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ANTIFUNGAL POTENTIAL OF *GARCINIA PARVIFOLIA* (MIQ.) MIQ. STEM BARK EXTRACT

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Abstract

The use of natural plant products that are effective against many pathogenic fungi in humans is one way to avoid fungal infections and reduce the impact of resistance caused by synthetic drugs. The plant *Garcinia parvifolia* (Miq.) Miq. is empirically utilized by the local community as a medicinal plant. One of them is by the Mului community, who use it as a remedy for athlete's foot caused by Candida albicans. This research aims to measure the antifungal activity of the Stem Bark Extract of *G. parvifolia* (Miq.) Miq. The extraction was carried out using three different solvents: Methanol, Ethyl Acetate, and n-hexane. The three types of extracts were then tested for their ability to inhibit the growth of *C. albicans*. The antifungal activity test results of the three G. parvifolia (Miq.) Miq. extracts showed that *G. parvifolia* (Miq.) Miq. can potentially be developed into a medicinal ingredient for treating *C. albicans* infections. It was found that extraction with ethyl acetate solvent has a powerful ability to inhibit the growth of *C. albicans* at four different concentrations. Therefore, using ethyl acetate solvent in the extraction process can be an appropriate method for extracting *G. parvifolia* (Miq.) Miq.

Keywords: *Candida albicans, Ekstrak Kulit Batang Garcinia Parvifolia (miq.) miq.*

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INTRODUCTION

The plant *Garcinia parvifolia* (Miq.) Miq. (G. parvilfolia (Miq.) Miq.) is empirically utilized by local communities as a medicinal plant. For instance, the local Lampung tribe in West Lampung Regency uses this plant to remedy postnatal recovery (Sovia Santi Leksikowati et al., 2020). The Dayak Iban community in Sungai Mawang Village, West Kalimantan, utilizes this plant as a remedy for jaundice in infants (Pradityo et al., n.d.), the Mului community in Paser Regency, East Kalimantan, use this plant to treat athlete's foot, combined with the plant Malotus lackeyi Elmer in its application (Salmitha, 2019) and the Ngaju Dayak community in Central Kalimantan use it as a remedy for back pain (Setyowati et al., 2005).

In the research on the potential of medicinal plants and traditional medicine of the Mului community, it was found that the plant *G. parvilfolia* (Miq.) Miq. and the plant Malotus lackeys Elmer holds high importance for the Mului community, as they are used to treat athlete's feet (Salmitha, 2019). One of the causes of athlete's foot is *Candida albicans*. (C. allbicans). *C. albicans* is an organism classified in the group of unicellular fungi; this yeast-shaped fungus can form budding cells to reproduce. Budding cells form when environmental conditions are less supportive. *C. albicans* is a normal flora in the human body, for example, on the surface of the skin, in the oral cavity, and the vagina, but *C. albicans* can become a parasite when the body's immune condition weakens. This microorganism is often found in skin folds such as the armpits, under the breasts, groin, buttocks, and between the toes and fingers. The infected skin appears red, somewhat moist, with fine scales, and has a clear boundary (Qanit & Nusadewiarti, 2023). *C. albicans* is the organism responsible for candidiasis.

Candidiasis is a disease with a high prevalence in tropical regions, especially Indonesia. Based on the research conducted by Amanda and Cut Mirsela on fish vendors in traditional markets in Medan City, it was found that 13.3% of the vendors experienced *C. albicans* infections. The research by Apriliana Puspita Sari et al. evaluated the picture and characteristics of candidiasis in patients with diabetes mellitus at RSUD Dr. Soetomo Surabaya, with 50.5% experiencing intertriginous candidiasis (Puspitasari et al., 2019). The research conducted at RS Abdul Wahab Syahranie, Indonesia, found that 22.22% of patients experienced oral candidiasis (Yunitasari, Yunita, & Toruan, 2018). Research conducted on fish vendors at Segiri Market in Samarinda found that 30% of the vendors experienced candidiasis between their finger webs(Suhartini et al., 2024) .

Candidiasis can be treated using synthetic drugs such as ketoconazole, fluconazole, Azoles, Polyenes, and allylamines(Fatril et al., 2020). Fluconazole is recommended as the primary treatment for C. albicans infections due to its high effectiveness, low toxicity, and affordability. However, the emergence of Candida strains resistant to fluconazole, especially in diabetic foot ulcers, has raised significant concern in public health (Moslemi et al., 2023). In the treatment of fungal infections, resistance to fluconazole is significant (Mayr et al., 2020; Rybak et al., 2020) In *C. albicans* that exhibit resistance to fluconazole, the mechanism involves increased expression of proteins encoded by the CDR1, CDR2, and MDR1 genes. (Keereedach et al., 2020)

To overcome resistance to fluconazole, traditional medicines can be used by utilizing local plants that contain active compounds with antifungal or antimicrobial properties. Active compounds that have antimicrobial and antifungal properties usually include alkaloids, flavonoids, tannins, saponins, and phenols. The active compounds contained in *G. parvifolia* (Miq.) Miq are alkaloids, flavonoids, steroids, tannins, and saponins (Salmitha, 2019).The active compound content of both types of plants supports their potential as a remedy for candidiasis caused by *C. albicans.* To determine the effectiveness of the active compounds contained in *G. parvifolia* (Miq.) Miq against the growth of *C. albicans*, research is needed on the Effectiveness of *Garcinia parvifolia* (Miq.) Miq. Stem Bark Extract against the Inhibition of *Candida albicans* Growth.

METHODS

1. Sterilization of Equipment

The equipment and materials to be sterilized include Petri dishes, Muller Hilton Agar media, and Sabouraud Dextrose Agar media. The petri dishes are wrapped in wrapping paper. The tools and materials were sterilized using an autoclave at a pressure of 2 Atm and a temperature of 121°C for 20 minutes.

2. Sample preparation

The stem bark of *G. parvifolia* (Miq.) . was thoroughly washed, then sliced thinly and air-dried for 1 week. The clean, dried stem bark sample of Garcinia parvifolia (Miq.) Miq., weighing 3000 grams, was ground using a Herb Grinder (Maksindo MKS-ML 2500) until it became powder. Subsequently, the powder of G. parvifolia (Miq.) Miq. The stem bark was sieved using a 65 mesh sieve.

3. Extraction of the Sample

They extract the stem bark of *G. parvifolia* (Miq.) miq using the maceration method. Soaking 3000 g of *Garcinia parvifolia* (Miq.) stem bark powder in 9000 ml of 90% ethanol at a temperature of 25-30°C. The soaking process is carried out for 3x24 hours, stirring every 1x24 hours. Filter the solution with a cloth to obtain the macerate. Collect the results of the maceration obtained from the filtration process. The evaporation process on the macerate was performed using a rotary evaporator at a temperature of 40°C at 56 rpm until all the ethanol evaporated, resulting in 600 g of concentrated ethanol extract. The subsequent macerate, each 200 g, is evaporated with a rotary evaporator (Buchy) using three different organic solutions: Methanol, Hexane, and Ethyl Acetate, each 2 L, until the fractions obtained are Methanol (14.36 g), Hexane (4.36 g), and Ethyl Acetate. (1,60 gr).

4. Media Preparation

Preparing the growth medium for C. albicans by dissolving SDA (Saboured Dextrose Agar) using Aquadest. Dissolve 40 grams of dextrose, 15 grams of agar, and 10 grams of water peptone in 1 L of aqua dest, then add 1% Amoxil. Next, sterilization

Preparation of Test Fungal Pure Culture.

Inoculate the pure culture of *C. albicans* with one loop on SDA media in a petri dish by aseptically streaking, then incubate for 1X24 hours at 37°C in an incubator. The pure culture is streaked on SDA media. (Saboured Dextrose Agar). Paper disks soaked with active compounds are placed at concentrations of 20%, 40%, 60%, 80%, and 100%. It is planting a positive control of 2% ketoconazole and a negative control that has not been treated. It was incubating the *C. albicans* culture for 1 X 24 hours. Measure the inhibition zone (clear zone) that forms on the media.

5. Preparation of Extract Sample Solution

The extract of *Garcinia parvifolia* (Miq.) stem bark with concentrations of 20%, 40%, 60%, 80%, and 100% (g/ml) with 5 repetitions. Prepare the test concentrates by weighing each extract: 0.20 grams, 0.40 grams, 0.60 grams, 0.80 grams, and 0.100 grams using an analytical balance. Dissolve each test concentrate using 1 ml of sterile aquades. Prepare the positive control using 2% ketoconazole, made by weighing 0.02 grams of ketoconazole dissolved in 1 ml of sterile aquades. Prepare the negative control using 1 ml of sterile aquades without extract and testing each experimental unit according to the work procedure (Pulungan, 2017).

6. Microbiological Testing

They are determining the antifungal activity of *C. albicans* using the Kirby-Bauer disc diffusion method. Pour the SDA medium into 12 Petri dishes with a volume of 20 ml each and let it solidify. Make a smear of *C. albicans* on the surface of the SDA medium with a sterile swab until evenly distributed. Soaking sterile paper disks in *Garcinia parvifolia* (Miq.) miq. Stem bark extract with various concentrations for 15 minutes. Placing the paper disks on the agar plates using tweezers at a distance of 2 cm apart on the petri dishes and incubating for 1x24 hours at 37ºC. The inhibition zone formed around the paper disk indicates the inhibitory activity of the *Garcinia parvifolia* (Miq.) Miq. Stem Bark extract. The inhibition zones formed were then measured using a micrometer.

7. Data Analysis

To analyze the effectiveness of *G. parvifolia* (Miq.) Miq. Stem Bark extract against the growth inhibition of *C. albicans* fungus, the measurement results of the inhibition zones were compared to the inhibition zone response criteria. The fungal growth inhibition response was classified based on the diameter of the clear zone as follows:

< 10 mm = weak
10-15 mm = moderate
16-20 mm = strong
> 20 mm = very strong (Alioes, Kartika, Zain, & Azzura, 2019)

RESULTS AND DISCUSSION

Candida albicans Growth Inhibition Test

Inhibition test results of 3 extracts, namely methanol, ethyl acetate, and N-hexane from G. parvifolia (miq.) miq extract. The growth of *C. albicans* can be seen in the following table.

Table of Inhibitory Zone Test Results of methanol extract, ethyl acetate, and N-hexane against *C. albicans*

Extract	Concentration	Mean Inhibitory Zone (mm))	Inhibitory Zone Category
Methanol	20%	0	Weak
	40%	18,2	Strong
	60%	19,4	Strong
	80%	23	Very Strong
Ethyl acetate	20%	26,4	Very Strong
	40%	26,6	Very Strong
	60%	23,4	Very Strong
	80%	21	Very Strong
N Hexane	20%	8,8	Weak
	40%	9,8	Weak
	60%	20,8	Very Strong
	80%	27	Very Strong

Methanol Extract

Based on the data above on the methanol extract, the growth inhibition power of *C.albicans* in the five repetitions carried out showed varying abilities at each concentration of the methanol extract. At a concentration of 20% methanol extract, there was no growth inhibition zone for C. albicans. At a concentration of 40% methanol extract, an average growth inhibition zone of C. albicans was formed of 18.2 mm (strong). At a concentration of 60% methanol extract, an average growth inhibition zone of *C. albicans* was formed of 19.4 mm (strong) and at a concentration of 80% methanol extract, an average *C. albicans* growth inhibition zone was formed of 23 mm (very strong).

Ethyl Acetate Extract

In the inhibitory power test of ethyl acetate extract on the growth of *C. albicans*, the ability to inhibit the growth of *C. albicans* at 4 different concentrations, namely 20%, 40%, 60% and 80% had the same ability. At a concentration of 20% ethyl acetate extract, an average growth inhibition zone of *C. albicans* was formed of 26.4 mm (very strong). At a concentration of 40% methanol extract, an average growth inhibition zone of *C. albicans* was formed of 26.6 mm (very strong). At a concentration of 60% methanol extract, an average growth inhibition zone of *C. albicans* was formed of 23.4 mm (very strong) and at a concentration of 80% methanol extract, an average *C. albicans* growth inhibition zone was formed of 21 mm (very strong).

N-Hexane Extract

In the test of the inhibitory power of hexane extract on the growth of *C.albicans* at four different concentrations, namely 20%, 40%, 60%, and 80%, it had other abilities. At a concentration of 20%, an average growth inhibition zone of *C. albicans* was formed of 8.8 mm (weak). At a 40% methanol extract concentration, an average growth inhibition zone of *C. albicans* was composed of 9.8 mm (weak). At a concentration of 60% methanol extract, an average growth inhibition zone of C. albicans was composed of 20.8 mm (very strong), and at a concentration of 80% methanol extract, an average *C. albicans* growth inhibition zone was formed of 27 mm (very strong).).

In other research related to the growth inhibitory activity of *C.albicans*, Ethanol Extract from Curcuma longa formed an inhibitory zone of 19 mm at a concentration of 0.75mg/mL, Ethanol Extract from Alpinia galanga formed an inhibitory zone of 25 mm at a concentration In of 0.75mg/mL, Extract Ethanol from Zingiber Officinale var rubrum forms an inhibition zone of 15 mm at a concentration of 0.75mg/mL, Ethanol Extract from Zingiber Officinale var officinarum forms an inhibition zone of 13 mm at a concentration of 0.75mg/mL, Ethanol Extract from Zingiber Officinale var amarum forms a zone inhibition of 12 mm at a concentration of 0.75mg/mL (Muhammad Evy Prastiyanto et al., 2021). Kabau fruit peel extract (*Archidendron bubalinum*) forms an inhibitory zone of 4 mm at a concentration of 80% (Fitria Ningrum et al., 2021). Kabau fruit peel extract (*Archidendron bubalinum*) forms an inhibitory zone of 15.8 mm at a concentration of 50% (Rahmawati et al., 2022). When compared with the inhibitory activity of *C.albicans*. at a concentration of 60% or 60mg/mL, the inhibitory power of methanol extract, ethyl acetate extract, and N-hexane extract from *G. parvifolia* (miq.)miq extract had a larger zone of inhibition.

Results of the third antifungal activity test Extract from *G. parvifolia* (miq.)miq extract. showed that *G. parvifolia* (miq.)miq. Has the potential to be developed into a medicinal ingredient, namely to treat *C.albicans* infections. From the results of the antifungal activity test of the three extracts, namely methanol, ethyl acetate, and N-hexane, it was found that the ethyl acetate extract had a powerful ability to inhibit the growth of *C.albicans* at four different

concentrations. So, the use of ethyl acetate solvent in the extraction process can be an appropriate method for extracting *G. parvifolia* (miq.) miq.

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