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Original Article

Antifungal Activity of Tithonia diversifolia Leaf Ethanol Extract Against Candida albicans: A Dose-Response Study

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ABSTRACT

This study aimed to evaluate the antifungal activity of *Tithonia diversifolia (Hemsl.) A. Gray* leaf ethanol extract against *Candida albicans*, focusing on identifying both the minimum inhibitory and maximum effective concentrations. Twelve treatments were applied using the paper disk diffusion method, including extract concentrations ranging from 10% to 100%, a positive control (ketoconazole), and a negative control (1% DMSO). Inhibition zone diameters were measured and statistically analyzed using one-way ANOVA followed by Duncan's multiple range test. The extract exhibited antifungal activity at a 50% concentration, with the strongest inhibition observed at 90%. These findings indicate the potential of *T. diversifolia* as a natural antifungal agent and support the exploration of plant-based alternatives to conventional antifungal drugs. This approach contributes to improving public health through the development of accessible, affordable treatments and highlights the importance of conserving biodiversity by utilizing locally available medicinal plants in biomedical research.

Keywords: *Tithonia diversifolia*, *Candida albicans*, antifungal, ethanol extract, medicinal plants

Introduction

Candidiasis, a disease caused by an overgrowth of the *Candida species*, particularly *Candida albicans*, can lead to illnesses in both animals and humans, causing immune suppression or debilitating diseases, with the predisposing factors to this overgrowth including young birds that are not fully immunocompetent, prolonged antibiotic use, concurrent immunosuppressive conditions (e.g., debilitation, PBFD, malnutrition), poor hygiene in the bird's environment and food preparation, failure to clean excess formula from the skin or mouth of hand-reared chicks, high concentrations of sugar in fruit and hand-rearing formula providing an optimal medium for the growth of yeast, and alkaline crop contents, observed when crop stasis occurs for any reason, enhancing yeast overgrowth (Al-Abedi, et al., 2024; Talazadeh, et al., 2022). This fungal infection has been reported in both domestic and wild birds, with confirmed cases in Galliformes (chickens, turkeys, quail), Anseriformes (ducks, geese), Psittaciformes (parrots), Passeriformes (songbirds), Columbiformes (pigeons), and Guinea fowl (Talazadeh, et al., 2022), *candida* infection also leading to decreased growth rates, poor feed conversion, and increased mortality, all of which contribute to economic losses. The cost of treatment and preventive measures further adds to the financial burden (Dias Carneiro, et al., 2024; Domán, et a., 2023; Talazadeh, et al., 2022).

Azole compounds, including ketoconazole, are the primary antifungal agents used to treat candidiasis. These compounds function by targeting the fungal cell membrane, specifically by inhibiting the enzyme lanosterol 14 α-demethylase, which is crucial for ergosterol synthesis, a key component of the fungal cell membrane (Suat, et al., 2020; Bruneau, et al., 2003). While ketoconazole is effective against various pathogenic yeasts, The efficacy of azoles is often compromised by the development of resistance in Candida species. Resistance mechanisms include overexpression of efflux pumps and mutations in the target enzyme CYP51 (Nishimoto, et al., 2020).

Due to concerns regarding drug safety, resistance, and accessibility, attention has turned to medicinal plants as alternative antifungal agents. *Tithonia diversifolia (Hemsl.) A. Gray*, commonly known as tree marigold, has garnered attention for its potential as an alternative antifungal agent due to its rich composition of bioactive phytochemicals such as flavonoids, tannins, alkaloids, and saponins, contributing to its antimicrobial properties especially the phenolic compounds and flavonoids (Barboza, et al., 2018). Flavonoids are known to exert antifungal effects through multiple mechanisms. Compounds such as sophoraflavone G and (-)-epigallocatechin gallate have been shown to disrupt cytoplasmic membrane function, leading to fungal cell death. In addition, flavonoids inhibit key enzymes like DNA gyrase, interfere with the energy metabolism of yeasts and fungi, and block ATP-dependent drug efflux pumps, which are often responsible for antifungal resistance. Microscopic analyses using SEM and TEM have further demonstrated that flavonoid derivatives induce abnormal hyphal growth, distorted cell walls, and a reduction in mitochondrial numbers in fungi such as Rhizoctonia solani (Peng, et al., 2024; Cushnie and Lamb, 2005)

Given its phytochemical profile and prior indications of antifungal activity, this study aimed to evaluate the inhibitory effect of ethanol extracts from *T. diversifolia* leaves against Candida albicans. The specific objectives were to determine the minimum concentration required to inhibit fungal growth and to identify the concentration with the maximum inhibitory effect.

Materials and methods

Research design

This study utilized a true experimental design with a post-test control group format to investigate the antifungal effects of ethanol extract derived from *Tithonia diversifolia (Hemsl.) A. Gray* leaves against Candida albicans, employing the disk diffusion technique.

Sample and test organism

The test organism used was *Candida albicans* ATCC 10231, obtained from Balai Besar Laboratorium Kesehatan (BBLK), Surabaya, Indonesia. The suspension was prepared to match the turbidity of 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/mL).

Preparation of plant extract

Fresh *Tithonia diversifolia (Hemsl.) A. Gray* leaves were cleaned, air-dried, and finely ground into powder form. The extraction process involved maceration in 96% ethanol for 72 hours with occasional stirring. The resulting solution was then concentrated using a rotary evaporator at a temperature range of 50–60°C to produce a viscous ethanol extract. This extract was subsequently diluted in 1% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany; Cat. No. 102952) to prepare a series of concentrations: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

Antifungal assay

The antifungal effect was evaluated using the paper disk diffusion technique on Sabouraud Dextrose Agar (SDA; Merck, Darmstadt, Germany; Cat. No. 105438) plates containing chloramphenicol. Plates were inoculated with a suspension of Candida albicans and treated with sterile paper disks loaded with 20 µL of each extract concentration. Ketoconazole (0.125 µg/disc) served as the positive control, while 1% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany; Cat. No. 102952) was used as the negative control. All assays were conducted in triplicate.

Incubation and observation

The inoculated plates were incubated at 37°C for 24 hours. Antifungal activity was determined by measuring the diameter of the

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clear zones of inhibition around the paper disks in millimeters. Each zone was measured in three different directions, and the average value was calculated.

Data analysis

The data were subjected to one-way ANOVA analysis, followed by Duncan's multiple range test to identify statistically significant differences between treatment groups, with a significance level set at $p < 0.05$.

Result

The antifungal potential of ethanol leaf extracts from *Tithonia diversifolia (Hemsl.) A. Gray* against *Candida albicans* was investigated through the measurement of inhibition zones on Sabouraud Dextrose Agar (SDA) using the disk diffusion method. This experiment involved twelve treatment groups, comprising ten extract concentrations ranging from 10% to 100%, as well as a positive control (ketoconazole 0.125 µg/disc) and a negative control (1% dimethyl sulfoxide, DMSO).

The data revealed that lower extract concentrations of 10% and 20% exhibited no visible inhibition zones, with average diameters of 6.00 ± 0.00 mm, identical to the negative control, indicating a lack of antifungal activity at these levels. Beginning at a concentration of 30%, a slight antifungal effect was observed (6.22 ± 0.39 mm), which gradually increased with higher extract concentrations. Notably, extracts at 90% and 100% produced the largest inhibition zones among all extract groups, measuring 15.44 ± 2.88 mm and 16.67 ± 2.22 mm, respectively. Despite this, both values remained lower than the inhibition diameter produced by the positive control ketoconazole, which showed a consistent zone of 20.00 ± 0.00 mm.

Furthermore, the negative control (1% DMSO) showed no inhibitory activity, confirming that the solvent alone did not contribute to fungal growth suppression. Statistical analysis using one-way ANOVA followed by Duncan's multiple range test demonstrated significant differences ($p < 0.05$) among most treatments, particularly when comparing extract concentrations of 50% and above to the negative control. The analysis indicated that 50% was the minimum effective concentration that produced a statistically significant antifungal effect. The antifungal efficacy continued to improve with increasing concentration up to 90%, beyond which the effect plateaued.

A summary of the inhibition zone diameters observed across all treatments is presented in Table 1.

Table 1. The summary of the average inhibition zone diameters observed in each treatment.

Treatments	Mean Inhibition Zone (mm) ± SD
P (+)	20.00 ± 0.00^{e}
P (-)	6.00 ± 0.00^{a}
P1	6.00 ± 0.00^{a}
P2	6.00 ± 0.00^{a}
P3	6.22 ± 0.39^{a}
P4	7.43 ± 0.21^{ab}
P5	8.55 ± 0.69^{bc}
P6	9.00 ± 0.58^{bc}
P7	10.56 ± 1.90^{c}
P8	10.76 ± 1.71^{c}
P9	15.44 ± 2.88^{d}
P10	16.67 ± 2.22^{d}

Note: Distinct superscript letters denote statistically significant differences among groups at the 5% significance level.

These findings indicate a dose-dependent relationship between the concentration of *Tithonia diversifolia (Hemsl.) A. Gray* ethanol leaf extract and its antifungal activity against *Candida albicans*. The results support the conclusion that the extract begins to exert significant inhibitory effects starting from 50% concentration, with optimal performance observed at 90%.

Discussion

The present study demonstrates that the ethanol extract derived from the leaves of *Tithonia diversifolia (Hemsl.) A. Gray* possesses

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antifungal properties against *Candida albicans*, showing a clear dose-dependent pattern. While concentrations below 30% did not exhibit observable inhibitory effects, a measurable inhibition zone was detected at 30%, becoming more pronounced at 50% and reaching its maximum at 90%. Nonetheless, the antifungal activity of the extract remained consistently less effective than that of ketoconazole

Candida albicans is a commensal yeast frequently found on mucosal surfaces in humans and animals, which may transition to a pathogenic state when host immunity is impaired or the normal microbiota is disrupted. In veterinary medicine, candidiasis has been associated with considerable mortality and economic losses, particularly among avian populations (Benarrós & Salvarani, 2024; Jacobsen et al., 2021; Basmaciyan et al., 2019).

Ketoconazole, used as the positive control in this study, exerts its antifungal action by inhibiting the synthesis of ergosterol, an essential component of fungal cell membranes. This disruption compromises fungal cell integrity and viability (Lisa et al., 2022). However, despite its efficacy, ketoconazole is linked to several adverse effects, including liver toxicity, reproductive toxicity in males, and teratogenic outcomes (Adis Medical Writer, 2020). Furthermore, the emergence of antifungal-resistant *Candida* species such as *C. auris*, *C. glabrata*, *and C. tropicalis* has intensified the challenges in managing fungal infections (Raposa & Vazquez, 2025; Murphy & Bicanic, 2021). Resistance mechanisms may involve modifications in drug target enzymes, overexpression of efflux pumps, and biofilm formation, all of which hinder treatment success and highlight the urgency of developing alternative antifungal agents (Chauhan et al., 2025; Shivarathri et al., 2020). The rising incidence of azole resistance, including resistance to ketoconazole, further emphasizes the importance of identifying novel antifungal candidates (Jangir et al., 2023).

Plant-derived antifungal agents have gained attention due to their potential efficacy and lower toxicity profiles. Bioactive compounds such as terpenoids, phenolics, and essential oils have exhibited antifungal effects against a variety of *Candida species* (Sharma et al., 2025). The antifungal activity observed in *Tithonia diversifolia* is likely attributable to its diverse phytochemical constituents, including flavonoids, tannins, alkaloids, saponins, and phenolic compounds (Babii et al., 2021; Barboza et al., 2018). Specifically, flavonoids have been shown to impair fungal cell membranes, inhibit efflux pump activity, disrupt cell wall integrity, and induce programmed cell death (Rodriguez et al., 2023; Babii et al., 2021; Barboza et al., 2018). These mechanisms are likely responsible for the inhibition observed in C. albicans.

The positive association between extract concentration and antifungal activity corroborates findings from prior research (Marbun et al., 2022), emphasizing the critical role of compound concentration in determining antimicrobial effectiveness. Although the extract did not exceed the efficacy of ketoconazole, its notable inhibitory effect at higher concentrations indicates promising potential as a natural antifungal agent.

Future investigations should aim to isolate and characterize the bioactive constituents responsible for the antifungal effect of *Tithonia diversifolia*, followed by in vivo evaluation. Such studies are essential to support the development of accessible and low-toxicity antifungal therapies, especially in the context of increasing resistance to conventional drugs.

Conclusion

The ethanol extract of *Tithonia diversifolia (Hemsl.) A. Gray* leaves demonstrated dose-dependent antifungal activity against *Candida albicans*. Observable inhibition began at 30%, with significant effects at 50%, and maximum activity at 90% concentration. Although its efficacy was lower than ketoconazole, the extract's antifungal potential suggests it contains bioactive compounds worthy of further investigation. Future research should aim to isolate these compounds and assess their effectiveness in vivo to support the development of safe, natural antifungal therapies.

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