

# Anticandidal Effects of Ethanolic Extract of Lada Katokkon (*Capsicum annuum chinense*) Against Emerging Fungal Pathogens

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## Abstract

*Candidiasis* is an opportunistic mycotic infection with a high prevalence, caused by *Candida* species, especially *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. The increasing resistance of *Candida* spp. to conventional antifungals such as fluconazole poses a challenge in the management of *candidiasis*, thus the need for natural-based alternatives, such as Lada Katokkon. This condition encourages the need to explore alternative therapeutic agents based on natural ingredients. Lada Katokkon (*Capsicum annuum chinense* Jacq), a local chili variety cultivated in the Toraja region, is known to contain secondary metabolite compounds such as *capsaicin*, flavonoids, saponins, and alkaloids that have potential as antifungals. This study aims to evaluate the antifungal activity of the ethanol extract of Lada Katokkon against the growth of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* in vitro using the disc diffusion method. The extract was tested at five concentrations: 1%, 3%, 5%, 7%, and 9%. There was a positive relationship between extract concentration and inhibition zone diameter. The highest activity was recorded at 9% concentration, particularly against *C. parapsilosis* ( $16.89 \pm 4.74$  mm), followed by *C. albicans* ( $9.03 \pm 5.96$  mm) and *C. tropicalis* ( $8.07 \pm 0.81$  mm). In contrast, 1% concentration produced the lowest zone of inhibition in all species tested. These results suggest that the ethanol extract of Lada Katokkon has potential as an alternative natural antifungal agent in candidiasis therapy.

**Keywords:** Antifungal *Candidiasis*; *Capsicum annuum chinense* Jacq; Lada Katokkon.

**Abbreviations:** *C. albicans*: *Candida albicans*, *C. tropicalis*: *Candida tropicalis*, *C. parapsilosis*: *Candida parapsilosis*, SHU: Scoville Heat Unit.

## INTRODUCTION

*Candidiasis* is an opportunistic fungal infection caused by *Candida* species, with *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* as the most common pathogens (Murugesh et al., 2019). This infection can affect various organ systems, especially in individuals with low immunity such as HIV/AIDS patients, cancer patients undergoing chemotherapy, organ transplant recipients, and individuals taking immunosuppressive therapy (Bayas et al., 2024). According to the World Health Organization (WHO), *candidiasis* affects approximately 10% to 15% of women globally, and remains a significant problem in neonatal and geriatric groups due to high morbidity and mortality rates. Meanwhile, the Centers for Disease Control and Prevention (CDC) estimates that there are approximately 400,000 cases of invasive candidiasis each year worldwide (Arya and Rafiq, 2023).

Among the various *Candida* species, *C. albicans* is known to exhibit overexpression of efflux pump genes

(CDR1, MDR1), point mutations in ergosterol biosynthesis genes (ERG11, ERG3, ERG6), as well as mutations in glucan wall building genes, which contribute to its pathogenicity in the oral mucosa and genital area (Sahetapy et al., 2022). Meanwhile, *C. tropicalis* is associated with deeply invasive infections and is often isolated from patients with diabetes mellitus and those undergoing immunosuppressive treatment (Talapko et al., 2021). *C. parapsilosis*, although rarer, is increasingly reported in nosocomial cases and has been associated with cardiac pump catheter infections (Sahetapy et al., 2022; Silva et al., 2017). A major concern today is the increasing resistance of *Candida* species to conventional antifungal agents, particularly the azole class such as fluconazole. Mechanisms of resistance include overexpression of efflux pump genes (e.g., CDR1, MDR1), point mutations in ergosterol biosynthesis genes (ERG11, ERG3, ERG6), and mutations in glucan synthase complex genes (FKS1, FKS2) (Sahetapy et al., 2022).

This increase in resistance emphasizes the need for alternative therapeutic strategies, including the exploration of natural bioactive compounds that have potential as antifungal agents. Some traditional medicinal phytochemicals have attracted attention due to their side effects and relatively low availability. Some phytochemicals, such as flavonoids, alkaloids, saponins, tannins, and terpenoids, have shown antimicrobial effects through various mechanisms, including disruption of microbial cell membranes, inhibition of biofilm formation, and interference with cell division (Behbehani et al., 2023; Hassain et al., 2008; Reyes Escogido et al., 2011).

Lada Katokkon is a typical chili pepper variety from the highlands of Toraja, South Sulawesi, Indonesia (Al Amanah et al., 2022). Although known to be rich in active compounds such as capsaicin and flavonoids, scientific studies on its antifungal potential, particularly against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, are still very limited. Lada Katokkon contains flavonoids, carotenoids, vitamin C, and saponins. *Capsaicin* has been shown to have antimicrobial, analgesic, and anti-inflammatory properties that can kill microbes, while flavonoids such as quercetin and kaempferol have shown antifungal activity, including inhibition of biofilm formation of *Candida* spp. (Lubis et al. 2022; Damayanti and Utami, 2024; Omolo, 2014; Fattori et al., 2016; Al Aboody, 2020; Azis, 2023; Susilawati et al., 2023; Hersila et al., 2023).

Previous studies have shown the antifungal potential of chili extracts against *Candida albicans*, including a study using green chili extract (*Capsicum annuum*) that showed an inhibition zone of up to 11.2 mm (Lubis et al. 2022). Another report from Brazil showed antifungal

activity of Katokkon extract at 2% concentration. However, research on Katokkon chili as an antifungal agent especially against *C. albicans*, *C. tropicalis*, and *C. parapsilosis* is still limited in Indonesia (Behbehani et al., 2023).

Based on this background, this study aims to evaluate the antifungal activity of the ethanol extract of Katokkon pepper (*Capsicum annuum chinense* Jacq) against the growth of *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* in vitro.

## MATERIALS AND METHODS

### Study Area

This study was conducted in May 2025, with different stages conducted in several locations. Laboratory activities, including media preparation, isolate rejuvenation, and inhibition tests, were carried out at the Microbiology Laboratory, Faculty of Medicine, Pattimura University, Ambon, Indonesia. Sampling of simplisia was conducted at the Rantepao Morning Market, North Toraja Regency, South Sulawesi. Plant identification was carried out at UPT Laboratorium Herbal Materia Medica, Batu City, East Java, while pathogen confirmation tests were carried out at the Maluku Provincial Health Laboratory Center.

### Materials

#### Plant Material

Fresh katokkon pepper symplisia was obtained from the highland area of North Toraja, South Sulawesi, Indonesia.

**Table 1.** Data etnobotani Lada Katokkon.

Etnobotani	Types of Plants
Family	: <i>Solanaceae</i>
Species	: <i>Capsicum annuum chinense</i> Jacq
Variety	: <i>Capsicum</i>
Common Name	: Cabai Katokkon
Regional Name	: Lada Katokkon
Part of plant used	: Fruit Flesh
Weight of Fresh Katokkon Pepper	: 3000 g
Weight of Dried Katokkon Pepper	: 200 g
Weight of Simplia	: 160 g
Extract Yield	: 77,6 g
Extract yield (%)	: 32,62
Shape	: Viscous Extract
Color	: Dark Red
Characteristic Odor	: Typical LadaKatokkon (Spicy Aroma)

#### Microorganisms

The test isolates consisted of *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 750, and *Candida parapsilosis* ATCC 22019. Isolates were obtained from

the collection of the Surabaya Public Health Laboratory Center. Species confirmation was carried out using the VITEK 2 Compact system at the Maluku Provincial Health Laboratory Center.

### Reagents and Media

- Sabouraud Dextrose Agar (SDA)
- Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5  $\mu$ g/mL methylene blue
- Ethanol 70% (technical grade)
- Fluconazole (positive control)
- Sterile distilled water (negative control)

### Extraction Procedure

The chili fruit was washed, thinly sliced, and then dried using an oven at 50°C for seven days.<sup>7</sup> After drying, the material was ground into powder, then macerated in 70% ethanol in a ratio of 1:10 for 72 hours at room temperature. The filtrate from maceration was filtered and evaporated using a rotary evaporator at 40°C to obtain a thick extract. The extract was stored at 4°C until use (Ananta and Anjasmara, 2022).



Figure 1. A: Fruit Lada Katokkon B: Simplicia Lada Katokkon.

The ethanol extract of Katokkon pepper was dissolved in sterile distilled water to produce five concentration variations: 1%, 3%, 5%, 7%, and 9%.



Figure 2. Ethanol extract of pepper cathocon in several concentrations

### Rejuvenation procedure

Rejuvenation was performed by planting colonies of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* from storage media to sterile SDA media using the zig-zag technique. Incubation was carried out at 35-37°C for 24-48 hours.

The entire procedure was performed aseptically to prevent contamination (Sophia and Adinegoro, 2023).

### VITEK 2 Compact Confirmation Test

Species confirmation was performed with the VITEK 2 Compact system using a pure suspension of each isolate. This system analyzes biochemical patterns with special reaction cards automatically to generate specific identification and confidence levels of each isolate (Ligozzi et al, 2002; (Ling and Chen, 2003)

### Phytochemical Screening

Phytochemical analysis of the extract was carried out to identify the presence of secondary metabolites, where this test was carried out to determine whether there were levels of *capsaicin* and if so what quantity. The method used was thin layer chromatography densitometry (KLT-Densitometry). The general procedure includes the preparation of standard solutions of *capsaicin* and test samples, application on GF254 silica gel plates, development using a mobile phase (usually a combination of organic solvents such as chloroform ethanol or toluene acetate), and detection at a specific wavelength (usually 280-290 nm) using a densitometer. After development, the plate is dried and analyzed with a densitometer to measure the intensity of the compound spot based on the absorbance of UV light. The data obtained is the optical density of each spot, which is then converted to *capsaicin* concentration based on a calibration curve. This method has been widely used in the determination of *capsaicin* levels in chili extracts and their derivative products because the validation of the KLT-Densitometry method has been proven to meet analytical parameters such as linearity, precision, accuracy, and good detection limits. Its advantages include low cost, short analysis time, and it does not require complicated sample preparation as in HPLC (Gantait, 2012).

### Antifungal Assay

Antifungal activity test was performed using the disc diffusion method (Lubis et al. 2022). *Candida* spp. suspension was adjusted to 0.5 McFarland turbidity standard. The suspension was spread evenly onto the MHA-glucose-methylene blue surface (Luz et al., 2021). Sterile discs of 6 mm diameter were soaked with 20  $\mu$ L of each extract concentration, then placed on the agar. Fluconazole (25  $\mu$ g) was used as a positive control, while distilled water was a negative control. Incubation was carried out at 37°C for 24-48 hours, and the inhibition zone was measured using a digital caliper (Manghadam et al., 2023).

### Data Analysis

Data were presented as mean  $\pm$  standard deviation. Analysis was performed descriptively to evaluate the relationship between extract concentration and the diameter of the inhibition zone formed.

## RESULTS AND DISCUSSION

### Results

#### Phytochemical Screening

The results of phytochemical screening of the ethanol extract of Lada Katokkon showed the presence of the bioactive compound, *capsaicin*. This compound is

known to have antimicrobial activity that has the potential to inhibit the growth of pathogenic fungi.

The KLT-densitometry test showed that the capsaicin content in the chili powder samples reached an average of 9,128.55 mg/kg or equivalent to 0.91% dry weight. This value indicates that the Lada Katokkon has a fairly high capsaicin content, which contributes to its potential biological activity.

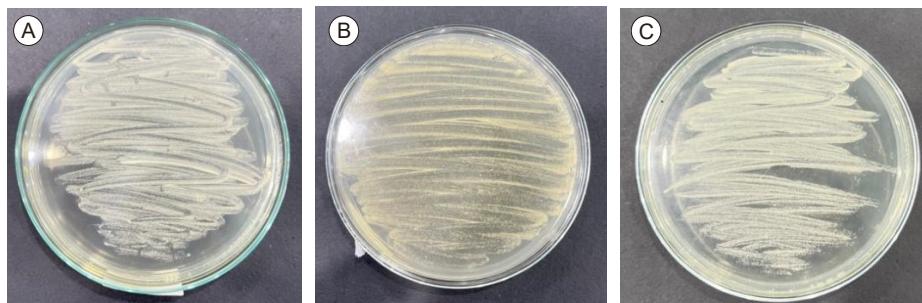
**Table 2.** Capsaicin Level Measurement Results on Lada Katokkon (*Capsicum annuum chinense* Jacq).

Sample Name	Weight sample	Volume spotting	Sample final add	Amount of spotting in sample	Capsaicin in sample Tool results	Capsaicin content in sample	Average	
	(g)	(µl)	(mL)	(µl)	(ng)	mg/Kg	mg/Kg	% (b/b)
Bubuk Cabai	0,1021	1	1	102,1	982,91	9.626,93	9.128,55	0,91
Lada Katokkon	0,108	1	1	101,8	878,55	8.630,16		

#### Rejuvenation of *Candida* Isolates

The rejuvenation process of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* on SDA media showed success in

obtaining fresh and active cultures. All isolates grew well and were ready to be used for antifungal activity tests.



**Figure 3.** Rejuvenation A: *C. albicans*, B: *C. tropicalis* and C: *C. parapsilosis*.

#### VITEK 2 Compact Confirmation Test

Confirmation tests using the VITEK 2 Compact system showed that all test isolates were successfully identified as *Candida* species with a high level of confidence. *C. albicans* and *C. tropicalis* showed a probability level above 95% with an "Excellent" level, while *C. parapsilosis* showed a level of 86% and was rated "Acceptable".

#### Antifungal activity

The ethanol extract of Lada Katokkon showed antifungal activity against all three *Candida* species in a concentration-dependent manner. The higher the concentration of the extract, the larger the diameter of the inhibition zone formed.

**Table 3.** Confirmation Test Results of Test Pathogenic Isolates

Fungal Isolate	Probability (%)	Confidence Level
<i>C. albicans</i>	96	Excellent
<i>C. tropicalis</i>	99	Excellent
<i>C. parapsilosis</i>	86	Acceptable

**Table 4.** Mean Inhibition Zone Diameter (mm) of Katokkon Pepper Extract Against *Candida* spp.

Concentration (%)	<i>C. albicans</i> (mean + SD)	<i>C. tropicalis</i> (mean + SD)	<i>C. parapsilosis</i> (mean + SD)
1%	4.36 ± 0.62	3.48 ± 0.43	4.82 ± 0.88
3%	6.18 ± 0.44	5.21 ± 0.58	8.22 ± 1.14
5%	7.94 ± 0.61	6.47 ± 0.35	12.11 ± 2.38
7%	8.67 ± 1.06	7.32 ± 0.67	14.89 ± 1.27
9%	9.03 ± 5.96	8.07 ± 0.81	16.89 ± 4.74
Fluconazole (Control)	15.74 ± 0.91	14.53 ± 1.04	19.26 ± 1.29
Aquadeest (Control)	0	0	0

The highest zone of inhibition was achieved at 9% extract concentration, with the strongest effect against *C. parapsilosis* ( $16.89 \pm 4.74$  mm), followed by *C. albicans* ( $9.03 \pm 5.96$  mm), and *C. tropicalis* ( $8.07 \pm 0.81$  mm). Meanwhile, at 1% concentration, the inhibition effect was at a minimal level for all species tested. For comparison, fluconazole showed higher antifungal

activity than Lada Katokkon extract, but the extract still showed a clear pattern of increasing effect as the concentration increased. There was no zone of inhibition in the negative control (distilled water), indicating that the inhibitory activity came from the active compounds in the extract.

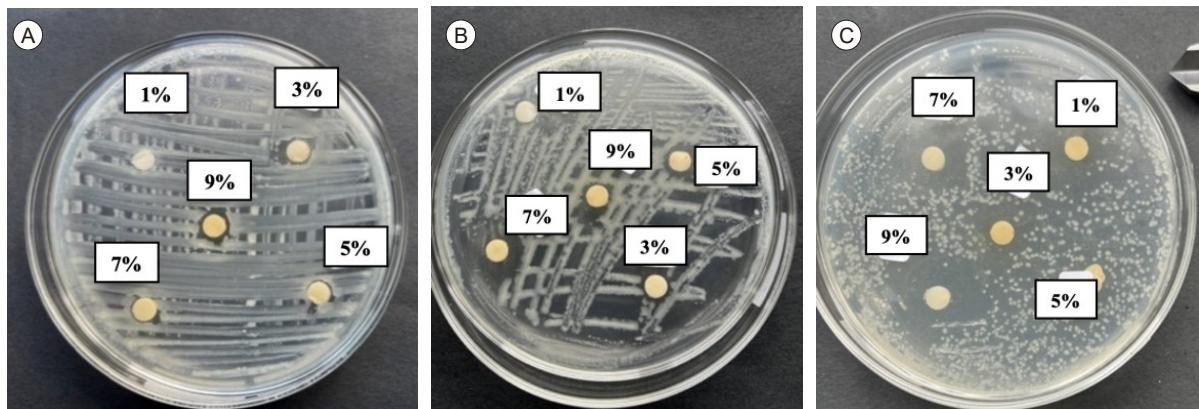


Figure 4. Inhibition test on isolates A: *C. albicans*, B: *C. tropicalis* and C: *C. parapsilosis*.

## Discussion

The inhibition test of ethanol extract of Lada Katokkon (*Capsicum annuum chinense Jacq*) against *Candida albicans* showed an increase in antifungal activity as the concentration of the extract increased. At a concentration of 1%, the inhibition zone formed is relatively small ( $4.36 \pm 0.62$  mm) and is classified in the weak category. Increasing the concentration to 3% and 5% gave a greater inhibitory effect, although still in the weak to moderate category. More pronounced antifungal activity appeared at a concentration of 7% ( $8.67 \pm 1.06$  mm), and reached its peak at 9% ( $9.03 \pm 5.96$  mm), both classified as moderate. These results indicate that the extract began to show significant inhibitory potential at high concentrations.

The test against *Candida tropicalis* also showed a similar trend. Low concentrations (1% to 5%) produced relatively small zones of inhibition ( $<6.5$  mm) and were categorized as weak. Increasing the concentration to 7% produced a more pronounced effect ( $7.32 \pm 0.67$  mm), and the highest at 9% ( $8.07 \pm 0.81$  mm), which fell into the moderate category. This gradually increasing effectiveness suggests that the inhibition against *C. tropicalis* is dose-responsive.

*Candida parapsilosis* showed the most prominent results. At 1% concentration, the zone of inhibition reached  $4.82 \pm 0.88$  mm (still weak), but the increase in concentration consistently had a significant effect. Concentrations of 5% and 7% produced moderate inhibition, and at 9%, an inhibition zone of  $16.89 \pm 4.74$  mm was achieved, which is classified as strong. These data suggest that *C. parapsilosis* is more sensitive to the ethanol extract of Lada Katokkon than the other two species.

The results of this study support previous findings that capsaicin compounds have antifungal activity through several mechanisms, including disruption of the cell membrane, interference with ergosterol biosynthesis, and induction of cell lysis. In addition, research by Jawad et al. (2023) showed that capsaicin can inhibit fungal membrane integrity by decreasing ergosterol content, causing osmotic disturbances, and ultimately cell death.

Flavonoids contained in the extract also contribute to the antifungal activity. Flavonoids such as quercetin and kaempferol are known to inhibit biofilm formation and hyphal growth of *Candida* spp., especially in the early phase of colonization. Research by Saleh et al. (2020) supports that flavonoids play a role in inhibiting the growth of *C. albicans* hyphae, although not significantly against mature biofilms.

Other components such as tannins are also known to have antifungal potential. Martins et al. (2019) reported that the tannin fraction from *Stryphnodendron adstringens* was able to inhibit the growth of *C. albicans* and *C. tropicalis*, and increase survival rates in animal models without tissue toxicity. This effect is thought to play a role in the positive results against these two species in this study.

Rocha et al. (2019) also showed that flavonoids can cause biofilm damage, metabolic disturbances, and an increase in the number of dead cells through the mechanisms of oxidative stress and mitochondrial dysfunction. Therefore, the flavonoid content in Lada Katokkon extract is likely to work synergistically with capsaicin in producing antifungal activity.

Overall, the results of this study indicate that the ethanol extract of Lada Katokkon has potential as a natural antifungal agent with concentration-dependent

effects. The strongest activity was achieved against *C. parapsilosis*, indicating a possible specific sensitivity of this species to the active compounds contained in the extract.

However, the effectiveness of the extract is still lower than fluconazole, especially at low concentrations. This suggests that for clinical application, formulation optimization, concentration increase, or combination with other agents are required. Further research is needed to examine the molecular mechanisms underlying the activity of this extract, as well as its potential toxicity and stability in pharmaceutical formulations.

## CONCLUSIONS

Ethanol extract of Lada Katokkon (*Capsicum annuum chinense Jacq*) showed concentration-dependent antifungal activity against three *Candida* species, namely *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. The most potent inhibitory activity was achieved against *C. parapsilosis* at 9% concentration, suggesting a potential specific sensitivity of this species to the active compounds in the extract.

These findings provide preliminary evidence that Lada Katokkon extract has the potential to be developed as a candidate natural ingredient-based antifungal agent. However, its effectiveness, which is still below that of fluconazole, suggests the need for further formulation, toxicity testing, and exploration of the molecular mechanism of action before it can be applied clinically.

By utilizing local biodiversity, this study also supports the importance of phytopharmaca development as an alternative therapy for fungal infections that are increasingly resistant to conventional drugs.

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**Author's Contributions:** Allen Mangoting is responsible for conceptualization and design of the study, analysis and interpretation of data, drafting the article, assembling data and providing study materials. Eka Astuty is responsible for data analysis and interpretation, article drafting, revision of the article for important intellectual content, final approval of the article, administrative, technical and logistic support, provision of study materials. Amanda Gracia Manuputty is responsible for drafting the article, revising the article for important intellectual content, administrative support and also final approval of the article.

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