

**Antifungal Activity of Ethanol Extract from Sungkai Leaves (*Peronema canescens* Jack.)
Against *Candida albicans*: An in Vitro Study**

**Efek Antijamur Ekstrak Etanol Daun Sungkai (*Peronema canescens* Jack) Terhadap
Pertumbuhan *Candida Albicans*: Studi In Vitro**

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ABSTRACT

Oral candidiasis is a disease that affects the tongue and oral mucosa and is caused by the growth of the fungus *Candida albicans*. Secondary metabolites found in ethanol extracts of Sungkai leaves (*Peronema canescens* Jack.) have been shown to inhibit fungal growth. The purpose of study was to test the antifungal activity of an ethanol extract of Sungkai leaves on *C. albicans* growth in vitro. The study used the disc diffusion research design and a post-test-only control group design. *C. albicans* was cultured on Sabouraud Dextrose Agar (SDA) medium and treated with ethanol extracts of Sungkai leaves at concentration of 0.02, 0.04, 0.08, 0.16, and 0.32 g/mL. Ketoconazole at 0.02g/mL was used as a positive control, and 10% dimethyl sulfoxide (DMSO) used as a negative control. The process is repeated five times. The results showed that the ethanol extract of Sungkai leaves did not have antifungal activity against the growth of *C. albicans* which was indicated by the absence of an inhibition zone around the disc paper on the SDA media used. The conclusion was the ethanol extract of Sungkai leaves did not show antifungal activity against the growth of *C. albicans* fungus.

Keywords: Oral candidiasis, Antifungal, *Peronema canescens* Jack., *Candida albicans*.

ABSTRAK:

Kandidiasis oral merupakan salah satu penyakit yang menginfeksi lidah dan daerah mukosa rongga mulut yang umumnya ditandai dengan pertumbuhan jamur *Candida albicans*. Ekstrak etanol daun Sungkai (*Peronema canescens* Jack.) diketahui mengandung zat kimia yang disebut metabolit sekunder yang dapat menghentikan pertumbuhan jamur. Tujuan penelitian ini untuk menetapkan khasiat anti-jamur ekstrak etanol daun Sungkai terhadap pertumbuhan jamur *C. albicans* secara *in vitro*. Penelitian menggunakan metode *disc diffusion* dengan desain penelitian *post-test-only control group design*. Jamur *C. albicans* ditumbuhkan pada media *Sabouraud Dextrose Agar* (SDA) dengan diberikan perlakuan menggunakan ekstrak etanol daun sungkai dengan konsentrasi 0.02, 0.04, 0.08, 0.16, 0.32 g/mL. Ketokonazol 0.02g/mL sebagai kontrol positif dan Dimetil Sulfoksida (DMSO) 10% sebagai kontrol negatif Pengulangan dilakukan sebanyak 5 kali. Hasil penelitian menunjukkan bahwa ekstrak etanol daun Sungkai tidak memiliki aktivitas antijamur terhadap pertumbuhan *C. albicans* yang ditandai dengan tidak terbentuknya zona hambat di sekitar kertas cakram pada media SDA yang digunakan. Kesimpulannya adalah ekstrak etanol daun Sungkai tidak menunjukkan aktivitas anti-jamur terhadap pertumbuhan jamur *C. albicans*.

Kata Kunci: Kandidiasis oral, Antijamur, *Peronema canescens* Jack., *Candida albicans*.

INTRODUCTION

Fungal infections in the oral cavity are primarily caused by *Candida spp*, which are common commensals but can become opportunistic pathogens.¹ These infections can manifest as superficial, skin, or mucosal infections, particularly in immunocompromised individuals or those with underlying health conditions.¹ Fungal infections in the oral cavity,² commonly referred to as oral candidiasis are primarily caused by *Candida spp*.

Oral candidiasis is an infection that impacts the oral cavity particularly the oral mucosa, and is induced by the proliferation of the fungus *C. albicans*.³ *Candida albicans* is an opportunistic microbe typically found as a component of the natural flora in the oral cavity. However, under certain conditions, such as in individuals with weakened immune systems, *C. albicans* can multiply uncontrollably, causing a local infection called oral candidiasis.⁴ The characteristic symptoms of the infection include white plaques on the tongue, palate, and mucosa, which are sometimes accompanied by pain and inflammation.⁵

Oral candidiasis is generally treated with synthetic antifungal agents, such as ketoconazole, fluconazole, or nystatin.^{6,7} Ketoconazole, for example, is a broad-spectrum antifungal that inhibits the biosynthesis of ergosterol an essential component of the fungal cell membrane. Although effective long-term use of synthetic antifungals is often associated with problems such as fungal resistance and side effects.⁸ Therefore, there is an urgent need to find alternative antifungal agents from natural sources that are safer, more effective, and have minimal side effects.⁹

Kalimantan residents have traditionally used Sungkai leaves (*Peronema canescens Jack.*) as a natural cure for a variety of ailments.¹⁰ Sungkai leaves have been traditionally used for their immunomodulatory properties, particularly during the COVID-19 pandemic. The leaves contain high levels of vitamin C, polyphenols, and exhibit strong antioxidant activity especially in fresh shoot leaves.¹¹ This plant has been shown to contain bioactive compounds flavonoids, saponins, and tannins.^{12,13} The aim of this research was to see how effectively an ethanolic extract of Sungkai

leaves inhibited *C. albicans*. This method provides for a direct assessment of the leaf extract's antifungal potential without regard for human body variables.

This study is designed to provide scientific data supporting the use of Sungkai leaves as a safe and potentially effective alternative antifungal medication for treating oral candidiasis. The antifungal activity of the Sungkai leaf (*Peronema canescens Jack.*) has not been tested, prompting the researcher to be interested in initiating a study related to this medicinal plant native to Kalimantan as an alternative antifungal material.

RESEARCH METHODS

This study used a laboratory method utilizing the post-test-only control group technique to determine the effect of an ethanolic extract of Sungkai leaves (*Peronema canescens Jack.*) on the growth of *C. albicans* after treatment.

This study involved the preparation of essential materials and equipment, including the *C. albicans* strain ATCC® 10231 as the test organism Sungkai leaf extract as the test substance along with 96% ethanol, Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB), ketoconazole as the positive control, and 10% Dimethyl Sulfoxide (DMSO) as the negative control. This study has obtained ethical approval from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Mulawarman University, with the approval number NO. 143/KEPK-FK/IX/2022.

The research process began with the preparation of simplistic and Sungkai leaf extract. Fresh dark green Sungkai leaves were washed clean under running water and then dried at room temperature to preserve their active compounds¹⁴ (Figure 1). The desiccated leaves were pulverized into a powder utilizing a laboratory mixer. Next, the maceration process was used for extraction. Two liters of 96% ethanol were used to soak 120 grams of simplistic powder for three days at room temperature, with occasional stirring. Afterward, the Whatman No. 42 filter paper filtered the resulting maceration solution. Then, the resultant filtrate was evaporated using a rotary evaporator at 50°C until a viscous extract was achieved.^{15,16} (Figure 2). The extract was subsequently subjected to

drying in an oven at 60°C aiming to diminish the water content to below 10%.¹²

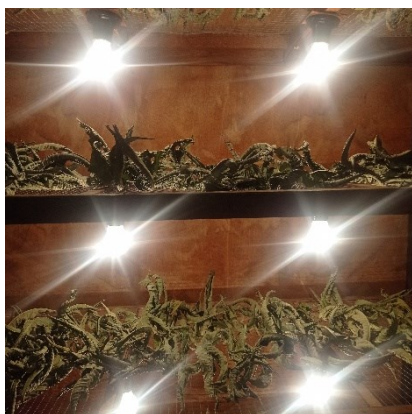


Figure 1. Dried leaves of Sungkai (*Peronema canescens* Jack.)



Figure 2. Evaporated filtrate of Sungkai leaves (*Peronema canescens* Jack.) using a rotary evaporator

After the extraction process, the thick Sungkai leaf extract was diluted using 10% DMSO to obtain various test concentrations, namely 0.02, 0.04, 0.08, 0.16, and 0.32 g/mL. The antifungal activity test was conducted using the disc diffusion method. The first step was preparing the agar medium, sixty-five grams of SDA powder were dissolved in one liter of distilled water and heated until fully dissolved. The medium underwent sterilization via autoclaving at 121°C for a duration of 15 minutes. Following sterilization, the medium, was cooled to a temperature range of 45-50°C, transferred into sterile Petri dishes and allowed to solidify.¹⁷

The *C. albicans* acquired from cryopreserved stock was revitalized by culturing on SDA slant media, followed by incubation at 35°C for, 24-hours. Following rejuvenation, a fungal suspension was prepared by combining the *C. albicans* culture with SDB media until the suspension achieved an absorbance of 0.39, as measured by a spectrophotometer, corresponding to a concentration of 1.25×10^7 CFU/mL. Discs measuring 6 mm in diameter were subsequently immersed in Sungkai leaf extract at different concentrations, alongside the positive control (ketoconazole 0.02g/mL) and negative control (DMSO 10%). The saturated discs were positioned on SDA media that had been inoculated with a *C. albicans* suspension via a sterile swab.^{18,19}

After placing the discs on the media, The Petri plates were incubated at 35°C for a duration of 24 to 48 hours. Following the incubation time, the diameter of the inhibitory zone surrounding the disc was measured with millimeter (mm) calipers. The data obtained from five repetitions for each concentration were analyzed descriptively to evaluate whether Sungkai leaves showed antifungal activity against the growth of *C. albicans*.¹⁸

RESULT

Based on the research using the disc diffusion method the ethanolic extract of Sungkai leaves (*Peronema canescens* Jack.) at various concentrations (0.02 to 0.32 g/mL) did not show any zone of inhibition around the test disc against the growth of *Candida albicans*. This indicates that the extract did not exhibit an apparent antifungal activity against *C. albicans* at the measured concentrations.

Conversely, the positive control using ketoconazole at a concentration of 0.02 g/mL successfully formed a clear zone of inhibition, demonstrating its effectiveness as an antifungal against *C. albicans*. On the other hand, the negative control using 10% Dimethyl Sulfoxide (DMSO) did not produce a zone of inhibition, indicating that DMSO itself does not have antifungal activity.

Table 1. Results of the antifungal activity test, measured by the zone of inhibition diameter

Concentration g/mL	Zone of Inhibition (mm)					Mean
	R1	R2	R3	R4	R5	
0.02 g/mL	0	0	0	0	0	0
0.04 g/mL	0	0	0	0	0	0
0.08 g/mL	0	0	0	0	0	0
0.16 g/mL	0	0	0	0	0	0
0.32 g/mL	0	0	0	0	0	0
C+	31.61	25.72	24.08	27.83	25.15	26.87
C-	0	0	0	0	0	0

Notes: R, Replication; C, Control

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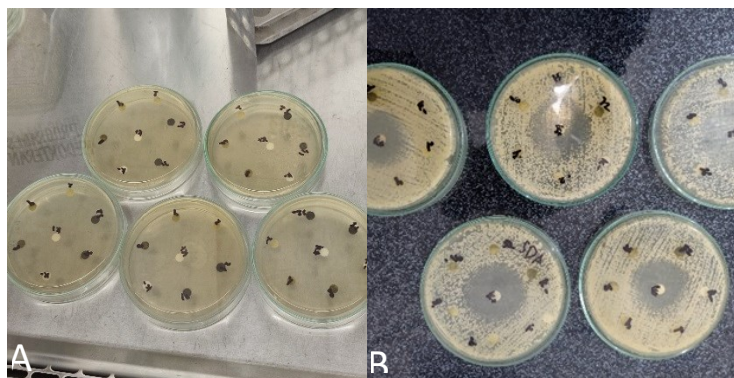


Figure 3. Results of the Sungkai leaf extract (*Peronema canescens* Jack.) assay on Sabouraud Dextrose Agar (SDA) media. Figure (A) Before incubation; (B) After incubation

DISCUSSION

The research findings indicate that the ethanolic extract of Sungkai leaves was not effective in inhibiting the growth of *C. albicans* in vitro even though Sungkai leaves are known to contain bioactive compounds such as flavonoids, saponins, tannins, and alkaloids, which theoretically have antimicrobial potential.^{15,20-22} This study's absence of a zone of inhibition could be due to several factors. One possible cause is that the concentration of active compounds in the extract may be too low to achieve a significant antifungal effect. Previous research has shown that flavonoid and tannin compounds can disrupt the permeability of fungal cell membranes and inhibit important enzymes involved in fungal growth.^{23,24} However, a specific concentration is required to achieve effective activity, as reported by Ibrahim and Kuncoro (2012), who stated that the concentration of active compounds significantly affects antimicrobial activity.

The stability of the active compounds during the extraction process may contribute to the results obtained. The maceration method using ethanol solvent can affect the integrity of

bioactive compounds, especially compounds that are sensitive to high temperatures or improper storage. Flavonoids, for example, can degrade during this process, losing their potential as antifungal agents.^{14,25,26}

Another factor to consider is the specific nature of *C. albicans* as an opportunistic pathogen that has strong defense mechanisms, including the ability to form biofilms.²⁷ Biofilms can protect *C. albicans* from the effects of antifungal compounds, thereby reducing the effectiveness of natural extracts. Tenover's research (2015) mentioned that biofilms can increase resistance to various antimicrobial agents, including natural compounds. Resistance can also occur if the concentration of the antifungal compound used is insufficient to inhibit fungal growth effectively.²⁸

Despite this, the bioactive compounds saponins and tannins in Sungkai leaves, for example, still have antifungal properties. A study by Swandiyasa et al. (2019) explained that saponins can damage fungal cell membranes, causing leakage of intracellular components and ultimately triggering cell death. Tannins can also inhibit enzymes

important for fungal metabolism. However, this activity highly depends on the adequate concentration and extraction method. If the concentration of active compounds in the Sungkai leaf extract is insufficient, the expected antifungal effect may not be observed.²³

In comparison, the positive control with ketoconazole at a concentration of 0.02 g/mL demonstrated a significant zone of inhibition. Ketoconazole works by inhibiting the enzyme lanosterol 14 α -demethylase, which leads to cell death. The effectiveness of ketoconazole as an antifungal has been well documented, although its use can cause side effects such as resistance and allergic reactions in some individuals.²⁸

The results of this investigation show that the ethanolic extract of Sungkai leaves at the investigated concentrations cannot be employed as an effective antifungal agent against *C. albicans*. To further explore the antifungal potential of Sungkai leaves, additional research is needed using different extraction methods, increased concentrations, or combinations with other compounds that may enhance its biological activity. In addition, the isolation and purification of specific active compounds from Sungkai leaves can help understand and maximize its pharmacological potential.

CONCLUSION

The findings indicated that the ethanol extract of Sungkai leaves, at concentrations between 0.02 g/mL and 0.32 g/mL, failed to demonstrate any antifungal activity, as evidenced by the lack of a zone of inhibition surrounding the test disc. This indicates that the bioactive compounds present in the extract, including flavonoids, tannins, and saponins, did not demonstrate adequate efficacy in suppressing the-growth of *Candida albicans* within the examined conditions and concentrations.

SUGGESTION

In light of these findings, it is imperative that additional research be conducted to investigate the antimicrobial properties of Sungkai leaves. This could involve augmenting the concentration of the extracts, employing more efficient extraction techniques, or isolating and purifying

particular bioactive compounds. Furthermore, it is advisable to evaluate the efficacy of the extract against various microorganisms or to perform combination assays with synthetic antifungals to investigate the potential for synergistic interactions. Additional investigations may encompass in vivo assessments and the formulation of topical applications derived from Sungkai leaf extract to ascertain its viability as a potential alternative therapeutic approach.

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