escherichia coli bacteria showed that there was antibacterial activity and the most effective concentration to inhibit bacteria was a concentration of 80%. There is no research information about A. hispida flower as an antifungal to inhibit the growth of fungal members of the species C. albicans. Therefore, it is necessary to do this research to determine the antifungal activity of the methanol extract of A. hispida flower on the growth of fungi in the species of C. albicans.

2. Material And Methods
2.1. Time and Study area
This research was conducted in September-December 2019. Extract preparation was carried out at the Biochemistry Laboratory, State Polytechnic of Pontianak. Inhibition testing was carried out at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University Pontianak, West Kalimantan.

2.2. Materials
The materials used in this study were sterile distilled water, methanol extract of A. hispida, C. albicans (Y116) fungus isolate obtained from the InaCC Laboratory, Biological Research Center - LIPI, DMSO 10% (Dimethyl Sulfoxide), ketoconazole, Mc Farland 0.5 solution, PDA (Potatos Dextrose Agar) medium, and methanol pro analysis (pa).

2.3. Procedure
2.3.1. Sampel Preparation. The sample used was a sorted 3 kg A. hispida flowers. The sample of A. hispida flower is dried in a place that is not exposed to direct sunlight for ± 1 week. After drying the flowers were crushed into simplisia, then weighed as much as 100 g, then put in a container covered with plastic material.

2.3.2. Extraction Preparation of A. hispida Flower. Methanol extract of A. hispida flower was prepared by maceration method using methanol as a solvent. 100 grams of A. hispida flower powder soaked in 1000 mL of methanol at room temperature and protected from direct sunlight. The process was carried out for ± 5 days until the solvent clear. stirring was conducted every 1x24 hours. The solution was filtered using filter paper to obtain macerate. All the macerates from the filtering results were collected and evaporated by a rotary evaporator until a thick extract was obtained. The extract was stored in a sterile container, then stored in a silica gel desiccator.

2.3.3. Medium Preparation. A total of 200 grams of potatoes are cut like cubes and then boiled on a hot plate in 1 L of distilled water until boiling. After boiling, potato extract was filtered and put into a beaker glass. Potato extract was added 20 g of sugar, 15 g of agar and ciprofloxacin. Then distilled water was added and matched it up to 1 L. After evenly mixed, the PDA media was sterilized using an autoclave at a temperature of 121°C, 1 atm pressure for 15 minutes.

2.3.4. C. albicans Culture Preparation. Pure culture of C. albicans isolate was collected from InaCC Laboratory, Center for Biological Research - LIPI was inoculated on PDA media in a petri dish by means of scratches. Fungi were incubated for 48 hours at 37 °C.

2.3.5. C. albicans Suspension Preparation. Pure culture of C. albicans which has been recultured, is put in 10 ml of saline solution, then homogenized with vortex until the turbidity of the inoculum is equal to the turbidity of the Mc Farland standard solution of 0.5. Preparation of Mc Farland’s standard solution using 9.95 mL 1% H$_2$SO$_4$ solution then mixed with 0.05 ml of BaCl 1% in a test tube.
2.3.6. *Extract Solution Preparation.* The extract of *A. hispida* flower was made with 6 different concentrations, namely 0; 0.4; 0.6 0.8 and 1.0 g / mL. The test concentration was made by weighing the extract respectively 0.4; 0.6; 0.8 and 1.0 g with analytical scales, then dissolved with 1 mL of 10% DMSO. Positive control using ketoconazole was prepared by weighing $1.5 \times 10^{-7}$ g / mL (15 µg / mL) then dissolved in 1 mL sterile distilled water. The negative control used 1 mL of 10% DMSO without extract.

2.3.7. *Antifungi Activity Testing.* Determination of antifungal activity was carried out by agar diffusion method using disc paper (disc diffusion method). This method is done by pouring the media for PDA into 8 pieces of petri dishes and leaving them until they are solid. The fungal cultures of *C. albicans* species were then evenly wiped on the surface of the PDA media using a sterile cotton swab. Sterile disc paper was inserted into the vial bottle containing *A. hispida* flower extract with different concentration levels and left for 15 minutes. After that, the disc paper was placed on the agar plate using tweezer, then incubated for 1x24 hours at 37°C [3]. The formed inhibition zone was measured using a caliper, to see the response of the extract to the fungi, whether it was very strong, strong, medium and weak [21]. Determination of growth inhibition response categories according to Rios *et al.* [21] can be seen in Table 1.

| Clear zone diameter | Categories |
|---------------------|------------|
| >20 mm              | Very strong|
| 11-20 mm            | Strong     |
| 6-10 mm             | Intermediate|
| ≤ 5mm               | Weak       |

2.3.8. *Thin Layer Chromatography Analysis.* The bioactive compounds content of *A. hispida* flower methanol extract were monitored using thin layer chromatography. The stationary phase is silica gel GF254 using various eluents. Chromatogram profile was visualized using visible light, UV light $\lambda = 254$ nm and UV light $\lambda = 366$ nm, and sprayed with some reagents. Identification of alkaloid compounds using dragendorff reagent with eluent of methanol: chloroform = 2: 8, flavonoid compounds using AlCl3 reagent with eluent of chloroform: ethyl acetate = 8: 2), tannin compounds using AlCl3 reagent with eluent of methanol : water = 6: 4), and terpenoid compounds using Liebermann-Burchard reagent with eluent of hexane : chloroform = 2: 8

2.4. *Data Analysis*
The data were analyzed by one-way ANOVA using SPSS 22. If the analyzed data showed a significant difference, then proceed with the Duncan test at the 95% confidence level [26].

3. *Result And Discussion*
3.1. *Result*
Based on the results of ANOVA analysis with the Duncan test, it showed that the inhibition zone diameter of the methanol extract of *A. hispida* flower was significantly different from each concentration with the positive control treatment, namely ketoconazole $15 \mu$g L and DMSO 10%. The largest zone of inhibition was at 1.0 g/mL which is significantly different from ketoconazole. The smallest zone of inhibition was 0.4 g/mL which is significantly different from DMSO 10% (Table 2).
Table 2. Average diameter of inhibition zone of A. hispida extract against C. albicans for 24 hours

| Concentration (g/mL) | Inhibition zone diameter (mm) | Categories  |
|----------------------|------------------------------|------------|
| 0                    | 0,00 ± 0,00 a                | -          |
| 0,4                  | 10,59 ± 0,05 b               | intermediate |
| 0,6                  | 11,19 ± 0,13 b               | strong     |
| 0,8                  | 11,78 ± 0,05 b               | strong     |
| 1.0                  | 23,48 ± 0,00 c               | very strong |
| 1,5x10^-7            | 27,72 ± 0,07 d               | very strong |
| Ketokonazol          |                              |            |

Note: Numbers followed by different letters show significantly different results with a confidence level of 0.05%.

The results of the analysis of the activity zone of A. hispida flower extract showed a significant difference in inhibiting the growth of C. albicans. The positive control treatment was significantly different from all extract concentrations. The extract concentration of 0.4 g/mL was not significantly different from 0.6 and 0.8 g/mL but was significantly different from the extract concentration of 1.0 g/mL.

Phytochemical test results showed that there were several secondary metabolites in the methanol extract of A. hispida flower. Based on the results of phytochemical tests carried out by methanol extract of A. hispida flower, it showed that the extract contained secondary metabolites consisting of flavonoids, phenols, alkaloids, and terpenoids (Table 3).

Table 3. Phytochemical test of methanol extract of A. hispida flower

| Chemical content | Method            | Result |
|------------------|-------------------|--------|
| Flavonoid        | AlCl₃             | +      |
| Alkaloid          | dragendorff       | +      |
| Fenol            | FeCl₃             | +      |
| Terpenoid        | liebermann-burchard | +     |

(+) contains secondary metabolites

3.2 Discussion

Based on the tests that have been carried out, the methanol extract of A. hispida flower provides antifungal activity, namely inhibiting the growth of C. albicans which is indicated by the presence of an inhibition zone around the disc paper. The size of the resulting inhibition zone is influenced by the concentration of the extract given[18]. The antifungal activity test was carried out for 24 and 48 hours, the resulting inhibition zone diameter was reduced during the incubation period (Table 2). This showed that the methanol extract A. hispida flower is fungistatic or can only inhibit the growth of C. albicans without killing it.

Based on the results of ANOVA analysis showed that the concentration of methanol extract of A. hispida flower of 1.0 g/mL (23.48 mm) was the best concentration in inhibiting the growth of C. albicans, while the extract concentration was 0.4 g/mL (10.59 mm) had the smallest zone of inhibition (Table 2). This is due to the difference in concentration given at the time of the test. The increase in the inhibition zone formed can be caused by the increase in the concentration and composition of the active compound in the extract. Oktaviana et al. [18] stated that the higher the concentration of the extract given would increase the inhibition zone. This is due to the increased content of the active ingredient in the extract which functions as an antifungal. The high inhibitory activity occurs because of the extract that diffuses into the fungal cells. The amount of extract that diffuses into fungal cells at high concentrations is also high (Sari et al., 2008).
The positive control for ketoconazole 15 µg/mL showed better results than all treatments of methanol extract of *A. hispida* flower (Table 2). This is because ketoconazole is an azole class of drugs that are best at inhibiting the growth of *C. albicans* [3]. This is in accordance with research conducted by Arifin *et al.* [4] stated that the best antibiotic in inhibiting the growth of *C. albicans* is ketoconazole 15 µg/mL with an inhibition zone value of 29.3 mm, compared to other antibiotics such as fluconazole (11.32 mm), nystatin (8.36 mm), and itraconazole (0 mm). Ketoconazole is a broad spectrum antifungal drug and is water soluble at acidic pH. The mechanism of action of ketoconazole is by inhibiting the demethylation of lanosterol into ergosterol which is an important sterol for fungal membranes. This inhibition disrupts membrane function and increases permeability so that fungal growth is inhibited [16].

Negative control using DMSO 10% did not produce an inhibition zone, this condition proves that DMSO 10% has no effect on antifungal activity. DMSO 10% was used as a negative control because at this concentration it is expected that it does not have an antifungal effect on *C. albicans*, this is consistent with research conducted by Kumar [12] which states that DMSO does not have antifungal activity at concentrations below 15%. DMSO is a polar aprotic solvent that can dissolve both polar and non-polar compounds and dissolves in various organic and water solvents [7]. DMSO 10% which is used to dilute the methanol extract of *A. hispida* flower has no effect on the inhibition zone.

The solvent used in the extraction of *A. hispida* flower will affect the content of the compounds in the extract. The solvent used was methanol solvent. Methanol is a compound that has a molecular structure of CH3OH, is polar because it has a hydroxyl group (-OH) and is non-polar because it has a methyl group (-CH3) [20]. Methanol is universal so it can dissolve both polar and non-polar compounds. Methanol can attract alkalioids, steroids, saponins, and flavonoids from plants [29]. According to Suryanto & Wehantouw [27], methanol is able to attract more secondary metabolites, namely phenolic compounds, flavonoids, and tannins in breadfruit leaves (*Artocarpus altilis* F.) compared to ethanol.

The inhibited growth of *C. albicans* in the concentration of the extract was due to the activity of the active compound from the methanol extract of *A. hispida* flower. According to Salni *et al.* [22], who isolated the antifungal compounds from white galangal rhizomes, said that the active compounds affect fungal growth through several ways, inhibiting fungal cell wall synthesis, inhibiting cell membrane function, inhibiting protein synthesis and inhibiting nucleic acid synthesis. Based on the results of the phytochemical test of methanol extract of *A. hispida* flower, the secondary metabolites identified were flavonoids, phenols, alkaloids, and terpenoids (Table 3) which are thought to cause antifungal activity. This is in accordance with the research of Abad *et al.* [1] who isolated secondary metabolites from several plants, which showed that flavonoids, phenols, alkaloids, terpenoids and saponins were active compounds found in plants and had antifungal activity.

Flavonoids are a group of compounds known as antioxidants that have antibacterial and antifungal effects because they contain phenol groups. Flavonoids containing phenol groups can also coagulate proteins and reduce the surface tension of microbial cells [30]. Flavonoids can form complex compounds against extracellular proteins that interfere with the integrity of membranes and cell walls. Flavonoids can also interfere with cell metabolism by inhibiting nutrient transport. The lipophilic nature of flavonoids disrupts the microbial membrane, this situation will slowly inhibit *C. albicans* [17]. Phenols are fungistatic compounds that can denature proteins. Denaturation of fungal cell wall proteins will cause brittleness of the fungal cell walls so that they are easily penetrated by other active substances that are fungistatic. If the denatured protein is an enzyme protein, the enzyme cannot work, it will cause metabolism and nutrient absorption to be disrupted [24].

Alkaloids are the most common secondary metabolite compounds that have nitrogen found in plant and animal tissues. The mechanism of action of the alkaloid compounds in the extract inhibits fungal cell respiration and inhibits the synthesis of nucleic acids, proteins, and membrane phospholipids. Tannins have the ability to inhibit the synthesis of chitin which is used to form cell walls in fungi and damage cell membranes so that fungal growth is inhibited [3]. Terpenoids are bioactive compounds that have antifungal functions. Terpenoids can inhibit fungal growth, either through the cytoplasmic membrane or interfere with the growth and development of fungal spores [14]. According to Subhisha
steroids function as antifungal because of the lipophilic properties of steroids which can inhibit spore germination in fungi. Secondary metabolites contained in the methanol extract of A. hispida flower can inhibit the growth of the fungus C. albicans.

4. Conclusion
The concentration of methanol extract of A. hispida flower offers an antifungal activity against the growth of C. albicans. The concentration of A. hispida methanol extract was the best in inhibiting the growth of C. albicans by 1.0 g/mL. Methanol extract of A. hispida flower contains alkaloids, flavonoids, terpenoids and fenols.

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