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The Study of Antifungal Activity of Betadine (*Jatropha multifida*) Stem Sap Extract against *Candida albicans* Growth *In Vitro*

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ABSTRACT

Indonesia has many medicinal plants that can be used to treat various diseases, especially infectious diseases. Several infections are caused by a fungus (yeast) of Candida albicans. Candida albicans is a normal oral cavity flora, but it is also an opportunistic pathogen that causes diseases such as candidiasis. Betadine stem sap (Jatropha multifida) has been widely used in humans to heal wounds and treat fungal infections. This study evaluated the antifungal activity of betadine stem sap extract at concentrations of 25, 50, 75, and 100% with nystatin as positive control and DMSO as negative control. Betadine stem sap extract was obtained using the maceration method with DMSO as a solvent. This study is a true experimental study using a post-test-only control group design. The method used is Kirby-Bauer disk diffusion using potato dextrose agar as the medium. The data obtained were tested using one-way ANOVA and post hoc LSD test. As a result, the betadine stem sap extract at 100% concentration showed the highest mean diameter of the inhibition at 20.8 mm, and the lowest at 25% concentration was 17.5 mm. This study concluded that betadine stem sap extract showed antifungal activity in which 100% of the extract inhibited the growth of Candida albicans yeast the most compared to nystatin ($p < 0.05$).

Keywords: *Candida Albicans*, Candidiasis, *Jatropha Multifida*, Sap

ABSTRAK

Banyak tanaman obat di Indonesia yang dapat digunakan untuk mengobati berbagai macam penyakit, terutama yang disebabkan oleh infeksi. Beberapa infeksi tersebut dapat disebabkan oleh jamur *Candida albicans* yang merupakan flora normal rongga mulut dan merupakan patogen oportunistik yang dapat menyebabkan kandidiasis. Getah batang betadine (*Jatropha multifida*) banyak digunakan oleh masyarakat untuk menyembuhkan luka dan infeksi jamur. Penelitian ini merupakan penelitian *true experiment* dengan rancangan penelitian *post-test only control group*. Penelitian ini menggunakan jamur *Candida albicans* yang diberi ekstrak getah batang betadine pada konsentrasi 25, 50, 75 dan 100% dengan nistatin sebagai kontrol positif dan DMSO sebagai kontrol negatif. Ekstrak getah diperoleh dengan metode maserasi menggunakan pelarut DMSO. Metode uji antijamur yang digunakan adalah metode *Kirby-Bauer* menggunakan media *potato dextrose agar*. Analisis data dilakukan dengan uji ANOVA *one-way* dan *post hoc* LSD. Penelitian menunjukkan ekstrak getah batang betadine 100% memiliki rata-rata diameter zona hambat tertinggi yaitu 20,8 mm, sedangkan yang terendah sebesar 17,5 mm pada konsentrasi 25%. Ekstrak getah batang betadine memiliki aktivitas antijamur dengan konsentrasi 100% paling efektif dalam menghambat pertumbuhan *Candida albicans* dibandingkan dengan nistatin ($p<0,05$).

Kata kunci: *Candida Albicans*, Kandidiasis, *Jatropha Multifida*, Getah

1. Introduction

Indonesia is a country rich in medicinal plants and has been used by most of the Indonesian population for generations.[1] The number of medicinal plants known to the world is about 40000 species but only 1200 plant species are used as raw materials and medicinal herbs.[2] WHO in 2003 recommended the use of traditional medicine for public health in the prevention and treatment of diseases especially for chronic diseases, degenerative diseases and cancer.[3] In regard to Indonesia, a tropical country, infection is one of the main causes of diseases.[1] Infection is the invasion of the host by microorganisms that includes the reproduction of the invading organism and the hosts' response to the invading organism.[4] Infection can be caused by various microorganisms such as viruses, bacteria, rickets, protozoa, and fungi.[4] In the human body, *Candida sp* is a normal flora that can turn into opportunistic pathogens in the digestive tract, mucous membranes of the oral cavity, urogenital, and skin.[5,6,7]

Candida species pose a major threat to public health as they are the leading cause of morbidity and mortality worldwide. Diseases that can be caused by candida are vaginitis, oral candidiasis, cutaneous candidiasis, candidemia and systemic infections. Candidemia is a nosocomial infection that represents 15% of bloodstream infections, and *Candida* is responsible for 50 to 70% of systemic infections. *Candida albicans* is the pathogenic species most often isolated followed by *C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. famata, C. guilliermondii,* and *C. lusitaniae*.[7] The prevalence of oral thrush with the majority of *C. albicans* is 45% in infants, 45-65% in children, 30-45% in healthy adults, 50-65% in adults wear a long-term denture, 65-88% in those living in acute and long-term facilities, 90% of people with leukemia receive chemotherapy, and 95% of people living with HIV.[8] The incidence of oral candidiasis is associated with predisposing factors such as age, sex, smoking habits, use of oral antibiotics, and antirertoviral treatment. According to the research of Shiboski et al, the incidence of oral candidiasis increases at the age of more than 35 years.[9]

Antifungal agents for candidiasis treatment caused by *C. albicans* include fluorinated pyrimidine cytosine (5-FC) which targetting RNA synthesis and DNA replication, polyene which can affect cell membrane integrity, azole with target of biosynthetic pathway ergosterol, and echinocandin which can affect biosynthesis cell wall.[10] Use of antifungal agents increases with the increasing number of *C. albicans* infections. This raises of certain clinical consequences such as widespread usage of azole causing isolates that are resistant to azole. Cases of treatment failure with fluconazole due to the development of *C. albicans* resistance have been found since 1990. In subsequent studies, *C. albicans* resistance was not only found in fluconazole therapy, but also in other antifungals.[10] As a result of increased antimicrobial resistance and antifungal, several studies have been developed to identify and evaluate efficient alternative therapies.[11]

Jatropha sp, typically *Jatropha multifida*, well-known as betadine *plant*, has antibacterial, anti-inflammatory and antioxidant properties.[12,13] Each part of this plant has benefits such as its own leaves can be used to treat scabies. The leaves and stem bark of this plant can be used as a remedy for neurodermatitis, itchy skin, and skin eczema. The plant sap is used to treat sores and wounds, and the stems are used in Nigeria as chew sticks for dental care.[14] Falodun et al (2014) found that betadine stem sap contains metabolites in the form of monocyclic lathyrane diterpenoids, multifidone and multifidinol with the highest antifungal activity observed in multifidone and multifidinol.[15] However Chairani et al mentioned that betadine stem sap does not have an antiseptic effect on *Candida albicans* with 20%, 40%, 60%, 80%, and 100% concentrations.[12] Based on the above description, the authors want to conduct scientific experiment on the Antifungal effect of betadine (*Jatropha multifida*) stem sap extract against the growth of *Candida albicans*.

2. Materials and Methods

This study is a true experimental study that uses a post-test only control group design. This study was approved by research ethics committee of Faculty of Medicine, Universitas Sumatera Utara/H. Adam Malik General Hospital Medan with ethical certificate No.42/TGL/KEPKFKUSU-RSUPHAM/2020. The preparation and dilution of betadine stem extract was carried out at the Laboratory of Traditional Medicine, Faculty of Pharmacy, University of Sumatera Utara. Identification, breeding and testing of samples were carried out at the Microbiology Laboratory of the Faculty of Pharmacy, University of Sumatera Utara. The research sample to be used was the fungus *Candida albicans* ATCC 10231. The betadine plant was obtained from Gg. Madirsan, Tj. Morawa, Deli Serdang Regency, North Sumatra.

The number of *Candida albicans* samples was 24 divided into 6 groups (n=4): positive control (nystatin), negative control (DMSO), and experimental groups: 25%, 50%, 75%, and 100% concentration of betadine stem sep extract. The tools used in this study are gloves, masks, autoclave, UV-VIS spectrophotometer, glass cuvettes, refrigerator, incubator, vortex mixer, analytical scales, aromatic glass, mortar and pestle, glass petri plates, shelves with test tubes, inoculating loop/ose, bunsen lamp, 10 ml measuring cup, erlenmeyer, spatula, tweezer, sliding caliper, Laminar Air Flow, oven, and rotary evaporator. The research materials used were concentrated preparations of betadine stem sap extract, culture culture of *Candida albicans*, DMSO solution, nystatin, distilled water, nutrient broth powder, label paper, sterile paper discs, aluminum foil, markers, sterile cotton swabs, sterile cotton, and tissue.

The extract was made using maceration method. Betadine sticks are sliced with a knife and the sap that comes out is collected in a sterile container. The sap is soaked with DMSO and stirred for the first 6 hours then let the sap still for 18 hours while occasionally stirring. The sap is filtered using cotton and filter paper until macerate is obtained. The macerate was evaporated with a rotary evaporator at 40^{o}C to obtain a thick extract which would be diluted with DMSO to obtain 25%, 50%, 75%, and 100% concentrations.

Antifungal activity test was carried out using the diffusion method with Kirby-Bauer disk. Blank discs soaked in extract, nystatin, and DMSO are placed on potato dextrose agar filled with *Candida albicans*. After that, the petri dishes are put into an incubator to be incubated at 37˚C and observed after 24-48 hours. The inhibition zone formed around the paper disk is measured by a sliding caliper. Statistical analyzes were performed and analyzed using the one-way ANOVA and post hoc LSD tests. One-way ANOVA test was performed to distinguish the antifungal activity of each concentration of betadine stem sap extract against *Candida albicans*. A post hoc LSD test was performed to determine if there was a significant difference between Betadine stem sap extract concentration groups.

3. Results

The inhibition zone area of each group was evaluated by calculating the average clear zone diameter of each repetition that was shown in Table 1. The study showed significant differences in each treatment group. Table 2 showed 100% concentration has the greatest inhibitory effect on the growth of *Cadida albicans* (20.8 ± 0.096) mm while 25% concentration has the smallest inhibition (17.5 ± 0.330) mm. The 25% extract concentration also has a greater inhibition compared to nystatin (10.850 ± 0.772) mm.

Based on the ANOVA test results (Table 2), it was shown that p value was 0,000 meant that there was a significant difference in *C. albicans* growth inhibition activity among 6 treatments. Post hoc LSD results (Table 3) showed significant differences between groups of 25% with 50%, 75%, 100%; between 50% with 75% and 100%, and 75% with 100% ($p < 0.05$).

Table 1. Results of measurement of inhibitory zone diameters of betadine stem sap extracts against *Candida albicans*.

Isolation	Groups	Inhibitory Zone Diameter (mm)			
		1	2	3	4
Candida albicans	25%	17.5	17.9	17.1	17.4
	50%	18.3	18.5	18.2	17.9
	75%	18.5	19.7	186	19.7
	100%	20.9	20.7	20.8	20.7
	Control +	10.5	10.1	11.9	10.9
	Control -	0			

Table 2. Results of the one-way ANOVA test and the average measurement of the clear zone diameters of each group against *Candida albicans*.

Groups	n	Mean ± SD Inhibitory Zone	*P-Value* (ANOVA)
25%	4	17.475 ± 0.330	p = 0.000
50%	4	18.225 ± 0.250	
75%	4	19.125 ± 0.665	
100%	4	20.775 ± 0.096	
Control +	4	10.850 ± 0.772	
Control -	4	0.000 ± 0.000	

Table 3. Results of the post hoc LSD test among concentration groups towards *Candida albicans*.

Concentration	25%	50%	75%	100%	Control +	Control -
25%	-					
50%	0.030	-				
75%	0.000	0.011	-			
100%	0.000	0.000	0.000	-		
Control +	0.000	0.000	0.000	0.000	-	
Control -	0.000	0.000	0.000	0.000	0.000	-

4. Discussion

The extract of betadine stem sap has antifungal activity related to secondary metabolite compounds. The study of Chairani and Harfiani on phytochemical content of betadine stem sap showed the presence of alkaloid, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides.[12] According to Freiesleben and Jager research, alkaloids can inhibit nucleic acids synthesis and affect ergosterol on *C. albicans*. Saponins can inhibit nucleic acid synthesis by lysing microbial cell membranes and inhibiting DNA polymerase. Flavonoids can form a combination with proteins that affect the integrity of cell membranes and cell walls, resulting in disruption cell metabolism by inhibiting nutrient transport.[16,17]

According to Sulistyawati and Mulyani, phenol has antifungal properties as it can bind to cell membrane proteins resulting in cell membranes disruption allowing phenol to enter the cell nucleus.[18] Phenolic compounds through hydroxy groups can bind to sulfhydryl groups in fungal proteins and can change the shape of cell membrane. The position and number of hydroxyl groups in phenols are related to its toxicity to microorganisms, and the more phenol is oxidized, the higher its inhibitory activity. Tannins as antifungals will react to cell walls and penetrate cell membranes because they can damage proteins. The antifungal properties of tannins can be related to the hydrolysis of ester bonds of gallic acid, which affect the biosynthetic process in the connection between cell walls and cell membranes. Changes in cell membrane permeability can lead to a decrease in cell volume.[17] Other compounds that can be found in betadine stem sap are labaditin, multifidenol, multifidol, multifidol glucoside. These compounds are considered to have antifungal properties but the mechanism of action has not fully known clinically yet.[19]

The resulted inhibition zone diameter found in this research was categorized as strong to very strong activity, based on Davis and Stout inhibition zone sensitivity criteria. It can be seen that 25-75% concentration have diameters of 17.1-19.7 mm which is included in the category of strong antifungal (10-20 mm) and 100% concentration has ± 20 mm diameter which is included in the category of very strong antifungal (>20 mm). The large increase in inhibition zone diameter in agar media is due to the concentration of sap increasing from 25% to 100%. The concentration of the compound affects the rate of diffusion, where the higher the betadine stem sap concentration, the faster the diffusion occurs, consequently the greater the antifungal power and the wider the inhibitory zone diameter produced.[12]

Dimethyl sulfoxide (DMSO, ((CH3)2SO)) can dissolve polar and nonpolar compounds and pass-through hydrophobic barriers such as plasma membranes. This property is important for carrying pharmocological compounds that work intracellularly. Due to its high solubility, DMSO is widely used as a solvent for many drugs and is often used as a solvent for in vitro and in vivo studies. Additionally, DMSO is considered as a less toxic solvent.[20]

This study results are in good agreement with the study by Aransiola which showed that betadine stem sap has antifungal activity against *candida sp* with broth dilution method and potato dextrose agar as media with concentration of 263 mg/ml, 525 mg/ml, and 1050 mg/ml. [14] The study results also agree with Adesola and Adetunji's research who examined the effects of betadine stem sap in treating oral candidiasis (thrush) in children under 1 year of age. The results showed that betadine stem sap had antifungal effects in the first 24 hours after application whereas nystatin 100,000 IU showed an effect in 48 hours after the first dose, so it showed betadine stem sap was more effective in treating oral candidiasis compared to nystatin 100,000 IU.[21] However, these results contradicted the study by Chairani and Harfiani, which showed that betadine stem sap has no antifungal effect against *Candida albicans in vitro*. However, Chairani and Harfiani used the Kirby-Bauer method with Sabaroud dextrose media which was then incubated at 37oC.[12] Meanwhile, it was known that the optimum growth temperature for *Candida albicans* is 37oC in an aerobic atmosphere.[22]

According to the research results, it can be concluded that betadine stem sap extract has antifungal properties against the growth of *Candida albicans* in vitro at all concentrations compared to nystatin. This study can be used as a basis for further research to determine the therapeutic dose in a form of topical dosage to find the most effective antifungal concentration of betadine stem sap extract with minimum toxicity, as research show that concentrations of 25-75% alone have shown strong antifungal activity.

5. Conclusion

Based on the research results, Betadine stem sap extract was effective against candida albicans at all concentrations with better inhibition zone results than nystatin.

6. Acknowledgements

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7. Conflict of Interest

There is no conflict of interest.

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