

Ethanol Extracted *Carica papaya* L. Leaves: In Vitro Antifungal Activity Against *Candida albicans*

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ABSTRACT

Invasive fungal infections caused by *Candida albicans* remain a significant clinical concern, particularly among immunocompromised individuals, while the increasing occurrence of resistance to azole antifungals has encouraged the exploration of plant-based antifungal agents. *Carica papaya* L. leaves contain bioactive compounds, including flavonoids, alkaloids, tannins, and saponins, which may contribute to antifungal activity; however, evidence regarding their activity against *C. albicans* remains limited. This study aimed to evaluate the in vitro antifungal activity of ethanolic *C. papaya* leaf extract against *C. albicans*, determine the concentration producing the greatest inhibitory effect, and identify the minimum inhibitory concentration (MIC). Papaya leaves were extracted by maceration using 90% ethanol. Extract concentrations of 25%, 50%, 75%, and 100% were evaluated against *C. albicans* using the disc diffusion method to determine inhibition zones and a microdilution method to determine the visual MIC. Fluconazole and distilled water were used as positive and negative controls, respectively. All treatments were tested in triplicate, and the data were analyzed using one-way ANOVA and the Kruskal-Wallis test, as appropriate. The extract demonstrated concentration-dependent antifungal activity, with the 100% concentration producing the largest inhibition zone among the extract treatments. The MIC was observed at a concentration of 25%. Statistical analysis indicated significant differences in antifungal activity among the tested concentrations ($p < 0.05$). Fluconazole produced greater inhibition than all extract concentrations, whereas the negative control showed no detectable inhibition. These findings demonstrate that ethanolic *C. papaya* leaf extract exhibits in vitro antifungal activity against *C. albicans*, with activity increasing with extract concentration. The extract may therefore serve as a potential source of plant-derived antifungal compounds, although further studies are required to characterize its active constituents and evaluate its efficacy under in vivo conditions.

Keywords: Antifungal agent, *Candida albicans*, *Carica papaya* L., Inhibition Zone

INTRODUCTION

Candida albicans are known to be the cause of most candidiasis case, with accounting for approximately 78.7% of all candida isolates in patients [1]. As a commensal opportunistic pathogen, *C. albicans* asymptotically colonizes the GI tract and mucosal surfaces (oral and vagina). However, when host immune defenses are compromised, this fungus can penetrate epithelial layers and migrate to deeper anatomical sites, triggering a spectrum of clinical manifestations ranging from mucosal infections, such as oral and vaginosis candida to more concerning systemic infections, including candidemia, endocarditis, and hepatosplenic candidiasis [2], [3].

Globally, *Candida* species remain the leading cause of systemic fungal infections, particularly among immunocompromised patients and those in intensive care units. Data from 2010–2020 indicate that *C. albicans* accounted for the highest proportion of

candidemia cases, at 57.42% [4]. In Indonesia, the prevalence of *C. albicans* infections has been reported to range between 25–50%, with significant local incidence rates, further underscoring the substantial public health burden of this pathogen in the region [5], [6].

Current clinical management of candidiasis relies heavily on synthetic antifungal agents, including fluconazole, itraconazole, and nystatin. Fluconazole is frequently employed as the first-line therapy due to its ease of administration, relatively favorable safety profile, and approximately 70% efficacy [7]. However, its widespread use has precipitated increasing cases of antifungal resistance [8]. Although itraconazole demonstrates higher efficacy (~80%) and is recommended for severe or refractory cases, its clinical utility is limited by complex drug interactions via CYP3A4 inhibition and pH-dependent absorption, which can diminish its effectiveness when co-administered with proton pump inhibitors or rifampicin [9].

Moreover, long-term antifungal therapy is fraught with challenges, including significant side effects such as hepatotoxicity and nephrotoxicity, as well as the rising incidence of multidrug-resistant strains [10]. These limitations underscore the urgent need to explore alternative antifungal agents with improved safety and efficacy profiles.

Natural products have increasingly gained attention as promising sources of novel antifungal compounds. Among these, the leaves of the papaya tree (*Carica papaya* L.) have been traditionally used in Indonesian herbal medicine, particularly in Timor-Leste, to treat infectious and parasitic diseases [11], [12]. Phytochemical analyses have shown that papaya leaves are rich in secondary metabolites, including flavonoids, tannins, alkaloids, saponins, and terpenoids [13]. Many of these compounds are well-documented for their antimicrobial and antifungal properties. It is believed that they disrupt fungal cell walls, inhibit enzyme activity, or interfere with fungal DNA synthesis, thereby contributing to their antifungal effects [14]. While recent studies have demonstrated the potent antifungal activity of papaya seeds extract against *C. albicans*, with a minimum inhibitory concentration (MIC) of 2.5% [15], comprehensive investigations specifically evaluating the antifungal efficacy of papaya leaf extract remain conspicuously absent.

Up until now, no study has systematically determined the inhibition zones and MIC values of papaya leaf extract with varying concentrations against *C. albicans*, nor has any research directly compared its effectiveness with that of fluconazole as the gold standard. To address this scientific gap, this study aims to evaluate the antifungal activity of *Carica papaya* L. leaf extract at varying concentrations (25%, 50%, 75%, and 100%) against *C. albicans*. Specifically, this research seeks to measure the inhibition zone diameters, determine the MIC, and directly compare its efficacy to first-line synthetic fluconazole. The findings of this study are expected to provide foundational scientific evidence on the potential of papaya leaves as a viable, natural alternative for managing candidiasis.

RESEARCH METHODS

This study was based on methodologies from prior research [16], which helped select suitable methods for evaluating the antifungal activity of papaya leaf extract.

Study Design

An in vitro bivariate experimental study was conducted to assess the antifungal activity of papaya leaf extract against *Candida albicans* at concentrations of 25%, 50%, 75%, and 100%. Fluconazole (15 mg) served as the positive control, while sterile distilled water functioned as the negative control. All treatments were conducted in triplicate.

Extract Preparation

Fresh papaya leaves were washed to remove any impurities or contaminants. After washing, the leaves were sliced into smaller pieces to enhance the extraction process. The sliced leaves were then dried

using a fruit dryer until they reached a consistent dryness, ensuring that moisture content was minimized. Once dry, blender was used to grind the leaves, resulting in a uniform particle size simplicia. This simplicia was then macerated in 90% ethanol at a 1:10 ratio for 3 days. The mixture was then filtered to separate the liquid extract from the solid plant residue and concentrated using a rotary evaporator to remove excess ethanol, yielding a viscous extract rich in antifungal compounds. [17][18] Finally, this concentrated extract was diluted in sterile distilled water to achieve the specific concentrations (25%, 50%, 75%, and 100%).

Minimum Inhibitory Concentration (MIC) Test

The MIC was established using the broth microdilution method within 96-well plates containing Potato Dextrose Broth (PDB). A standardized *Candida albicans* suspension was inoculated into wells with each concentration of the extract, fluconazole, or distilled water. After 24 hours of incubation at 30°C, the lowest concentration that produced clear wells, indicative of no visible growth was recorded as the MIC [19].

Disk Diffusion Susceptibility Test

The disk diffusion method was performed on Potato Dextrose Agar (PDA). A standardized suspension of *Candida albicans* was spread evenly across agar plates. Each sterile paper disks were saturated with different concentrations of papaya leaf extracts, fluconazole, or distilled water as a control and carefully placed onto the inoculated agar. The plates were then incubated and measured with a vernier caliper, resulting clear inhibition zones indicate the extent of fungal growth suppression around the disk. Each procedure was triplicated to ensure the reliability and accuracy of the results [20].

Statistical Analysis

In this study, data were analyzed using SPSS version 27. Descriptive statistics such as means and standard deviations were computed for each group. The normality of the data was assessed, and a one-way ANOVA was conducted, followed by the Games-Howell post-hoc test to identify significant differences [21] MIC data were not subjected to statistical analysis because they are categorical. Hypothesis testing was conducted at a 95% confidence level ($\alpha = 0.05$) [22].

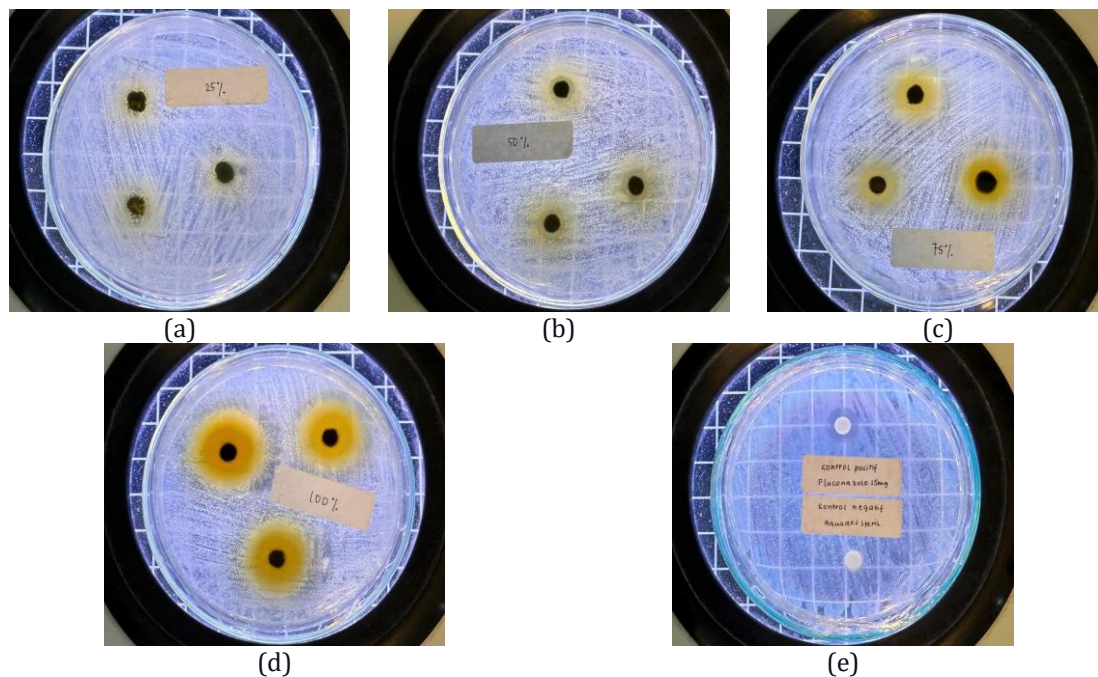
RESULT AND DISCUSSION

Inhibition Zone Diameter

The antifungal properties of papaya leaf extract (*Carica papaya* L.) against *Candida albicans* were evaluated using the agar diffusion method. The average diameters of the inhibition zones are summarized in Table 1.

Table 1. Inhibition zone diameters of papaya leaf extract against *Candida albicans*

Treatment	Mean $\pm$ SD (mm)
25% Extract	0.00 $\pm$ 0.00
50% Extract	7.71 $\pm$ 0.91
75% Extract	8.11 $\pm$ 0.22
100% Extract	8.99 $\pm$ 0.19
Fluconazole 15mg	9.33 $\pm$ 0.54
Distilled water	0.00 $\pm$ 0.00

**Figure 1.** Inhibition zone: (a) 25% extract; (b) 50% extract; (c) 75% extract; (d) 100% extract; and (e) fluconazole and distilled water

The inhibition zone diameter increased proportionally with extract concentration. One-Way ANOVA results indicated a statistically significant difference among treatment groups ($p < 0.05$). The Games-Howell post hoc test resulted that 25% concentration did not differ significantly from distilled water (negative control). Conversely, the 50%, 75%, and 100% concentrations exhibited significant inhibition zone differences from the distilled water, yet did not significantly differ from fluconazole (positive control). This finding suggests that the higher concentrations possess an efficacy approaching that of the positive control.

Minimum Inhibitory Concentration (MIC)

The extract at a concentration of 25% significantly inhibited the growth of the microorganisms, as evidenced by a noticeable clarity in the broth compared to the control sample, which showed turbidity indicating active growth. Furthermore, all higher concentrations (50%, 75%, and 100%) of the extract maintained this inhibitory effect, resulting in increasingly clearer media that demonstrated a pronounced lack of microbial growth.

Method Comparison

The 25% extract did not produce a visible inhibition zone in the diffusion test. However, it effectively inhibited bacterial growth in the dilution test. This

dissimilarity can likely be attributed to the extract's viscosity, which interfered its ability to diffuse properly through the agar medium during the diffusion test. As a result, although the extract was potent enough to prevent growth at 25% concentration, its physical properties limited its ability to produce a clear zone of inhibition in the diffusion test.

The results show a dose-dependent antifungal effect of papaya leaf extract against *C. albicans*, with increased inhibition zones at higher concentrations indicating that its activity is linked to bioactive compounds. The efficacy of the 100% extract is comparable to that of fluconazole, highlighting its significant antifungal potential.

Phytochemicals like flavonoids, saponins, and alkaloids in the extract disrupt fungal cell membranes and metabolic processes. Furthermore, the difference between diffusion and dilution methods points to a limitation of the agar diffusion test for viscous extracts, as it may underestimate their activity. Liquid-based tests provide a more accurate assessment of inhibitory potential.

The minimum inhibitory concentration (MIC) of 25% aligns with previous studies, although variations exist due to differences in extraction methods. Aqueous extraction may miss nonpolar compounds, thereby raising the MIC. Future research using organic solvents,

such as ethanol, could enhance extraction efficiency and reduce the MIC.

Study Limitations

This study has several limitations, including the use of distilled water as solvent, which may have restricted the extraction of nonpolar bioactive compounds. The broad concentration range (25–100%) made accurate determination of the minimum inhibitory concentration (MIC) difficult, and visual assessment of MIC is subjective and influenced by extract color. The in vitro study design does not adequately account for the complexities and variables present in living organisms (in vivo), which can significantly affect pharmacokinetic and immunological parameters. To more accurately assess the therapeutic potential of papaya leaf extract, future research should incorporate organic solvents, utilize narrower concentration ranges, and include in vivo validation. This approach will provide a more comprehensive understanding of its effects and efficacy.

CONCLUSION

Papaya (*Carica papaya* L.) leaf extract showed a dose-dependent antifungal effect against *Candida albicans*, as indicated by increasing inhibition zone diameters from 50% to 100% concentration, with the 100% extract resembling efficacy comparable to fluconazole ($p > 0.05$). While the 25% concentration failed to produce a zone in the agar diffusion test, it successfully inhibited fungal growth in the broth dilution method, establishing the MIC at 25% and underscoring that the diffusion technique minimizes activity for viscous extracts due to restricted agar penetration.

AUTHOR CONTRIBUTIONS

Conceptualization, Callishta Theodora Sutanto and Primta Bangun; formal analysis, Callishta Theodora Sutanto; investigation, Callishta Theodora Sutanto; resources, Callishta Theodora Sutanto; data curation, Callishta Theodora Sutanto; writing original draft preparation, Callishta Theodora Sutanto; writing review and editing, Primta Bangun and Irza Haicha Pratama; supervision, Primta Bangun and Irza Haicha Pratama; funding acquisition, Callishta Theodora Sutanto.

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