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Antifungal Effectiveness of Snakehead Fish (Channa striata) Meat Extract Against Candida albicans

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ABSTRACT

Background: Candida albicans is normal flora in the human oral cavity, but in the presence of predisposing factors Candida albicans can become a pathogen. Side effects of antifungal treatment can cause drug resistance, so alternative therapies are needed. Snakehead fish (Channa striata) is a freshwater species native to Indonesia and is a popular food fish in Asia. Its meat is reported to contain amino acids and fatty acid compounds that possess antifungal activity.

Objectives: The aim of this study was to determine the antifungal activity of various concentrations of Snakehead Fish (Channa striata) meat extract against Candida albicans.

Methods: This study was an in vitro experimental laboratory study ultilizing a post-test only control group study design. The test group consisted of Snakehead Fish (Channa striata) meat extract at concentrations of 25%, 50%, 75%, and 100% obtained using the maceration method. The control group consisted of a positive control (Nystatin) and a negative control (aquadest). The antifungal activity test of Snakehead Fish (Channa striata) meat extract was dilution method for the minimum inhibitory concentration (MIC) test, solid dilution for the minimum bactericidal concentration (MBC) test, and the disc diffusion method for the inhibition zone test.

Results: The results of this study showed that Snakehead Fish (Channa striata) meat extract has antifungal against Candida albicans with both the MIC and MBC at a concentration of 100%. The largest average inhibition zone was observed at a concentration of 100% (16.10 mm).

Conclusion: Snakehead Fish (Channa striata) meat extract has antifungal potency against Candida albicans.

***Keywords*:** Antifungal, Candida albicans, Snakehead fish meat extract.

INTRODUCTION

Candida albicans is part of the normal flora in the human oral cavity. Based on prevalence data in the United Kingdom, Candida albicans is found in the oral cavity of 45% of neonates, 45-65% of children, 30-45% of adults, and 74% of the elderly. In general, this fungus lives as a commensal organism, but in the presence of predisposing factors, Candida albicans can become pathogenic. Predisposing factors that may support the growth of Candida albicans include long-term antibiotic use, impaired immune function, uncontrolled diabetes, denture use, xerostomia, iron deficiency, and phosphate deficiency.

Oral candidiasis is a fungal infection of the oral cavity caused by Candida albicans.

Candidiasis can be classified based on its clinical presentation into acute pseudomembranous candidiasis, chronic atrophic candidiasis, acute atrophic candidiasis, chronic hyperplastic candidiasis, and angular cheilitis. Oral candidiasis lesions caused by Candida albicans can be found in parts of the oral cavity such as the oral mucosa, oropharynx, and tongue. The treatment of oral candidiasis is based on the type of candidiasis and the severity of the infection.

A common treatment for Candida albicans infection is Nystatin. Nystatin is the first-line drug for the topical treatment of oral candidiasis. Nystatin inhibits fungal growth through interaction with ergosterol in the fungal cell membrane, causing a selective reduction in membrane permeability and fungal cell death. Antifungal treatment may lead to drug resistance in Candida albicans. Therefore, traditional medicines are an alternative option that can be used in the development of new antifungal drugs. One alternative ingredient suspected to have antifungal effects is snakehead fish.

Snakehead fish, or Channa striata, also known as striped snakehead, is a freshwater fish native to Indonesia and is a popular food fish in Asia. In Indonesia, especially in Sumatra, Kalimantan, and Java, snakehead fish has many benefits for the community, both economically and in the health sector. Snakehead fish inhabit river estuaries, lakes, and swamps, and can even live in low-oxygen waters. The nutritional content of snakehead fish is not much different from that of other freshwater fish, seving as a source of protein beneficial to the body, with protein digestibility reaching more than 90%. Snakehead fish (Channa striata) is also a potential food source that can be utilized as an antioxidant source because of its high nutritional content.

The nutritional content in 100 grams of snakehead fish includes protein (76.9%), fat (1.70%), water (13.61%), and carbohydrates (3.53%). Several studies have shown that snakehead fish has a much higher protein level compared to other freshwater fish. The high protein content, especially albumin, essential amino acids, and essential fatty acids found in snakehead fish meat, is very beneficial for health. In the health sector, snakehead fish is referred to as a medicinal freshwater fish and is used to accelerate wound healing. According to [1], snakehead fish oil emulgel at concentration F3, namely 15%, had faster burn wound healing ability with a healing percentage of 100% on day 15. Snakehead fish also has highly beneficial biomedical properties such as anti-inflammatory, antinociceptive, and antimicrobial effects, including significant mild antibacterial and antifungal activity.

The amino acids contained in snakehead fish meat include leucine, lysine, alanine, histidine, valine, arginine, threonine, and other amino acids. [2] reported that the antifungal

activity of the amino acids D-lysine and D-alanine was also evaluated against Candida albicans. Both amino acids showed moderate antifungal activity with MIC values of 39 and 18 μg μL^{-1}, respectively. The combination of these two amino acids with amphotericin B reduced the MIC by up to six-fold. Amino acids can exert antifungal effects by causing enzyme damage in fungal cells. Meanwhile, the fatty acid content in snakehead fish meat can damage membrane integrity, thereby increasing membrane fluidity, which causes uncontrolled intracellular protein leakage and cell death.

According to [3], collagen extract from snakehead fish (Channa striata) at a maximum concentration of 100 μL produced a broad antifungal spectrum and showed greater resistance against Candida albicans. This result is supported by [4], who showed that antifungal activity from snakehead fish meat extract with 95% ethanol produced higher results compared to the control group. A similar study by [5] using snakehead fish (Channa striata) fillet extract with two solvents, namely distilled water and 50% ethanol (distilled water:ethanol = 1:1), showed that distilled water extraction produced more high-molecular-weight protein than 50% ethanol extraction. This study aimed to determine the antifungal effect of snakehead fish (Channa striata) meat extract against Candida albicansin vitro.

METHODS

This research was an in vitro laboratory experimental study with a post-test only control group design to determine the antifungal effect of snakehead fish meat extract against *Candida albicans*. The study was conducted from March 15 to April 30, 2024, at the Chemical Engineering Laboratory of Politeknik Negeri Sriwijaya Palembang and the Microbiology Laboratory of the Faculty of Medicine, Universitas Muhammadiyah, Palembang, South Sumatra.

The research object was snakehead fish meat extract. The control and treatment samples were divided into 6 groups: snakehead fish meat extract concentrations of 25%, 50%, 75%, and 100%, a positive control with Nystatin 100,000 IU, and a negative control with sterile distilled water. Each treatment was repeated 4 times, for a total of 24 samples: 16 snakehead fish meat extract samples, 4 positive control samples, and 4 negative control samples. The inclusion criteria were snakehead fish aged 6-8 months (harvest age) with a weight of 100-500 grams per fish.

Sample Preparation

Snakehead fish meat was obtained from the Fish Cultivation Group (POKDAKAN) of the South Sumatra Provincial Marine and Fisheries Office. The snakehead fish used in this

study were 6-8 months old (harvest age), weighed 100-500 grams per fish, and were alive. The fish meat was separated from the skin, bones, and digestive organs to obtain the meat. The meat was then cleaned with running tap water, weighed, cut into small pieces, and finely ground using a blender.

Preparation of Snakehead Fish Meat Extract

A total of 500 grams of prepared snakehead fish meat was steamed with water at a ratio of 1:1 at 75°C for 20 minutes. Extraction was performed using the maceration method, with 96% ethanol added until the meat was submerged and stirred until evenly mixed with the ethanol solvent. It was then soaked for 72 hours at room temperature (25°C). The mixture was filtered using Whatman No. 40 filter paper to obtain the liquid filtrate. The filtrate was then evaporated to obtain a thick extract free from solvent and with a constant weight.

Preparation of Extract Concentration Variatons

Each final extract concentration was prepared to a total volume of 10 mL by mixing the concentrated extract with distilled water and 5% DMSO. The snakehead fish meat extract solution was prepared in a final volume of 10 mL using 5% DMSO in distilled water as the solvent. The extract concentrations used were 25%, 50%, 75%, and 100%. The 25% concentration was made by mixing 2.5 mL of extract with 7.5 mL of solvent, the 50% concentration by mixing 5 mL of extract with 5 mL of solvent, and the 75% concentration by mixing 7.5 mL of extract with 2.5 mL of solvent. The 100% concentration used 10 mL of pure extract without dilution.

Preparations of Growth Media

Sabouraud Dextrose Agar (SDA) medium was prepared by dissolving 6.5 g of SDA powder in 100 mL of distilled water, then heating until completely dissolved. The medium was covered with aluminum foil, sterilized using an autoclave at 121°C for 15 minutes, poured into sterile petri dishes, and allowed to cool and solidify. Meanwhile, Sabouraud Dextrose Broth (SDB) medium was prepared by dissolving 3 g of SDB powder in 100 mL of distilled water, heating until dissolved, and sterilizing in an autoclave at 121°C for 15 minutes before use.

A *Candida albicans* suspension was prepared by inoculating a fungal culture onto the SDA medium and incubating it at room temperature for 24 hours. The fungal colonies that grew were then taken using an inoculating loop and suspended in a test tube containing 0.9% NaCl solution. The fungal suspension was homogenized and its turbidity adjusted to the 0.5 McFarland standard, equivalent to 1–5 × 10^6 CFU/mL. If the turbidity exceeded the standard,

the suspension was diluted. Conversely, if the turbidity was below the standard, *Candida albicans* culture was added until the desired turbidity was reached.

Preparations of *Candida albicans* Suspension

A loopful of *Candida albicans* culture was inoculated into 1 mL of SDA medium and incubated at 37°C for 24 hours. A loopful of *Candida albicans* culture was then inoculated aseptically into SDB medium, homogenized, and incubated for 24 hours at 37°C.

Antifungal Activity Test of Snakehead Fish Meat Extract Minimum Inhibitory Concentration (MIC) Test

The minimum inhibitory concentration (MIC) was determined using SDB medium with the liquid dilution method. Six sterile tubes were prepared and labeled 1-6. Tubes 1-4 contained snakehead fish meat extract at concentrations of 25%, 50%, 75%, and 100%, respectively, while tubes 5 and 6 served as the positive control (nystatin) and negative control (distilled water). Each tube was added with 5 mL of SDB liquid medium. Tube 1 was then added with 10 mL of 25% snakehead fish meat extract and 100 µL of Candida albicans suspension. Tubes 2, 3, and 4 were each added with 10 mL of snakehead fish meat extract at concentrations of 50%, 75%, and 100%, respectively, and 100 µL of Candida albicans suspension. Tube 5 was added with 1 mL of nystatin and 100 µL of Candida albicans suspension, while tube 6 was added with 1 mL of distilled water and 100 µL of Candida albicans suspension.

Each treatment was repeated four times and incubated at 37°C for 24 hours. The MIC value was determined based on the observation of turbidity or clarity of the medium. The lowest concentration that showed no fungal growth, marked by a clear medium, was established as the MIC.

Minimum Fungicidal Concentration (MFC) Test

The MFC was determined by re-culturing the MIC test results on SDA medium. One loopful of suspension was taken and incubated for 24 hours at 37°C. Observation was then made for the presence or absence of fungal colony growth on SDA medium after incubation. The lowest concentration that showed no fungal growth visually on the SDA medium was established as the MFC.

Inhibition Zone Test of Snakehead Fish Meat Extract

The inhibition zone test was performed using the disc diffusion (Kirby-Bauer) method. Liquid SDA medium was poured into petri dishes (9 mL) and allowed to solidify. Then, 200 µL of *Candida albicans* inoculum suspension was added and spread evenly on the surface of the SDA medium. Filter paper discs with a diameter of 6 mm were soaked in 10 mL of

snakehead fish meat extract for 30 minutes, then placed on the medium surface using forceps. The same procedure was performed for the positive control (nystatin) and negative control (distilled water). Each treatment was repeated four times. The SDA medium was then incubated for 48 hours at 37°C, and fungal growth was observed by measuring the clear zone around the discs. A caliper in millimeters (mm) was used to measure the diameter of the inhibition zone, and each measurement was carried out four times. The average inhibition zone diameter was categorized according to the inhibitory strength classification of Davis and Stout: very strong (>=20 mm), strong (11-20 mm), moderate (5-10 mm), and weak (<=5 mm).

Data analysis in this study was conducted from inhibition zone measurements using a normality test with Shapiro-Wilk and a homogeneity test with Levene's Test. After meeting the requirements, a one-way ANOVA test was performed to determine whether there was an effect of various concentrations of snakehead fish (*Channa striata*) meat extract on *Candida albicans*. This was followed by a post-hoc Tukey test to identify significant differences between concentrations of snakehead fish meat extract and the control groups, with a p-value of < 0.05 considered statistically significant. If the data were not normally distributed and not homogeneous ($p<0.05$), a non-parametric Kruskal-Wallis test would be used.

RESULTS

Results of the Minimum Inhibitory Concentration (MIC) Test

The presence of white clumps in the test solutions was observed to determine turbidity. The results showed that the negative control (distilled water) and snakehead fish meat extract concentrations of 25%, 50%, and 75% still showed *Candida albicans* growth, indicated by turbidity similar to the fungal suspension or the presence of white clumps in the solution. The 100% snakehead fish meat extract and the positive control showed clarity; therefore, it was concluded that the MIC of the snakehead fish meat extract was at a concentration of 100%. The MIC test results using the liquid dilution method in test tubes showed that the snakehead fish meat extract had an antifungal effect in inhibiting *Candida albicans*.

Visual observation of clarity or the absence of white clumps in the tube was not sufficient to determine the MIC result because the extract color became darker as the concentration increased, especially at concentrations of 75% and 100%. Therefore, a follow-up MFC test was performed by subculturing on Sabouraud Dextrose Agar (SDA) medium.

Results of the Minimum Fungicidal Concentration (MFC) Test

The test results showed that only 100% snakehead fish meat extract and the positive control (nystatin) were able to kill *Candida albicans*, as indicated by the absence of fungal growth. *Candida albicans* growth was still found in the 25%, 50%, and 75% concentration groups, as well as the negative control (distilled water) group. Based on these results, the MFC of snakehead fish meat extract was established at a concentration of 100%; thus, it can be concluded that snakehead fish meat extract has antifungal activity against *Candida albicans*.

Results of the Inhibition Zone Test

The inhibition zones formed at concentrations of 25% and 50% were included in the moderate category. At concentrations of 75% and 100%, they were included in the strong category, but not stronger than the positive control group (Table 1).

Table 1. Inhibiton zones formed in each group

Treatment	Repeat				Mean Zone (mm)	Inhibition Diameter
	1	2	3	4		
Snakehead fish meat extract 25%	8.8	8,5	8.2	8.2		8.425
Snakehead fish meat extract 50%	10.2	10	9.8	10.2		10.050
Snakehead fish meat extract 75%	13.2	12.9	13.2	13		13.075
Snakehead fish meat extract 100%	16.25	15.9	16	16.25		16.100
Nystatin	20.1	19.9	20.1	20		20.025
Distilled Water	0	0	0	0		0

The results of the inhibition zone normality test showed that the data were normally distributed and homogeneous with significance value of > 0.05; thus, the statistical one-way ANOVA test was conducted (Table 2). The results showed that the treatment groups (snakehead fish meat extract at concentrations of 25%, 50%, 75%, and 100%, along with the positive and negative controls) had a significance value of 0.000 ($p < 0.05$); therefore, it was concluded that there was a statistically significant difference in the mean inhibition zone values among the treatment groups.

Table 2. Results of One Way ANOVA test for inhibition zones

	F	Sig.
Between Groups	7506.00	0.000*

The data were then analyzed using a post-hoc Tukey test to determine significant

differences among the groups. The results of the post-hoc Tukey test (Table 3) showed that the test groups of snakehead fish meat extract concentrations, positive control, and negative control against *Candida albicans* had significance values of less than 0.05 ($p < 0.05$). Based on the post-hoc Tukey test results, it can be concluded that there were significant differences among the test concentration groups of snakehead fish meat extract, the positive control, and the negative control.

Table 3. Results of Post Hoc Tukey test of snakehead fish meat extract inhibition zones as an antifungal against *Candida albicans*

Treatment Groups	25%	50%	75%	100%	Nystatin	Distilled Water
25%		0.000*	0.000*	0.000*	0.000*	0.000*
50%			0.000*	0.000*	0.000*	0.000*
75%				0.000*	0.000*	0.000*
100%					0.000*	0.000*
Nystatin						0.000*
Distilled Water						

DISCUSSIONS

The results showed that snakehead fish (*Channa striata*) meat extract has an antifungal effect against *Candida albicans*. The tests performed were the MIC test using liquid dilution, the MFC test using solid dilution, and the inhibition zone test using disc diffusion. The use of ethanol as a solvent effectively dissolves various organic compounds, such as proteins, amino acids, and fatty acids, in fish. In addition, ethanol provides a stronger effect because its properties allow it to attract both polar and non-polar compounds, making it a good choice for extracting various components from snakehead fish and other biological materials. This is in line with the study by [5], which showed that snakehead fish fillet extract using ethanol was able to extract lower-molecular-weight proteins better than distilled water.

The MIC test results showed that the 100% snakehead fish meat extract concentration was the lowest concentration able to inhibit the growth of *Candida albicans*. The MIC result in this study was obtained through visual observation, a method that has the weakness of the solution becoming darker as the extract concentration increases, resulting in a less accurate and more subjective assessment. Therefore, to obtain a more accurate MIC result, further testing should be performed by determining absorbance values as a marker of turbidity using a UV-Vis spectrophotometer. According to [6], testing with a UV-Vis spectrophotometer

provides higher precision and accuracy in determining MIC values. MIC values are obtained by comparing absorbance values before and after 24 hours of incubation, where the absorbance value before incubation is greater than after incubation.

The MFC test results showed that the 100% snakehead fish meat extract was the minimum concentration able to kill *Candida albicans*, as no colony growth was found on Sabouraud Dextrose Agar (SDA) medium. In comparison, concentrations of 75%, 50%, and 25% still showed fungal colonies because they did not kill at least 99.9% of the colonies. The minimum fungicidal concentration (MFC) of an antifungal compound itself may be equal to or greater than the MIC value. This is in line with the study conducted by Afrina et al. (2017), which stated that the higher the extract concentration, the greater the fungicidal activity against fungi.

The inhibition zone results in this study showed that all concentrations of snakehead fish meat extract had the ability to inhibit the growth of *Candida albicans*, as seen from the presence of a clear zone around the paper disc. The strength of inhibition zones according to Davis and Stout is categorized based on diameter as weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm). The inhibition zones formed in this study began at the 25% concentration and increased to the largest at the 100% concentration. This study proves that the higher the concentration of snakehead fish meat extract, the larger the inhibition zone formed. The antifungal content of the snakehead fish meat extract also increased as concentration increased, making it more effective in inhibiting fungal growth. These results are in line with [7], who reported that the amino acid threonine can inhibit fungal growth by inhibiting the enzyme homoserine dehydrogenase, which catalyzes organic reactions including those in *Candida albicans*. This is also in line with the study by [3], where collagen extract from snakehead fish (*Channa striata*) at a maximum concentration of 100 µL showed greater resistance against *Candida albicans*.

The test groups of snakehead fish meat extract at concentrations of 25%, 50%, 75%, and 100% had lower mean inhibition zone values than the positive control Nystatin. The difference in mean values between the positive control group and the snakehead fish meat extract was statistically significant. This study shows that snakehead fish meat extract has an antifungal effect capable of inhibiting and killing *Candida albicans*, but it has not yet matched the antifungal effect of Nystatin. This may be because nystatin inhibits fungal growth by entering the cell membrane and forming sterol-binding channels that serve as pathways for cytoplasmic components, especially potassium ions, to leak out. This process causes selective

changes in cell membrane permeability and eventually leads to the death of *Candida albicans* cells.

The antifungal effect of snakehead fish meat extract in inhibiting the growth of *Candida albicans* comes from its amino acid and fatty acid content. The amino acid content in snakehead fish, around 16.03%, can exert an antifungal effect by causing enzyme damage in fungal cells, thereby producing a fungistatic effect. D-lysine and D-alanine have been reported to have considerable antifungal activity against *Candida*, differing from the amino acids tested. Essential amino acids such as isoleucine and valine may influence the virulence of *Candida albicans*. This is in line with [2], who reported that both amino acids, D-lysine and D-alanine, showed moderate antifungal activity with MIC values of 39 and 18 ug μg μL^{-1}, respectively.

The fatty acid content in snakehead fish meat is divided into two groups: saturated fatty acids at around 22.54% and unsaturated fatty acids at around 21.72%. Fatty acids can be used as antifungal agents capable of inhibiting fungal growth through their mechanism of action on fungal metabolic pathways and cell membranes. Fatty acids can disrupt cell membranes, especially cells with low sterol content, thereby increasing membrane fluidity, causing the leakage of intracellular components and fungal cell death. Unsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid have inhibitory effects on mitochondrial metabolism and biofilm formation in *Candida albicans*. This is also in line with the study by [8], which showed that the saturated fatty acid lauric acid was effective in inhibiting *Candida albicans* growth with more than 75% biofilm inhibition.

Based on this study that has been conducted, snakehead fish (*Channa striata*) meat extract has the potential to be further developed as an agent against *Candida albicans*. Therefore, it can be further developed through additional research regarding snakehead fish meat extract as an agent against *Candida albicans*. However, attention needs to be paid to formulation processing and concentration distribution, as these factors will affect the results of the test preparation.

CONCLUSION

The results of this study showed that snakehead fish (*Channa striata*) meat extract has an antifungal effect, enabling it to inhibit and kill *Candida albicans*, with both the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) at 100%.

The mean inhibition zones of snakehead fish (*Channa striata*) meat extract against *Candida albicans* were as follows: the 25% concentration (8.425 mm) and the 50%

concentration (10.050 mm) showed moderate inhibition against *Candida albicans*. Meanwhile, the 75% concentration (13.075 mm) and the 100% concentration (16.100 mm) showed strong inhibition against *Candida albicans*. The greater the concentration of snakehead fish (*Channa striata*) meat extract, the larger the diameter of the inhibition zone against *Candida albicans*, although its effectiveness was still not as potent as nystatin (20.025 mm) used as the positive control.

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