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POTENTIAL OF WULUH LEAVE (*Averrhoal bilimbi* L.) AS ANTIFUCIAL *Candida albicans*

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ABSTRACT

Wuluh starfruit in Aceh commonly called "Boh Limeung" is a plant that is very often encountered, because star fruit is one of the spices in cooking. The Acehnese use star fruit as "SUNTI ACID", which is star fruit that is dried and then salted and used as a spice in cooking. The chemical compounds in star fruit leaves are flavonoids, saponins, sulfur, formic acid, steroid peroxides and tannins which are able to inhibit microbial activity and damage cell membranes so that, they can inhibit bacterial growth. The purpose of this study was to determine the potential of starfruit leaves (*Averrhoa bilimbi* L.) as antifungal *Candida albicans*. The method used in this research is experimental laboratory. The treatments given in this study were 30%, 40%, 50%, 60% methanol extract concentrations. Then for the positive control (+), ketoconazole was used and for the negative control (-) aquadest was used. Based on the results obtained from the methanol extract of starfruit leaves (*Averrhoa bilimbi* L.) at concentrations of 30%, 40%, 50%, 60%, the average inhibition zone values were 17.5 mm, 17.35 mm, 17.95. mm, 18.77 mm. In Table 4.2, it can be seen that the largest inhibition zone is in the positive control (+) which is 23.5 mm while the negative control (-) is 0 mm.

Introduction

Candidiasis is an acute or subacute fungal disease caused *by Candida albicans* and can affect the mouth, vagina, skin, nails, bronchi and lungs. One of the fungi that causes superficial infections is *Candida albicans*. The fungus *Candida albicans* is a normal flora whose presence is most abundant on the skin, mucous membranes, oral cavity, digestive tract, respiratory tract, and vagina. Initially, *Candida albicans* were non-pathogenic, but when there are predisposing factors, Candida albicans will act as a pathogen (Nuryanti et al., 2015). *Candida albicans* grows as a normal micro flora of the human body in the digestive tract, respiratory tract and female genital tract. The fungus *Candida albicans* is also often found in the oral cavity of healthy people,

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gastrointestinal tract, upper respiratory tract, vaginal mucosa and under the nails as a saprophyte without causing disease (Khatimah et al., 2018). Candidiasis can generally treated with antifungal drugs. The widespread use of antifungal drugs that are freely available in the market does not mean solving the problem because side effects of using these synthetic drugs (Tripathi, 2013).

Therefore, it is necessary to develop antifungal drug processing with natural ingredients. As we know Indonesia has biodiversity that can be developed as a potential natural resource to be used as a traditional medicine. One of the plants that have the potential as ingredients for traditional medicine is starfruit (*Averrhoa bilimbi* L.) (Sari & Suryani, 2014). Belimbing wuluh is widely used by Indonesian people from cooking ingredients to traditional medicine. This plant can grow in areas with an altitude up to 500 meters above sea level (Anggraeni Puspitasari & Ardiansyah, 2017). Because the chemical content in star fruit leaves is able to inhibit the growth of microbes, namely tannins, flavonoids, saponins, sulfur, formic acid, peroxides, and steroids.

Based on the results of research Hasim et al (2019) showed that the phytochemical compounds contained in the ethanol extract of leaves belimbing wuluh were saponins, tannins, steroids, flavonoids, and alkaloids. The total phenolic and flavonoid content of the ethanol extract of belimbing wuluh leaves was 39.03 and 97.28 g QE/mg, respectively (Hasim et al., 2019). Furthermore, based on the results of research conducted by Soleha et al (2019) concluded that starfruit leaf extract with minimal inhibition in negative control while maximum inhibition at 100% concentration and this study concluded that starfruit leaf extract (Averrhoa bilimbi L.) Antifungal activity against Candida albicans (Soleha et al., 2019). Furthermore, Puspitasari et al (2017) explained the results of the study that starfruit leaf extract had a minimal inhibitory concentration (MIC) a concentration of 12.5% while the minimum killing rate (KBM) a concentration of 100%, and this study concluded that starfruit leaf extract (*Averrhoa bilimbi* L.) Antifungal properties against *Candida albicans* (Anggraeni Puspitasari & Ardiansyah, 2017).

From the explanation above, the problem in this study is how the potency of starfruit leaf extract (*Avverhoal bilimbi* L.) in inhibiting the growth of the fungus Candida albicans. With the aim knowing the potential starfruit leaf extract in inhibiting the activity of the fungus *Candida albicans*.

Materials and Methods

This research will be carried out for 1 (one) year to achieve the planned target. The research was conducted by laboratory experiment. By using a completely randomized design. The treatment given in this study to fungi, namely with a concentration of methanol extract 30%, 40%, 50%, 60%. The positive control (+), ketoconazole was used and for the negative control (-) aquadest was used. Total treatment in this study was 6 treatments with 4 repetitions. So the replication in this study was 4 times. The stages of the research are as follows:

1. Making wuluh starfruit leaf simplicia
   Fresh star fruit leaves are washed using running water to separate dirt and foreign materials from the leaves. Then leaves are drained and cut into small pieces using a knife, leaves are dried at room temperature. After drying, the star fruit leaves are blended and using sieve until smooth.
2. Extraction
   Extraction was carried out by maceration, the sample was immersed in methanol as solvent, then filtrate the extract was concentrated, in order to obtain star fruit leave extract. Total of 500 grams of simplisia powder of star fruit leaves was inserted into the maceration tool then the methanol solution was slowly added into the maceration tool containing the sample and stirred until evenly distributed. The filtrate is filtered and the solvent is

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replaced with a new one, stirring occasionally. Solvent replacement was carried out until the liquid was clear, the extract was collected and evaporated with a water bath until a thick methanol extract was obtained

3. Phytochemical screening test, after obtaining the extract, the next stage is phytochemical screening to determine the content of secondary metabolites contained in the sample, including: flavonoid test, saponin test, tannin test, steroid test.
4. How to make it tilted
   The test containing 3 ml of Potato Dextrose Agar (PDA) medium was sterilized in an autoclave, then laid horizontally. After the medium thickens, and the test is erected, it is obtained that the medium is tilted.
5. Pure culture rejuvenation
   The test mushrooms were rejuvenated by scraping the mushrooms using an needle on Potato Dextrose (PDA), then incubated in an incubator at 21°C for 48 hours.
6. Making Mc. Farland
   9.5 ml of 1% H2SO4 solution was mixed with 0.5 ml of 1% BaCl2 solution in an flask and then shaken to form a turbidity solution equivalent to a concentration of 1.5 x 108 CFU/ml.
7. Testing antifungal activity
   Determination of antifungal activity was carried out using the streak plate method using disc paper. 20 ml medium was poured into a petri dish. One ml of the inokulum of the test fungus was poured into a petri dish. Slowly shake the petri dish with a figure eight motion without being lifted from the table surface, so that the test fungi material is evenly mixed in the agar medium. The agar medium is allowed to solidify. 6 mm disc paper was dipped into the test solution for ± 10 minutes, then aerated until no solution dripped. The disc paper is placed on the surface of the agar medium, the distance between the disc paper is 3 cm and from the edge of the media 2 cm. The petri dish was closed and then incubated for 48 hours in the incubator. The antifungal activity was observed based on the diameter of the inhibition zone indicated by the clear area formed around the paper disc. The diameter of the inhibition zone was measured using a caliper.

Results and Discussion

Results of Phytochemical Screening of Wuluh Starfruit Leaves (*Averrhoal bilimbi* L.)

Based on the results of phytochemical screening of the methanol extract of starfruit leaves (*Averrhoal bilimbi* L.) are as follows:

Table 1 Phytochemcal Screening of *Averrhoal bilimbi* L

Identical Compound	Result	Description
Flavonoid	+	Positif Flavonoid
Saponin	+	Positif Saponin
Tanin	+	Positif Tanin
Steroid	-	Negatif Steroid

Based on Table 1, the results of phytochemical screening of the methanol extract of belimbing wuluh leaves indicate the presence of flavonoids, which are indicated by a change in color to yellow-orange or red, saponins are indicated by the formation of stable foam, and tannins are indicated by the formation of a greenish black color.

The results of phytochemical screening that have been carried out on the methanol extract of belimbing wuluh leaves (*Averrhoal bilimbi* L.) showed that the leaf extract was positive for the content of

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flavonoids, tannins, saponins and steroids that have antifungal activity. In flavonoid compounds, it is done by adding starfruit leaf extract with concentrated Mg and HCl powder, the addition of concentrated HCl is used to hydrolyze flavonoids into glycos, namely by hydrolyzing O-glycosis. Glycosyl will be replaced with H+ from acid because of its electrophilic nature. Reduction with concentrated Mg and HCl can produce yellow-orange complex compounds. The color change to yellow-orange indicates a positive flavonoid. The tannin compound was carried out by adding leaf extract with 1% FeCl3 reagent. The results of the tannin compound test of the methanol extract of starfruit leaves (Averrhoal bilimbi L.) gave positive results indicated by the change in color to blackish green. The color change to blackish green occurs due to the formation of tannin compounds with FeCl3 (Ikalinus et al., 2015). The saponin test on the leaf extract was positive when hot aquadest was added, foam was formed. This is because saponins contain glycosyl groups that act as polar groups and steroid and triterpenoid groups that function as nonpolar groups. Compounds that have polar and nonpolar groups will be surface active so that when shaken with water, saponins can form micelles, where the polar structure will face out while the nonpolar group will face inward. In this condition, saponins will be shaped like foam, indicating the presence of glycosides that have the ability to form white foam in water (Anggraeni Puspitasari & Ardiansyah, 2017)..

Antifungi Activity Test Result

The results of the antifungal activity test of the methanol extract of starfruit leaf (*Averrhoal bilimbi* L.) against the growth of the fungus *Candida albican* showed that all concentrations of the methanol extract of starfruit leaf (*Averhoal bilimbi* L.) had antifungal activity against the fungus *Candida albican*. This can be seen by the formation of a clear zone around the top of the disc at all concentrations after being incubated for 48 hours at 37°C. Then the clear zone formed was measured using a caliper with accuracy (mm).

Table 2 Activity Test of Result Antifungi

Treatment and Control	Diameter of Average (mm)	Description
30%	17,5	Strong
40%	17,35	Strong
50%	17,95	Strong
60%	18,77	Strong
Positif Control	23,5	Very Strong
Positif Control	-	-

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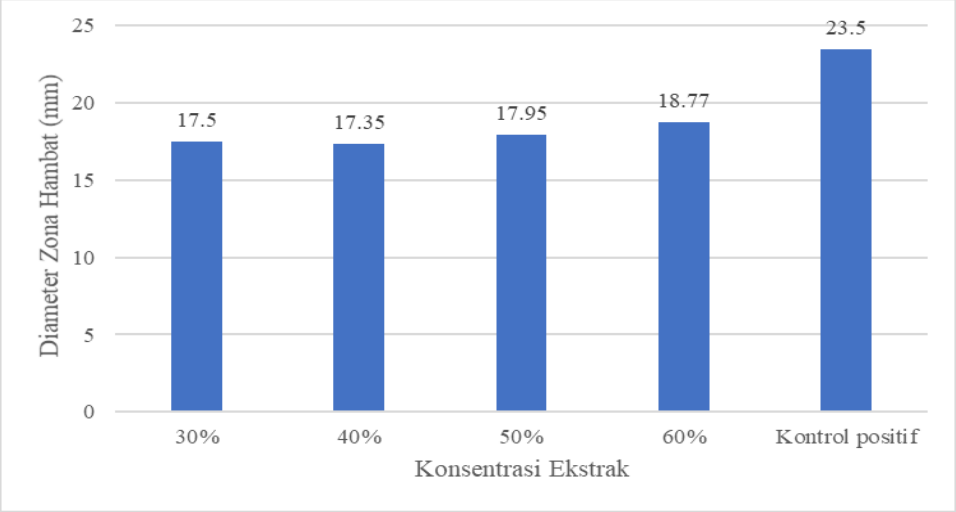

Figure 1 Barrier Zone Diagram

From the results obtained, it can be seen that all concentrations of methanol extract of starfruit leaves (*Averrhoal bilimbi* L.) have antifungal activity against the fungus *Candida albicans* which is included in the strong category. At a concentration of 30% of the inhibition zone formed was 17.5 mm, the concentration of 40% of the inhibition zone formed was 17.35 mm, the concentration of 50% of the inhibition zone formed was 17.95 mm, and a concentration of 60% of the inhibition zone formed was 18. .77mm. Then the control (+) has an inhibition zone of 23.5 mm very strong category, but the control (-) has no inhibition zone diameter because it was not given any treatment.

In this study, the most effective antifungal activity was found at a concentration of 60% with an inhibitory zone of 18.77, while the smallest inhibition zone was found at a concentration of 40%, which was 17.35. The decrease in antimicrobial activity is influenced by several factors such as the rate of diffusion of the extract, the concentration of the extract, the amount of antimicrobial active substances contained in the extract, the influence of incubation temperature, the concentration of the fungus, and the optimal pH of the medium for the growth of Candida albicans. The main factor that affects the growth of candida is the incubation temperature, because fungal growth requires an optimal temperature, and most fungi are mesophilic, namely microbes that like moderate temperatures so that they grow well at temperatures of 30-37 ° C (Sari & Suryani, 2014).

The formation of an inhibition zone or clear zone around the paper disc issecondary metabolites contained in the extract which act as antifungals. Secondary metabolite compounds produced by plants function to protect plants from attacks by other organisms, therefore the plant's living environment greatly affects the levels of secondary metabolites produced by these plants (Oktirisma, 2018). In addition, the nutrients contained in the soil also affect the content of secondary metabolites found in plants. Therefore, the more nutrients contained in the soil will cause plants to have better secondary metabolite quality.

Selection of the appropriate solvent is an important factor in the extraction process. The solvent used is a solvent it can be extract most of the secondary metabolites present in the simplicia. In this study, the solvent used was methanol because methanol is a universal solvent so that it can dissolve polar and non-polar analytes (Gedong et al., 2013).

The ability of methanol extract of starfruit leaves (*Averrhoal bilimbi* L.) in inhibiting the growth of *Candida albican* fungus is due to the content of secondary metabolites, namely

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tannins, saponins and flavonoids. These compounds play an important role in inhibiting antifungal activity in plants. The mechanism of action of tannins as antifungals is by inhibiting the biosynthesis of ergosterol which is the main sterol constituent of fungal cell membranes. Sterols are structural and regulatory components found in eukaryotic cell membranes. Sterols are the final product of sterol biosynthesis in fungal cells. Like cholesterol in mammalian cells, sterols are thought to play a role in the permeability of fungal cell membranes. Saponins as antifungals can work by forming complexes with sterols which are enzymes that make up fungal cell walls, causing loss of cell wall permeability. Flavonoids as antifungal activity compounds work by forming combinations with phospholipids from fungal cell membranes, causing damage to fungal cells which can inhibit cell growth and increase membrane permeability which causes fungal cells to denature (Arifin & Rahmayanti, 2018). The activity of an antimicrobial substance is influenced by concentration. the substance. Increasing the concentration of substances causes an increase in the content of active compounds that function as antimicrobials, so that the ability to kill a microbe is also greater (Roslizawaty et al., 2013).

Conclusions

The methanol extract the leaves of belimbig wuluh (*Averrhoal bilimbi* L.) has antifungal activity against the fungus *Candida albican*. Can be seen from the average value of inhibition zone at 30% concentration of 17.5 mm, inhibition zone at 40% concentration of 17.35 mm, inhibition zone at 50% concentration of 17.95 mm, inhibition zone at 60% concentration of 18.77 mm.

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