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Research paper

Phytochemical Analysis and Antifungal Activity of *Phoebe excelsa* Nees Leaf Extract
Analisis Fitokimia dan Aktivitas Antijamur Ekstrak Daun *Phoebe excelsa* Nees

Anis Nurohma*, Occa Roanisca, dan Ristika Oktavia Asriza

Department of Chemistry, Universitas of Bangka Belitung
Kampus Terpadu Universitas Bangka Belitung, Bangka, Bangka Belitung, 33172
* *Corresponding author: anisnurohma22@gmail.com*

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ABSTRACT

Candidiasis is an infection caused by the fungus *Candida albicans*. Candidiasis was recorded in the second-highest position as an infectious disease that accompanies HIV/AIDS, with as many as 266 cases. Candidiasis is the highest infection caused by fungi and is opportunistic. Infections caused by candida albicans can generally be treated with antifungal medications. However, the use of drugs in the long term can cause resistance to these drugs, besides antifungal drugs can also cause various side effects. Therefore, in this study, an assessment of the phytochemicals of the ethanol extract of medang sang's leaves and its bioactivity as antifungal *Candida albicans* will be carried out. Testing the content of secondary metabolites in medang's leaves was carried out qualitatively by observing changes in the sample when reacted with reagents. Testing the antifungal activity of the ethanol extract of medang sang's leaves was carried out in-vitro using the disc diffusion method. Results Based on the test, the ethanol extract of medang sang's leaves contains alkaloids, flavonoids, and tannins. The results of testing the antifungal activity of *Candida albicans* showed no inhibition, this was because the tang compound contained in the ethanol extract of medang sang's leaves did not have antifungal activity against *Candida albicans*.
***Keywords**: Phoebe excelsa Nees, Phytochemicals, Antifungal, Candida albicans*

INTRODUCTION

Fungi are one of the microorganisms that can cause infection. Candidiasis is the highest infection caused by fungi and is opportunistic (Mutiawati, 2016). About 80-90% of the causes of candidiasis are caused by *Candida albicans* fungal infections (Afifah, 2015). Infection by Candida albicans is often found in individuals with compromised immune systems and chronic diseases (Framasari *et al*., 2020). Based on data from the Ministry of Research and Technology/National Research and Innovation Agency in 2019, the prevalence of candidiasis in Indonesia is around 20 – 25% of cases.

Candidiasis can generally be treated with synthetic drugs. However, the use of these synthetic drugs often causes side effects. Therefore, the use of natural medicine is preferred because it is believed to be safer and does not cause side effects. Based on research on the genus *Phoebe*, the species *Phoebe cooperiana* contains secondary metabolites such as phenolics and flavonoids which have bioactivity as antioxidants. (Payum, 2013). One of the plants that have been used as medicine is Medang Sang (*Phoebe excelsa* Nees).

Medang sang (*Phoebe excelsa* Nees) is a type of plant from the genus Phoebe which is widespread in Indonesia, including in Bangka Belitung. Medang sang is used by the people of Bangka Belitung to treat various diseases including fungal infections. The use of Medang Sang as medicine must of course be accompanied by scientific research. However, research on the Sang field is still very minimal. So that in this study, identification of the

secondary metabolite content in Medang Sang leaves and their bioactivity as an antifungal against *Candida albicans* will be carried out.

METHODOLOGY

In this study, qualitative identification of secondary metabolites and antifungal activity of the ethanol extract of the leaves of Medang Sang (*Phoebe excelsa* Nees) will be tested in vitro.

Materials

The material used in this research is the simplicia leaves of Medang Sang (*Phoebe excelsa* Nees), technical ethanol (MKR Chemicals), distilled water, HCl, NaOH, Wagner reagent, Liebermann – Burchard reagent, amyl alcohol, HCl 37%: ethanol 95% (1:1), $CHCl_3$, $FeCl_3$ 1%, PDB (*Potato Dextrose Broth*) GM403, magnesium.

Tools

The equipment used in this study were Pyrex Iwaki test tubes, test tube racks, stirring rods, spatulas, dropper drops, vials, OXOID disc paper, Petri dishes, Pyrex measuring cups, Pyrex beakers, tripod, Bunsen, wire loop, blender Philips HR 2115, analytical balance Pioneer™, *rotary evaporator* IKA RV 10 DIGITAL V – GERMANY, LAF (*laminar airflow*), incubator Memmert INB 500, *autoclave* Hiramaya HVA – 110.

Procedure

Preparation and Extraction

The leaves of Medang sang (*Phoebe excelsa* Nees) are dried in the open air and then mashed with a blender until they become powder. Medang sang leaf powder was then extracted using the maceration method with ethanol as a solvent. The powder was immersed in ethanol with a sample and solvent ratio of 1: 10. Immersion was carried out for 3 x 24 hours. Then after 3 x 24 hours filtered to obtain the filtrate and evaporated using a rotary evaporator to obtain a thick extract.

Phytochemical Screening

Identification of secondary metabolites of the ethanolic extract of Medang Sang leaves included tests for alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins.

1. Alkaloid Test

Testing for alkaloids using Wagner's reagent. The 1 mL of the dissolved extract was added with a few drops of Wagner's reagent. The result is positive if a brown precipitate is formed.

2. Flavonoid Test

Flavonoid testing was carried out by adding 2-3 drops of HCl and 2-3 small pieces of magnesium metal into 1 mL of Medang Sang leaf extract. The test is positive if a yellow to orange color is formed.

3. Terpenoid and Steroid Test

1 mL of the extract was added with a few drops of Liebermann-Burchard reagent. The terpenoid test is positive if a brownish or violet ring is formed, while the steroid test is positive if a green to blue color is formed.

4. Saponin Test

1 gram extract was put in a test tube and warm water was added dropwise and then shaken vertically for 10 seconds. The formation of stable foam not less than 10 minutes indicates a positive result.

4. Tannin Test

The tannin test was carried out by adding 2 – 3 drops of 1% $FeCl_3$ in 1 mL of Medang Sang extract. A positive result is formed when a bluish-black or green color is formed.

Antifungal Test

a) Sterilization

The heat-resistant equipment for testing was sterilized using an autoclave at a temperature of 121^{o}C and a pressure of 1 atm for 15 minutes.

b) Media *Potato Dextrose Agar* (PDA) preparation

The agar medium was made by weighing 3 grams of agar and 3.6 grams of PDB and put in a 250 mL Erlenmeyer then added 150 mL of distilled water. Then the medium was heated to boiling, then allowed to stand and sterilized for 15 minutes in an autoclave at a temperature of 121^{o}C.

c) Rejuvenation of *Candida albicans*

PDA media is heated on a hotplate until it melts. Prepare 3 test tubes, then the melted SDA is inserted into each tube and allowed to stand until it hardens. Colonies of Candida albicans were taken using a needle aseptically from pure culture and then streaked on agar media and incubated at 37oC for 24 hours.

d) Inhibitory Test

The method used in the power test is the disc diffusion method. The media that has been heated is poured into a petri dish and allowed to harden. After hardening the rejuvenated Candida albicans culture was streaked on the media. Medang sang leaf extract solution was made with various concentrations of 25%, 50%, 75%, and 100%, w/v (mg/mL). Then, the paper discs were immersed in each of these solutions for 30 minutes. The soaked disc paper is placed on the PDA media. Ketoconazole 10% was used as a positive control and DMSO as a negative control. The clear zone was observed after 24 hours, the diameter was measured using a caliper. The value of antifungal inhibition can be done by measuring the clear zone formed in the following way:

$$Zona\ hambat = diameter\ zona\ bening - diameter\ cakram$$

RESULTS AND DISCUSSION

Extraction

Extraction was carried out by the maceration method with ethanol as solvent. The extraction of Medang Sang leaves uses the maceration method. The principle of the maceration method is the distribution of the active compound in the solvent-based on the level of polarity. The extract was then concentrated with a rotary evaporator aimed at evaporating the solvent so that a thick extract was obtained. The results of the extraction of Medang Sang leaves can be seen in table 1.

Table 1. The results of the extraction of the leaves of Medang Sang

Sample (g)	Extract (g)	Yield (%)
300	32,18	10,73

Medang sang leaf extract produced from 300 grams of Simplicia was obtained in as much as 32.18 grams (10.73% yield).

Phytochemical Screening

Phytochemical identification aims to determine the content of secondary metabolites in a test sample. Secondary metabolites identified in the qualitative test of Medang Sang leaf extract include alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins. Phytochemical test results can be seen in table 2.

Tabel 2. Phytochemical screening result

Fitokimia	Hasil	Ket.
Alkaloid	Chocolate precipitate	+
Flavonoid	orange	+
Terpenoid	No changes	-
Steroid	No green-blue color formed	-
Saponin	No stable foam is formed	-
Tanin	blackish green	+

Based on the test results in Table 2, the ethanolic extract of Medang Sang leaves contains secondary metabolites of alkaloids and phenolic compounds such as flavonoids and tannins.

Alkaloid

Testing for alkaloids on the ethanol extract of Medang Sang leaves showed the presence of alkaloids due to the presence of deposits. Alkaloids contain nitrogen atoms which are basic and will form a precipitate when reacted with Wagner's reagent. The reaction between alkaloids and Wagner's reagent can be seen in Figure 1.

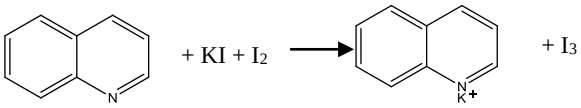

Figure 1. The reaction between alkaloids and Wagner's reagent (Marliana *et. al.,* 2005)

Flavonoid

The results of the flavonoid test of the ethanol extract of Medang Sang leaves showed a color change to orange, so it could be identified that the extract was positive for flavonoids. Concentrated chloride will protonate the carbonyl group of flavonoids and form flavonoid salts (red). Meanwhile, magnesium metal will reduce flavonoids to produce an orange color (Harborne, 1987). Figure 2 is the reaction between flavonoids with HCl and Mg

$$Mg + 2HCl \rightarrow MgCl_2 + 2H_2$$

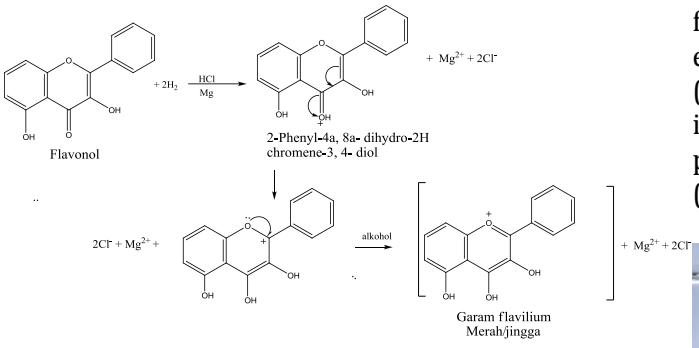

Figure 2. The reaction between flavonoids with HCl and Mg (Setiabudi *et. al.,* 2017)

Tanin

The identification of tannins in the ethanol extract of Medang Sang leaves showed positive results because a blackish green color was formed. The color change is due to the reaction between the –OH aromatic group in the tannin structure with $FeCl_3$ (Hayati *et. al.,* 2015). The reaction between tannin dan $FeCl_3$ showed in Figure 3.

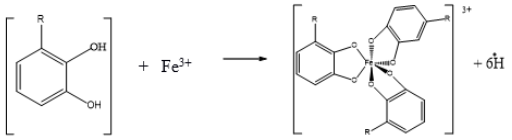

Figure 3. The reaction between tannin dan $FeCl_3$ (Perron & Brugmaghim, 2015)

Antifungal Test

The antifungal activity test was aimed to determine the inhibitory activity of the medang sang leaf extract against Candida albicans in vitro. The method used in this test is disc diffusion. Medang Sang leaf extract was made in several concentrations: 25%, 50%, 75%, and 100%, w/v (g/10mL), then used a positive control with ketoconazole and negative control with DMSO. Based on the test of Medang Sang leaf extract, showed that there was no antifungal activity against Candida albicans because there was no clear zone formed on the agar media. The results of phytochemical testing of the ethanolic extract of Medang Sang leaves were identified as containing phenolic compounds of hydroquinone/tannin, flavonoids, and alkaloids. The antifungal activity test is shown in Figure 4.

Generally, phenolic compounds have the potential as antifungals by denaturing proteins on the membrane and cell walls, causing lysis (Balafif *et. al.,* 2017). The mechanism of action of flavonoid compounds as antifungals is by forming complexes with soluble and extracellular proteins and fungal cell walls (Jalianto, 2015). Alkaloids act as antifungals by inhibiting the synthesis of nucleic acids, proteins, and phospholipid membranes (Adegoke dan Adebayo, 2009).

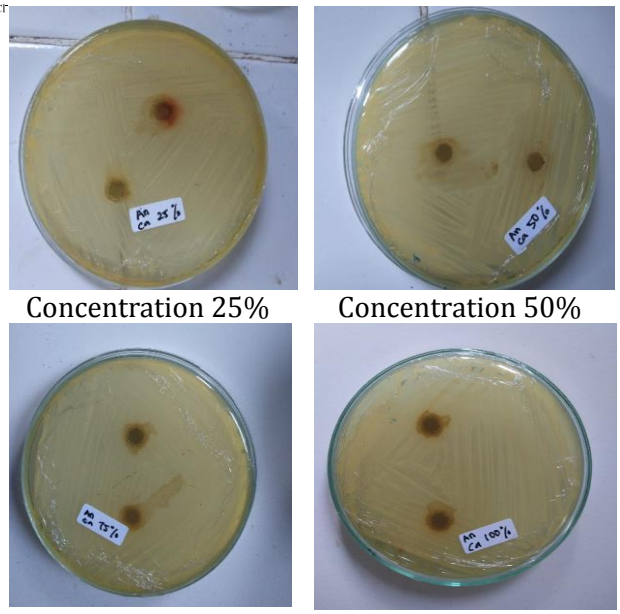

Figure 4. The antifungal activity test

The absence of antifungal activity in Medang Sang leaf extract was due to the compounds contained in the extract not having activity as antifungal Candida albicans although based on phytochemical tests there were flavonoid, phenolic, and alkaloid compounds. The bioactivity of a compound is influenced by the substituents bound to the compound, it is possible that the compounds contained in the extract do not have strong enough substituents to support their bioactivity as antifungal Candida albicans. The positive control of ketoconazole showed antifungal activity of 6.55 mm. Ketoconazole is an azole-derived antifungal drug that inhibits the enzyme C-14α-*demet*hylase thus does not form ergosterol and membrane plasma becomes permeable and damaged (Philips, 2001).

CONCLUSION

Based on the research data obtained, it can be concluded that qualitative phytochemical testing showed that the ethanolic extract of the leaves of Medang Sang (*Phoebe excelsa* Nees) contained alkaloids, flavonoids, and tannins. The results of the antifungal activity test of the leaf extract of Medang Sang (*Phoebe excelsa* Nees) against *Candida albicans* showed no

inhibition because the compounds contained in the extract did not have antifungal activity against *Candida albicans*

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