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The Activity of ethanol extract and aquadest of Jati Belanda (Guazuma Ulmifolia Lamk) leaves against candida albicans

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ABSTRACT
Fungi are the cause of infection, the development of fungal infections in Indonesia is influenced by environmental conditions so that microbes grow quickly and can increase significantly. Candida Albicans is a fungal disease that infects humans. As a country with a tropical climate, Indonesia has potential biodiversity as a medicine, one of which is Dutch teak (Guazuma ulmifolia Lamk) which has secondary metabolites that kill microbes that can be used as natural antifungals. The purpose of this study was to determine the antifungal activity of ethanol extract and distilled water of Jati Belanda leaves on the growth of Candida Albicans. The method used is maceration with ethanol and distilled water solvents. The anti-fungal activity was evaluated by the good diffusion method with SDA media. The results of the antifungal activity of ethanol extract and aquadest extract of Jati Belanda leaves against Candida Albicans were indicated by the formation of an inhibition zone around the wells on Candida Albicans ethanol extract concentrations of 25 mg / mL to 1000 mg / mL 8.6 mm - 15.6 mm, the distilled water extract has not formed a zone of inhibition at all concentrations. The conclusion is that the ethanol extract of Jati Belanda leaves can inhibit the growth of *Candida albicans*. while the distilled water extract was unable to inhibit the growth of *Candida albicans*.

INTRODUCTION

Infectious disease is a type of disease that is mostly suffered by residents of developing countries including Indonesia. The development of fungal infections in Indonesia is influenced by the humid, dusty, densely populated environment and low socioeconomic levels and warm temperatures, microbes can grow rapidly and these infections can increase significantly.[1]

Fungal diseases contribute significantly to morbidity and mortality in humans. Although recent research has improved our understanding of the complex and dynamic interplay that occurs between pathogenic fungi and the human host, much remains to be elucidated concerning the molecular mechanisms that drive fungal pathogenicity and host responses to fungal infections.[2]

Candida albicans is a commonly encountered fungal pathogen. It may cause diseases varying from superficial mucosal infections to systemic disorders with high

mortality rate. Notably, clinical available antifungal agents are limited, and drug resistance is a significant challenge.[3]

The increased incidence of Candida infections can be attributed to a variety of factors, either exogenous or (especially) endogenous. Over 100 species of Candida are known; C. albicans is the main representative. The frequency of distribution for Candida spp. varies in accordance with geographical location. Among the Candida species, it is C. albicans that is involved in bloodstream infections for 44% of Latin American and 62% of European cases.[4]

Conventional fungal infection treatments are unsatisfactory. Therefore, it has become essential to develop new drugs and alternative therapies (including natural products) for the treatment of C. albicans infections. Plants and their derivatives are known to be important in pharmacological research due to their great potential as a source for a variety of biologically active ingredients used in drug development.[4] Novel drugs with unique and targeted mode of action are very much need of the hour to treat and manage severe multidrug infections and other life-threatening complications. Plants and microbes are the major resources that we rely upon in our pursuit towards discovery of novel compounds of pharmacological importance with less toxicity.[5]

Natural product drug discovery has regained interest due to low production costs, structural diversity, and multiple uses of active compounds to treat various diseases. Attention has been directed towards medicinal plants as these plants have been traditionally used for generations to treat symptoms of numerous diseases. It is established that plants harbour microorganisms, collectively known as endophytes. Exploring the as-yet untapped natural products from the endophytes increases the chances of finding novel compounds. The concept of natural products targeting microbial pathogens has been applied to isolate novel antimycobacterial compounds, and the rapid development of drug-resistant.[6]

Indonesia is one of the countries with rich sources of medicinal plants. Its biodiversity is the second largest after Brazil. Thirty thousands types of plants, out of forty thousands, can be found in Indonesia and 940 of them are known to have restorative power. They have been used as traditional medicine by many generations of many ethnics in Indonesia. One of then are *Guazuma ulmifolia* Lamk. (in Indonesian is called *Jati Belanda*).[7] Plants in Indonesia that are commonly used as herbal medicines and have traditional medicinal properties are the leaves of Jati Belanda (G. ulmifolia Lamk) which is a family of Sterculiaceae. The use of G. ulmifolia herbal medicine has been used by public health services in seven provinces in Indonesia in the treatment of hyperlipidemia.[8] G. ulmifolia has the main content in the form of flavonoids which have anti-diabetes, anti-bacterial, hypertensive and anti-cancer properties.[9] Most of the studies examining the medicinal value were carried out using ethanol extracts. Previous research has shown that the ethanol extract of Dutch teak leaves has antimicrobial activity. Ethanol which has semi-polar properties is easier to dissolve the compounds contained in Jati Belanda. You can also make Jati Belanda leaf extract using distilled water. Although distilled water has polar properties so that it is not very effective in binding the compounds contained in Dutch teak leaves, making extracts with distilled water is easier.[10]

MATERIALS AND METHODS

The materials used in the study were: Jati Belanda leaves, miconazole, ethanol, distilled water, SDA media (Sabaroud Dextrose Agar), BHI media (Brain Infution), C.albicans fungal colonies, 70% physiological alcohol NaCl and 1% Mac Farland standard. This research design uses True-Experiment Design (pure experimental design) or laboratory experiments.

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Culture Preparation

The C. albicans fungal colonies that have been confirmed have been made a suspension in a test tube containing 3 mL of 0.90% NaCl solution. Turbidity compared to Mac Farland standard 1% solution. Jati Belanda leaves are taken in the second part with leaves that are not too old, washed with running water and dried at room temperature without direct sun exposure. After the leaves dry, they are grounded. The leaves of Jati Belanda were extracted by maceration method using 96% ethanol solution and Aquades. Jati Belanda leaf powder is soaked for 3 days, stirred every day, carried out 3 times of maceration until the colorless dutch teak leaf solution is added to each solution then filtered, and added again with a solution of ethanol and distilled water, then filtered and added again with the solution with the third immersion was cooled at room temperature. Then filtering is done using sterile gauze and filter paper until the Jati Belanda leaf filtrate is obtained. The filter results are evaporated until the Jati Belanda leaf extract is obtained.

Control Creation

Positive control for C. albicans. The suspension was taken and scratched using sterile cotton swabs on SDA media, let stand for ± 15 minutes, then perforated the media and put in mikonazole 1 gram in 1 ml aquadest. Negative control for C. albicans. The suspension is taken and scratched using sterile cotton swabs on SDA media, let stand for ± 15 minutes, then perforate the media and enter sterile aquadest.

Antifungal Activity Test

The C. albicans suspension was taken and scratched using a sterile cotton stick on SDA media, let stand for ± 15 minutes, then perforated the media with a cork borer at a distance of 2 cm each well and put in the ethanol extract and distilled water of Jati Belanda leaves with a weight of 25 mg, 50 mg. , 75 mg, 100 mg, 250 mg, 500 mg, 750 mg, 1000 mg in each well, then incubated at 37^{O}C for 24 hours and calculated the diameter of the inhibition zone formed using a ruler or calipers.

RESULTS AND DISCUSSION

Candida albicans is an opportunistic fungal pathogen that causes both superficial and systemic infection and an important candidate that contribute to high morbidity and mortality rates in immunocompromised patients. The ability of *C. albicans* to switch from yeast to filamentous form and thereby forming biofilms make them resistant to most of the antifungal drugs available today. Thus, the development of more effective antifungal drugs are essential and crucial at this point of time.[11]

Clinical use of antimicrobials faces great challenges from the emergence of multidrug-resistant pathogens. The overexpression of drug efflux pumps is one of the major contributors to multidrug resistance (MDR). In the life-threatening fungal pathogen *Candida albicans*, the major facilitator can excrete many structurally unrelated antifungals, leading to multidrug resistance.[12]

This study tested the activity of ethanol extract and aquadest of Jati Belanda leaves on C.albican growth at concentrations of 25 mg, 50 mg, 75 mg, 100 mg, 250 mg, 500 mg, 750 mg, and 1000 mg. The results of the inhibition zone diameter measured using a caliper can be seen in Table 1.

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Table 1. The diameter average of the inhibition zone of ethanol extract and aquadest of Jati Belanda leaves on *C.albican* growth

Extract concentration (mg)	Jati Belanda Leaves Ethanol Extract (mm)	Jati Belanda Leaves Aquadest Extract (mm)
25	8,6	0
50	9,3	0
75	9,6	0
100	10	0
250	10,6	0
500	11,6	0
750	14,6	0
1000	15,3	0
Mikonazole 1000	12	12

Based on Table 1. It was found that the ethanol extract inhibited the growth of *C. albican* and the aquadest extract did not inhibit *C. albican.*

A B C D

Figure 1. (A) Ethanol extract of Jati Belanda leaves against *C.albican* with a concentration of 25 mg, 50 mg, 75 mg, 100 mg. (B) Ethanol extract of Jati Belanda leaves against *C.albican* with a concentration of 250 mg, 500 mg, 750 mg, 1000 mg. (C) Teak leaf aquadest extract against C.albican with a concentration of 25 mg, 50 mg, 75 mg, 100 mg. (D) Jati Belanda aquades extract against *C.albican* with a concentration of 250 mg, 500 mg, 750 mg, 1000 mg.

This study used miconazole antibiotics as a fungal antibiotic to determine the inhibition zone formed including sensitive, intermediate, or resistant. miconazole enhanced the killing of C. albicans and induced prolonged fungistasis in organisms. The data suggest that miconazole could be used to increase the efficacy of PDT against C. albicans, and its mechanism of action is likely to be multifactorial.[13] The classification of the inhibition zone is shown in Table 2.

Table 2. Fungal performance according to CLSI

Antibiotik	Zona Hambat (mm)		
	Sensitiv	Intermediet	Resisten
Mikonazole	≥20	19 - 21	≤11

This study used a sample of Jati Belanda leaves extracted by maceration method using ethanol and aquades as solvents. The results of the ethanol extract research had a

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higher inhibition zone diameter, this happened because ethanol easily dissolved the compounds in Jati Belanda leaves, while the results in the aquadest extract were lower because the compounds that could be dissolved were flavonoids and tannins. Maceration is a type of solid-liquid extraction which is carried out by immersing the sample at room temperature using a specified solvent to dissolve the analyte in the sample.[14]

Extraction is the first step to recover and isolate the bioactive phytochemical compounds from the plant materials. The extraction efficiency from plant materials is influenced by several factors, including the chemical nature of phytochemicals, the extraction method used, size of sample particle, and the presence of interfering substances. The extraction yield depends on the pH, temperature, extraction time, solvent polarity, and sample compositi on. The sample composition and solvent polarity are regarded as the critical parameters under similar time extraction and temperature. The TPC (total phenolics content) of the ethanol extracts gives the highest value due to the possible complex formation of some phenolics compound in the extract that are soluble in this solvent. These phenolics compound may have higher molecular weights solubilized in the ethanol and contain more phenolics group than the phenolics in the water extracts.[15]

Ethanol is the best extraction solvent to dissolve phenolic compounds from the extract of G. ulmifolialeaves.[16] The chemical composition indicated the presence of flavan-3-ol derivatives and condensed tannins and glycosylated flavonoids showed free-radical scavenging antioxidant activity, antihemolytic activity.[17] The phytochemical analysis revealed the presence of phenols, tannins, saponins, steroids, resins, quaternary bases, quinones, flavonoids and triterpenoids.[18] Ethanol is a semi-polar volatile solvent that can dissolve polar and non-polar compounds so that it can dissolve saponins, alkaloids, flavonoids, steroids, and tannins.[19] Aquades are chemical substances that are polar due to the difference in charge, high dielectric constant and small size. The polar nature of distilled water can dissolve tannins and flavonoids.[20]

The results of ethanol extract and aquadest of Jati Belanda leaves on C.albican growth are in Table 1. In the ethanol extract with the interpretation of the results according to CLSI is a concentration of 25 mg to 250 mg is resistant, 500 mg to 1000 mg including intermediates. In the aquadest extract, there was no inhibition zone at all concentrations. The inhibition zone formed in the growth of C.albican is the working mechanism of the active substance of Jati Belanda leaves. In the ethanol extract of Jati Belanda leaves there are saponin compounds that react interfere with fungal cell membranes.[21] Flavonoid compounds can also damage cell membranes which can affect changes in cell permeability as well with saponins and tannins.[22]

At all concentrations of C.albican can not be inhibited with aquades extract of Jati Belanda leaves, the inhibition zone diameter is not formed, presumably the number of active compounds dissolved in aquadest is low so that it is difficult to penetrate the cell wall compared to ethanol extract which can dissolve more active substances in Jati Belanda leaves.[23] C. albican which has thick walls consisting of 6 layers with thin walls so that the active substance in the aquades extract of Jati Belanda leaves cannot penetrate C. Albicans.[24] Another cause of the absence of the inhibition zone is because the cell wall structure of C. Albicans consists of 80-90% carbohydrates, 6-25% protein, and 1-7% lipids.[25]

CONCLUSIONS

Based on the results of the research that has been carried out, the activity test of ethanol extract and aquadest of Jati Belanda leaves (Guazuma ulmifolia Lamk.) On the growth of C.albican can be concluded that the diameter of the inhibition zone of ethanol extracts on the growth of C.albican at a concentration of 25 mg, 50 mg, 75 mg, 100 mg,

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250 mg, 500 mg, 750 mg, and 1000 mg have an inhibition zone. While the aquadest extract did not get an inhibition zone.

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