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COMPARATIVE IN VITRO ANTIFUNGAL ACTIVITY OF SINGLE AND COMBINED EXTRACTS OF MICHELIA ALBA AND IMPATIENS BALSAMINA AGAINST CANDIDA ALBICANS

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Abstract

Background: Infections caused by Candida albicans are increasingly prevalent, particularly among immunocompromised individuals, and are further complicated by rising resistance to synthetic antifungal agents such as azoles. This has led to growing interest in traditional medicinal plants as alternative antifungal sources.

Objective: This study aimed to evaluate the in vitro antifungal activity of single and combined extracts of Michelia alba and Impatiens balsamina against Candida albicans.

Methods: Both plant extracts were obtained by ethanol maceration and phytochemically screened. Antifungal activity was evaluated using the disk diffusion method with 20 µL (20 mg/disc) of crude extract. Extract combinations (1:3, 1:1, and 3:1) were tested in triplicate, and data were analyzed using the Kruskal–Wallis and Mann–Whitney tests.

Results: Impatiens balsamina extract showed the largest inhibition zone (10.72 mm), followed by the 3:1 combination (9.91 mm) and Michelia alba extract (7.83 mm). All extracts exhibited moderate antifungal activity, with significant differences among groups ($p = 0.009$).

Conclusions: Both plant extracts, individually and in combination, demonstrated antifungal activity against Candida albicans, suggesting their potential as candidates for natural antifungal agents.

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INTRODUCTION

Candida albicans, a natural inhabitant of the oral, gastrointestinal, and urogenital microbiota, is typically regulated by host immunity but can overgrow in immunocompromised individuals and cause infections ranging from mild mucosal lesions to severe systemic disease (1). The WHO lists *C. albicans* as a critical fungal pathogen. Candidemia carries over 40% mortality in high-risk patients and remains a leading cause of severe invasive infections worldwide (2). Invasive candidiasis also imposes a substantial healthcare burden, particularly among critically ill and immunocompromised patients (3). The increasing incidence of antifungal resistance and limited therapeutic options further complicate the global management of *Candida* infections (4). *C. albicans* has several adaptive mechanisms that enhance pathogenicity, including biofilm formation, phenotypic switching, and the production of virulence-associated factors such as hyphal transformation and secreted aspartyl proteases (5). Of these mechanisms, biofilm formation is particularly important in promoting persistent infections and decreasing responsiveness to antifungal therapy (6).

Conventional management of candidiasis commonly relies on synthetic azole agents that act by inhibiting ergosterol synthesis and compromising the integrity of the fungal cell membrane (7). Prolonged and widespread use of these drugs has contributed to the emergence of resistant *C. albicans* strains, reducing treatment effectiveness (8). Resistance can develop through ERG11 gene mutations, increased drug efflux, and altered biofilm formation (9). This growing resistance has intensified the need to explore alternative antifungal agents, particularly plant-based sources that may act through multiple mechanisms, which could lower the likelihood of resistance development.

Medicinal plants have long been recognized as important sources of bioactive substances with established antimicrobial properties. White champaca flower (*Michelia alba*), a member of the *Magnoliaceae* family and used in Southeast Asian herbal medicine, contains bioactive constituents such as essential oils (linalool, methyl eugenol, and geraniol), benzoic acid derivatives, flavonoids, saponins, tannins, and phenolic compounds. These constituents have been associated with antimicrobial and antifungal activities (10). In parallel, garden balsam leaf (*Impatiens balsamina*) from the family *Balsaminaceae* is well-documented for flavonoids, saponins, tannins, alkaloids, and phenolic compounds that may disrupt fungal membrane integrity and inhibit enzymatic function (11). These complementary phytochemical profiles suggest that combining both plants may influence antifungal activity through interactions among their bioactive compounds. However, the nature of these interactions remains unclear and requires further investigation through controlled in vitro studies.

Despite growing evidence of the individual antifungal activity of *M. alba* and I. *balsamina* against *C. albicans*, no published study has systematically examined the combined application of their extracts or evaluated the interaction of their active compounds in a controlled in vitro setting. This gap is important because combinatorial approaches using herbal extracts may enhance efficacy, lower the required concentrations, and reduce the risk of resistance development. The combination of medicinal plant extracts may produce additive or complementary antifungal effects because different phytochemical constituents can act on multiple fungal targets simultaneously, potentially enhancing antifungal activity. However, these interactions require experimental investigation before definitive

conclusions can be drawn. However, these interactions cannot be assumed and require experimental investigation to determine whether the combined extracts provide greater antifungal activity than the individual extracts. Therefore, this study aimed to determine whether single and combination extracts of *M. alba* and *I. balsamina* produce measurable antifungal activity against *C. albicans* in vitro and to determine the most effective combination ratio, which serves as the primary research question of this investigation.

MATERIALS AND METHODS

This in vitro laboratory experiment utilized a post-test-only control group design. It was conducted in October 2025 at the Microbiology Laboratory of the Faculty of Medicine and Health Sciences, Warmadewa University, with ethical approval (No. 674/Unwar/FKIK/EC-KEPK/IX/2025) from the university's Health Research Ethics Committee.

The experiment included seven groups: a single extract of *M. alba* flower, a single extract of *I. balsamina* leaf, three combination ratios of (*I. balsamina* : *M. alba*) of 1:3, 1:1, and 3:1, a positive control using ketoconazole disk (25 µg), and a negative control using sterile distilled water. Each treatment was performed in triplicate.

Fresh plant materials consisting of *M. alba flowers* and *I. balsamina* leaves were collected from flower farmers in Kelurahan Beng, Gianyar Regency, Bali, Indonesia. Species identification was conducted at the Laboratory of Yayasan Generasi Biologi Indonesia to confirm botanical authenticity. The samples were verified as *M. alba* and *I. balsamina*.

Fresh flowers of Michelia alba and fresh leaves of Impatiens balsamina (1 kg each) were washed and oven-dried at 35°C for three days to preserve bioactive compounds and prevent heat-induced degradation. The dried materials were pulverized into fine powder using an electric grinder (Philips, Indonesia). Subsequently, 100 g of the dried powdered material from each species was separately extracted by maceration using 500 mL of 70% ethanol (1:5 w/v) for 72 hours with intermittent agitation to enhance solvent diffusion and extraction efficiency. The extracts were then filtered and concentrated using a rotary evaporator to obtain crude extracts. The extraction yielded 12.838 g (12.84%) of Michelia alba extract and 7.19 g (7.19%) of Impatiens balsamina extract. The concentrated extracts were stored at 4°C prior to use. Qualitative phytochemical screening was performed to detect alkaloids, flavonoids, tannins, phenolic compounds, steroids, terpenoids, and saponins using standard colorimetric methods before antifungal evaluation.

A pure culture of *Candida albicans* ATCC 14053 maintained at the Microbiology Laboratory of the Faculty of Medicine and Health Sciences, Warmadewa University, was grown on SDA at 37°C for 24 hours. The colonies were resuspended in sterile saline and standardized to a 0.5 McFarland turbidity (approximately 1–5 × 10^6 CFU/mL) to achieve consistent inoculum density (12). A standardized procedure is essential to obtain reliable and comparable susceptibility testing results (13). Antifungal activity was evaluated using the Kirby-Bauer disk diffusion technique. SDA plates were poured and allowed to solidify, inoculated with the standardized C. albicans suspension, and then fitted with antifungal-impregnated disks to evaluate growth inhibition (14).

Figure 1. Processing and extraction of plant materials: (A) fresh plant materials, (B) drying process, (C) grinding into powder, and (D) extraction using a rotary evaporator.

Sterile 6 mm filter paper disks were impregnated with 20 µL (20 mg/disc) of 100% crude extract. For the combination groups, the crude extracts were mixed at weight-to-weight (w/w) ratios of 1:3, 1:1, and 3:1 (Impatiens balsamina : Michelia alba), and 20 µL (20 mg/disc) of each mixture was applied to the paper disks. The impregnated disks were then positioned onto the inoculated agar surface. A 25 µg ketoconazole disk was used as the positive control, whereas sterile distilled water was applied as the negative control. The inoculated plates were incubated at 37°C for 24 hours prior to measurement.

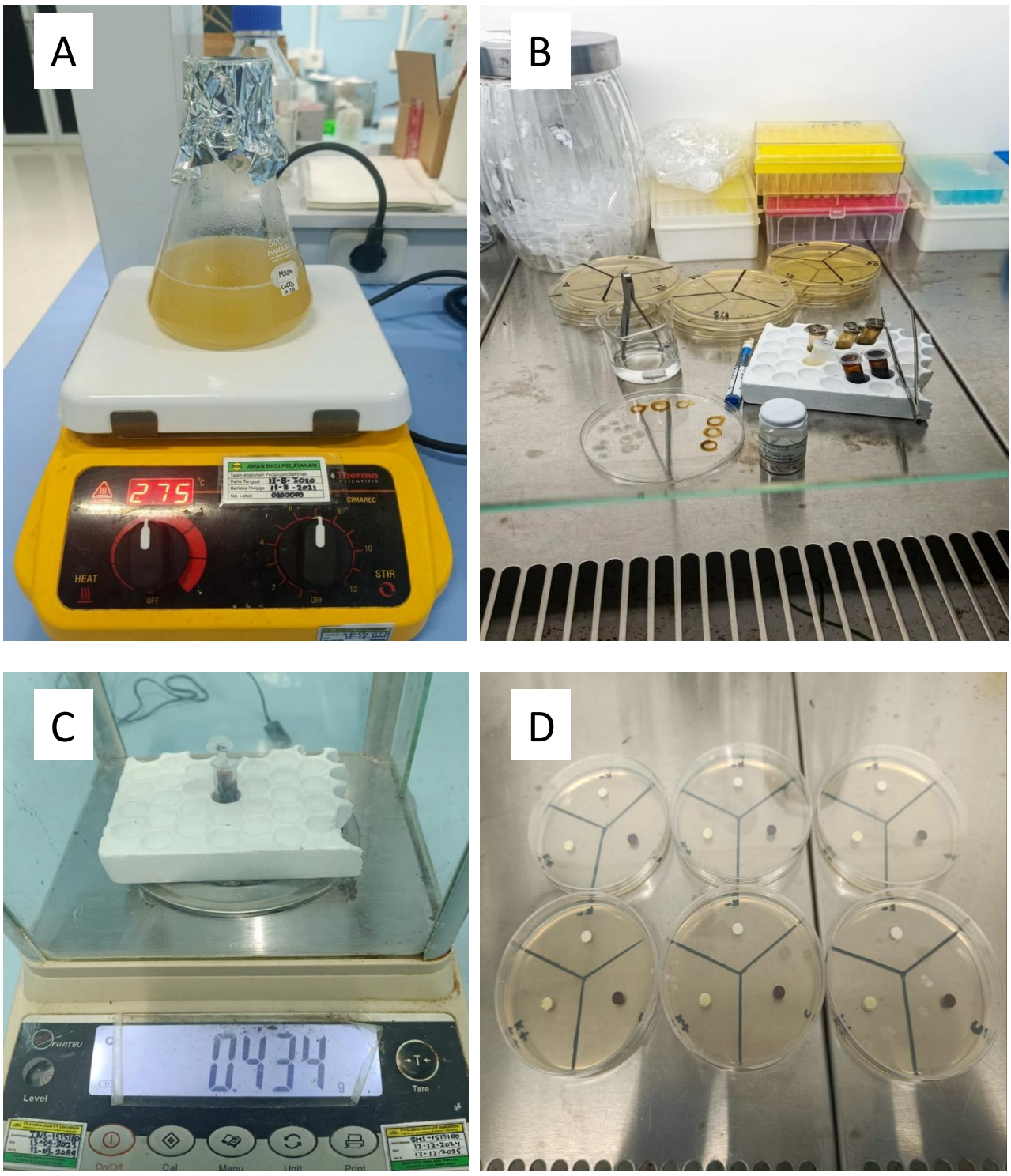

Figure 2. Preparation and antifungal testing procedures: (A) preparation of Sabouraud Dextrose Agar (SDA) media, (B) inoculation and disk placement on agar media, (C) weighing of crude extract, and (D) prepared agar plates with extract-impregnated disks prior to incubation.

After incubation, the clear zones surrounding each disk were measured in millimeters using a calibrated digital caliper. Antifungal activity was interpreted according to inhibition diameter as follows: <5 mm (inactive), 5–10 mm (moderate), 11–20 mm (strong), and >20 mm (very strong) (15). Data analysis was conducted using IBM SPSS Statistics version 30.0. The distribution of data was evaluated using the Shapiro–Wilk normality test. As the data were not normally distributed, group comparisons were performed using the Kruskal–Wallis test, followed by Mann–Whitney post hoc analysis with a significance threshold of $p < 0.05$.

RESULTS AND DISCUSSIONS

Following extraction, the extraction process yielded concentrated crude extracts with a viscous, semi-solid consistency and dark coloration, indicating successful extraction of bioactive compounds. Phytochemical screening of both ethanol extracts revealed distinct secondary metabolite profiles. Phytochemical screening of the ethanol extracts revealed distinct secondary metabolite profiles. The *M. alba* flower extract tested positive for flavonoids, tannins, phenolic compounds, and saponins, while alkaloids, steroids, and terpenoids were not detected. The *I. balsamina* leaf extract tested positive for tannins, phenolic compounds, and saponins, whereas alkaloids, flavonoids, steroids, and terpenoids were not detected. These findings confirm the presence of several bioactive secondary metabolites that may contribute to the observed antifungal activity.

All extract-treated groups generated measurable inhibition zones against *C. al*bicans, whereas the negative control (sterile distilled water) exhibited no inhibitory effect. This finding confirms that the observed activity was attributable solely to the extracts. The results are presented in Figures 3, 4, and 5.

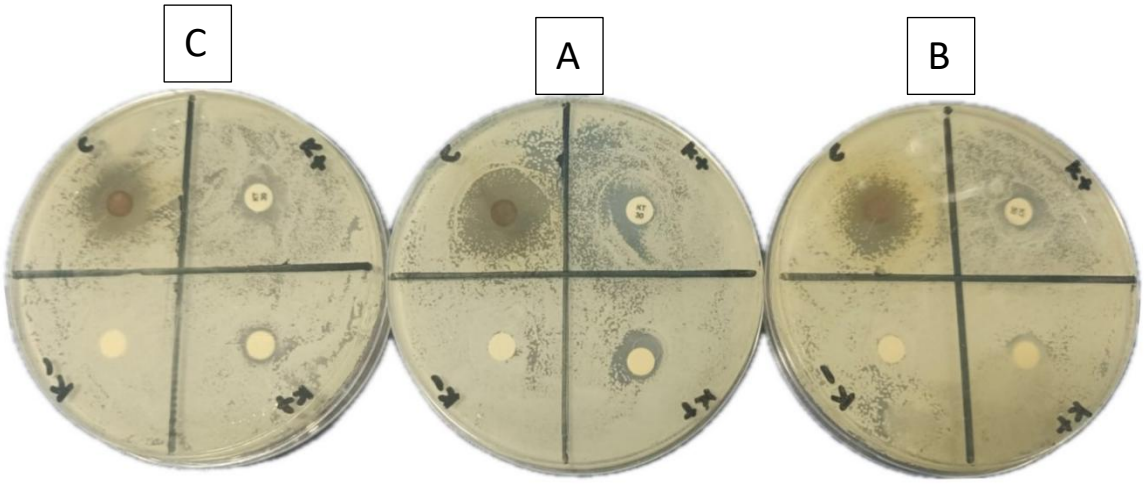

Figure 3. In vitro antifungal activity results of white champaca flower (*M. alba*) single extract against *C. albicans*. A. Replication 1; B. Replication 2; C. Replication 3.

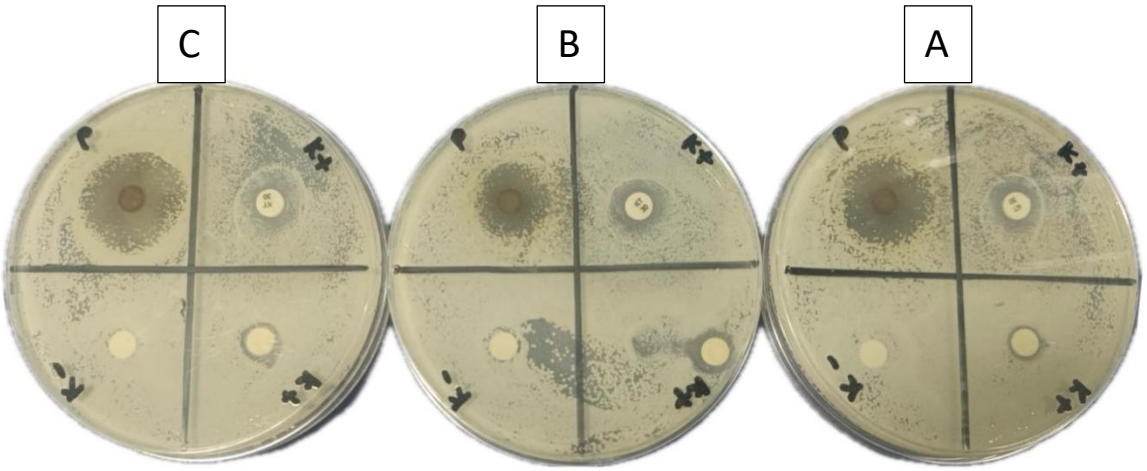

Figure 4. In vitro antifungal activity results of garden balsam leaf (*I. balsamina*) single extract against *C. albicans*. A. Replication 1; B. Replication 2; C. Replication 3.

The single extract of *M. alba* produced a mean inhibition zone of 7.83 ± 0.29 mm, classified as moderate, which was comparable to the ketoconazole positive control at 7.75 ± 0.25 mm. The single extract of *I. balsamina* produced the largest mean inhibition zone among all groups at 10.72 ± 0.25 mm. This value approached the upper boundary of the moderate category and exceeded both the M. alba extract and the ketoconazole control (Table 1).

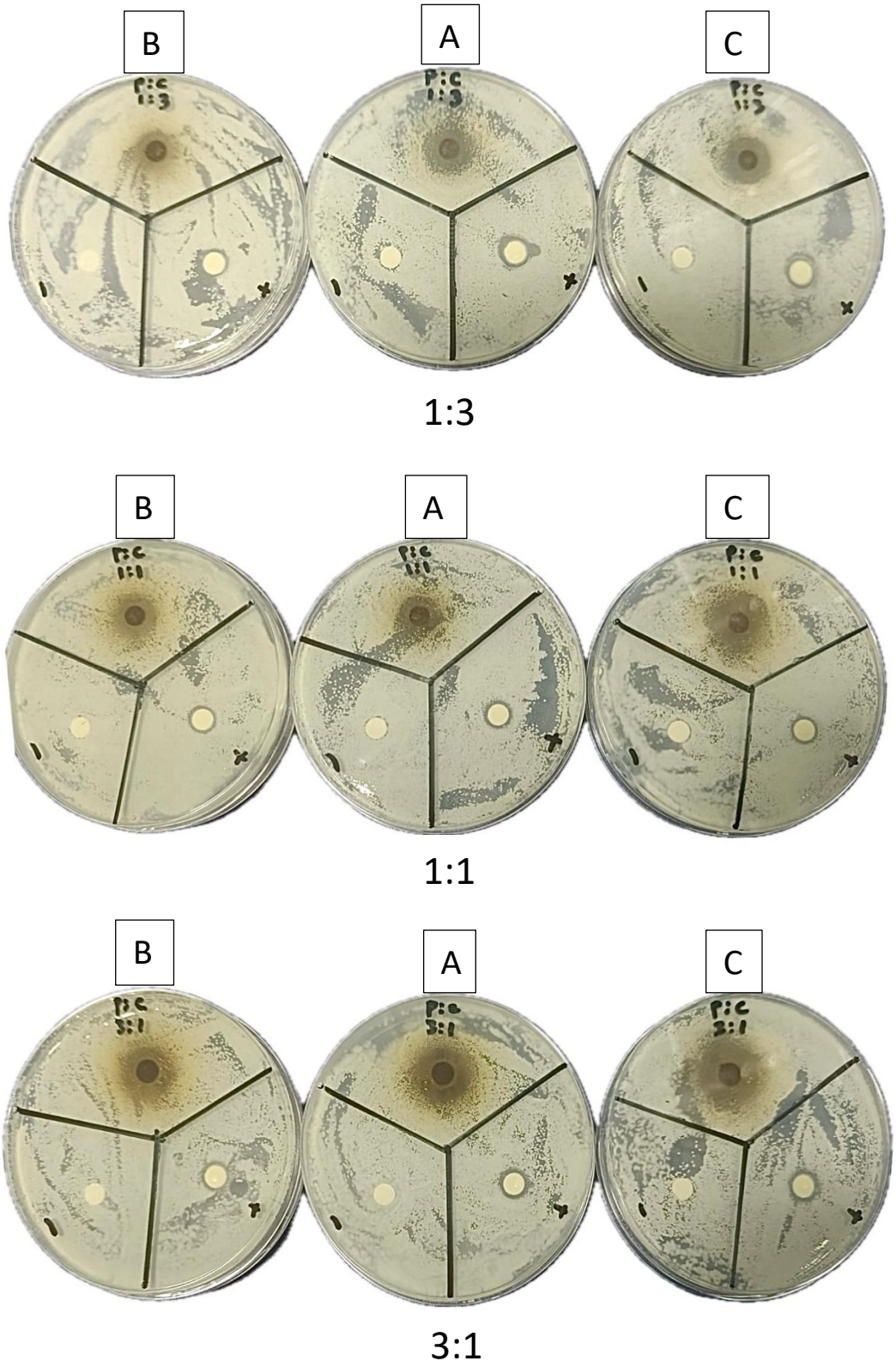

Figure 5. In vitro antifungal activity results of the combination extract of garden balsam leaf (*I. balsamina*) and white champaca flower (*M. alba*) at ratios of 1:3, 1:1, and 3:1 against *C. albicans*. A. Replication 1; B. Replication 2; C. Replication 3.

Among the combination groups, the inhibition zone diameters were 6.25 ± 0.25 mm for the 1:3 ratio, 7.25 ± 0.25 mm for the 1:1 ratio, and 9.91 ± 0.14 mm for the 3:1 ratio. All were classified as moderate (Table 1). A clear pattern emerged, as increasing the proportion of *I. balsamina* in the mixture corresponded with greater antifungal activity. The 3:1 ratio showed the highest activity among the combination groups and exceeded both the *M. alba* single extract and the ketoconazole control. In contrast, the 1:3 ratio showed the lowest activity among all extract-containing groups.

Table 1. Antifungal Activity Results of Single and Combined Extracts of *M. alba* and *I. balsamina* against *C. albicans*

Treatment Group	Mean (Rep. I, II, III) ± SD (mm)	Category
Single extract *M. alba*	7.83 ± 0.29	Moderate
Single extract *I. balsamina*	10.72 ± 0.25	Moderate
Combination 1:3 (*I. balsamina* : *M. alba*)	6.25 ± 0.25	Moderate
Combination 1:1	7.25 ± 0.25	Moderate
Combination 3:1	9.91 ± 0.14	Moderate
Positive control (ketoconazole)	7.75 ± 0.25	Moderate
Negative control (distilled water)	0	Inactive

Rep. = Replication; SD = Standard Deviation; Category based on inhibition zone classification.(15).

Table 1 shows variability in mean inhibition zone diameters across the experimental groups. Prior to comparative testing, normality was evaluated using the Shapiro–Wilk test, which demonstrated that the data did not follow a normal distribution ($p < 0.05$). Based on this finding, a non-parametric Kruskal-Wallis test was conducted and demonstrated a significant difference among treatment groups ($H = 17.051$; $p = 0.009$), as presented in Table 2.

Table 2. Kruskal-Wallis Test Results

Treatment Group	n	*Mean Rank*	Kruskal-Wallis H	df	P
Single extract *M. alba*	3	11.50	17.051	6	0.009*
Single extract *I. balsamina*	3	19.17	17.051	6	0.009*
Combination 1:3	3	7.33	17.051	6	0.009*
Combination 1:1	3	8.00	17.051	6	0.009*
Combination 3:1	3	17.83	17.051	6	0.009*
Positive control (ketoconazole)	3	11.17	17.051	6	0.009*

Negative control (distilled water)	3	2.00	17.051	6	0.009*

*n = number of samples; H = Kruskal-Wallis statistic; df = degrees of freedom; p = significance value; * = significantly different (p < 0.05).*

Because the Kruskal-Wallis test indicated a significant difference, pairwise comparisons were subsequently performed using the Mann-Whitney test to identify specific group differences. The detailed results are presented in Table 3.

Post hoc testing indicated that most extract-treated groups differed significantly from the negative control ($p < 0.05$). The single extract of *I. balsamina* and the 3:1 combination ratio differed significantly from the ketoconazole control ($p < 0.05$). Conversely, the *M. alba* extract and the 1:3 and 1:1 combination ratios were not significantly different from the positive control (Table 3).

Table 3. Mann-Whitney Post Hoc Test Result

Pairwise Comparison (Mann-Whitney)	p-value	Significance
M. alba vs Positive control	0.658	Not significant (ns)
M. alba vs Negative control	0.037	Significant*
I. balsamina vs Positive control	0.050	Significant*
I. balsamina vs Negative control	0.037	Significant*
Combination 1:3 vs Positive control	0.376	Not significant (ns)
Combination 1:3 vs Negative control	0.037	Significant*
Combination 1:1 vs Positive control	0.077	Not significant (ns)
Combination 1:1 vs Negative control	0.037	Significant*
Combination 3:1 vs Positive control	0.046	Significant*
Combination 3:1 vs Negative control	0.034	Significant*

*p-value = significance value; * = significantly different (p < 0.05); ns = not significant*

Overall, the results confirm that both individual and combined plant extracts inhibited the growth of *C. albicans*, although the extent of inhibition varied among treatment groups.

Antifungal Activity of *M. alba* Single Extract

This study demonstrated that the single extract of *M. alba* flowers exhibited moderate antifungal activity against *C. albicans*, reflected by a mean inhibition zone of 7.83 mm. This

finding is consistent with previous studies reporting the antimicrobial and antifungal potential of *M. alba* (16). The antifungal activity may be attributed to the presence of bioactive secondary metabolites identified in the phytochemical screening, particularly flavonoids, phenolic compounds, tannins, and saponins, which have been reported to interfere with fungal growth through multiple mechanisms. The moderate inhibition observed in this study is also consistent with reports noting that *M. alba* tends to show moderate antifungal potency in disk diffusion assays when whole-extract preparations are used rather than isolated essential oil fractions.

The mechanisms through which *M. alba* compounds inhibit *C. albicans* growth are multifaceted and involve disruption of multiple cellular targets. The antifungal activity of *M. alba* may be associated with the presence of flavonoids, phenolic compounds, tannins, and saponins detected in the phytochemical screening (10). Among these compounds, phenolic compounds are known to damage fungal membranes and induce organelle lysis, reflecting severe structural injury. Reduced ergosterol content and impaired membrane integrity increase membrane permeability and can ultimately lead to fungal cell death (17).

The phenolic and flavonoid compounds identified in the phytochemical screening may further reinforce the previously described antifungal effects by promoting oxidative stress in fungal cells, which impairs mitochondrial function and triggers cell death (18). Collectively, these multi-target mechanisms may contribute to the observed antifungal activity against *C. albicans*. However, their potential influence on antifungal resistance was not investigated in the present study and requires further research.

The inhibition zone produced by the M. *alba* single extract (7.83 mm) was comparable to that of the ketoconazole control (7.75 mm), and the Mann-Whitney test confirmed no statistically significant difference between the two groups ($p = 0.658$). This finding suggests that M. alba extract possesses measurable antifungal activity under the experimental conditions used in this study, consistent with previous reports of its antifungal potential (19). The literature also indicates that activity may be enhanced through isolation and concentration of specific bioactive fractions (20). Such strategies could be explored in future studies to optimize the antifungal formulation of *M. alba*.

The moderate antifungal activity observed may also reflect the crude nature of the extract used in this study. Crude ethanol extracts contain a complex mixture of compounds, some of which may interact in ways that reduce overall potency compared with isolated pure constituents (21). Future studies that apply fractionation and isolation techniques to identify specific bioactive compounds from *M. alba* may demonstrate greater antifungal activity and clarify the structure-activity relationships involved. Additionally, the use of solvents with different polarities may help extract more active fractions of the plant material. Although the activity observed was moderate, these findings provide a clear basis for the rational development of *M. alba*-based antifungal preparations.

Antifungal Activity of *I. balsamina* Single Extract

The single extract of *I. balsamina* leaf produced the largest mean inhibition zone among all treatment groups in this study, measuring 10.72 mm, which places it near the upper limit of the moderate category. This observation aligns with the pharmacological

review by (11), which describes the antifungal activity of *I. balsamina* against *C. albicans* and highlights the contribution of various bioactive secondary metabolites to its antifungal effects. The inhibition zone exceeded that of *M. alba* and ketoconazole, and the Mann–Whitney test indicated a statistically significant difference ($p = 0.050$), suggesting stronger antifungal activity under the tested conditions.

The superior antifungal activity of *I. balsamina* compared to *M. alba* may be associated with differences in the phytochemical composition of the extracts. Phenolic compounds, tannins, and saponins detected in the *I. balsamina* extract may contribute to its antifungal activity through multiple mechanisms. Phenolic compounds are known to disrupt fungal cell membrane integrity and increase membrane permeability, while tannins may interfere with fungal metabolism through interactions with cellular proteins. Saponins can alter membrane stability by interacting with membrane sterols, ultimately impairing fungal growth and survival (22). These mechanisms complement the membrane-targeting effects associated with *M. alba* compounds. Phytochemical analyses of *I. balsamina* extracts have identified various bioactive secondary metabolites with antimicrobial properties, and the strong antimicrobial effects observed are closely linked to this phytochemical profile (23).

The present results are consistent with and expand upon the findings of (24), who reported that *I. balsamina* extracts inhibited fungal pathogens under in vitro conditions. Although their study focused on *Rhizopus oryzae*, the antifungal mechanisms described, particularly membrane disruption and saponin-induced leakage of intracellular contents, are broadly relevant to *C. albicans* and align with the present results. (25) also confirmed the antifungal activity of *I. balsamina* against *C. albicans* using an in vitro antimicrobial screening method and reported inhibition zones within the moderate category, consistent with the current findings. The convergence of results across multiple studies and fungal species strengthens the evidence supporting *I. balsamina* as a promising antifungal plant. Previous studies have reported that *I. balsamina* contains various secondary metabolites with antimicrobial activity (25). In the present study, phytochemical screening confirmed the presence of phenolic compounds, tannins, and saponins, which may have contributed to the observed antifungal activity against *C. albicans*.

From a drug development perspective, the antifungal activity of *I. balsamina* demonstrated in this study, particularly its statistical superiority over both the positive control and the *M. alba* single extract, underscores its potential as a lead herbal candidate for candidiasis therapy. However, the moderate classification of the inhibition zone indicates that further optimization is necessary, possibly through bioassay-guided fractionation to identify and concentrate the most active antifungal constituents. The review by (26) identified more than 100 bioactive metabolites within the Impatiens genus, many of which have not undergone systematic antifungal evaluation, indicating considerable untapped pharmacological potential. Further studies incorporating quantitative assays, such as determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC), are needed to more precisely define its antifungal potency.

Antifungal Activity of Combination Extracts

The combination extracts at ratios of 1:3, 1:1, and 3:1 (*I. balsamina* : *M. alba*) produced mean inhibition zone diameters of 6.25 mm, 7.25 mm, and 9.91 mm, respectively, all classified as moderate antifungal activity. A clear dose-response pattern was observed, as increasing the proportion of *I. balsamina* in the mixture corresponded with a progressive increase in inhibition zone diameter. This pattern aligns with the stronger activity of *I. balsamina* as a single extract and suggests that its bioactive constituents largely determine the antifungal efficacy of the combination. Notably, the 3:1 ratio yielded a mean inhibition zone of 9.91 mm, exceeding that of the single *M. alba* extract (7.83 mm) and comparable to the ketoconazole positive control (7.75 mm), indicating that this proportion exhibited measurable antifungal activity.

The pattern of combination activity, in which the 3:1 ratio approaches but does not surpass the single *I. balsamina* extract, may suggest an additive-like interaction rather than a synergistic effect at this proportion. In pharmacological terms, a synergistic interaction produces an effect greater than the sum of individual effects, whereas an additive interaction results in an effect consistent with the combined contributions of each component. However, the nature of the interaction observed in the present study cannot be confirmed without specific interaction analyses. The slightly lower activity of the 3:1 combination compared with the single *I. balsamina* extract may reflect interactions between compounds from both extracts at the fungal cell membrane level or dilution of the most active *I. balsamina* constituents upon mixing.

The 1:3 combination, which contained a higher proportion of *M. alba* than *I. balsamina*, produced the lowest inhibition zone among the combination groups at 6.25 mm, even lower than the single *M. alba* extract alone at 7.83 mm. This reduction in activity compared with the single *M. alba* extract when a smaller proportion of *I. balsamina* was added requires careful interpretation. One possible explanation is that certain compounds in *I. balsamina*, when present in low concentrations relative to *M. alba*, may interact with *M. alba* constituents in ways that reduce the overall antifungal activity of the combination. Another possibility is that differences in polarity and solubility of the combined extracts at this ratio influence the diffusion of active compounds through the agar medium, thereby limiting the effective inhibition zone. These findings suggest that the proportion of each component substantially influences the overall antifungal activity of the combination.

Overall, the findings suggest that the interaction between *M. alba* and *I. balsamina* extracts under the tested conditions may exhibit an additive-like pattern rather than a synergistic effect. However, the nature of this interaction cannot be confirmed without specific interaction analyses. The 3:1 combination demonstrated measurable antifungal activity and produced a larger inhibition zone than the ketoconazole control. This observation may provide preliminary information for the future development of herbal antifungal formulations, as the 3:1 ratio may represent a balanced preparation in terms of efficacy and ingredient availability. Future studies should systematically assess synergism, additivity, and antagonism using methods such as the checkerboard assay or calculation of the fractional inhibitory concentration index (FICI), which would provide clearer mechanistic evidence regarding the interaction between these two plant extracts.

CLINICAL IMPLICATION

The findings of this study suggest that both *Michelia alba* and *Impatiens balsamina* extracts have potential as alternative antifungal agents against *Candida albicans*. Given the increasing resistance to conventional antifungal drugs such as azoles, these plant-derived extracts may serve as promising candidates for future antifungal development. However, additional pharmacological, toxicological, and clinical studies are required before therapeutic application can be considered.

Additionally, the observed antifungal activity of the combined extracts supports further investigation of herbal extract combinations as potential antifungal formulations. However, further studies, including determination of the minimum inhibitory concentration (MIC), toxicity evaluation, and in vivo investigations, are required before any clinical application can be considered.

LIMITATIONS

This study has several limitations that should be considered when interpreting the findings. First, the disk diffusion method used in this study is highly influenced by the ability of active compounds to diffuse through the agar medium. Therefore, the observed antifungal activity may not fully reflect the quantitative antifungal potency of the extracts, particularly for compounds with larger molecular sizes or lower solubility. Second, antifungal activity was evaluated only against *Candida albicans*, and thus the findings cannot be generalized to other pathogenic fungal species. Third, the phytochemical screening performed was qualitative in nature and did not provide information regarding the concentration of individual bioactive compounds present in the extracts. In addition, this study did not include further analyses to specifically evaluate the interactions among compounds in the extract combinations, including whether the interactions were synergistic, additive, or antagonistic. Therefore, the effectiveness and interaction profile of the combined extracts require further investigation using more comprehensive analytical methods.

CONCLUSIONS

Both single extracts of *M. alba* flower and *I. balsamina* leaf demonstrated moderate antifungal activity against *C. albicans* in vitro. The *I. balsamina* single extract showed the highest antifungal activity among all treatment groups, with a mean inhibition zone of 10.72 mm, and was statistically superior to both the *M. alba* extract and the ketoconazole positive control. Among the combination groups, the 3:1 ratio (*I. balsamina* : *M. alba*) was the most effective, producing a mean inhibition zone of 9.91 mm. The observed pattern of activity may suggest an additive-like interaction; however, this could not be confirmed without specific interaction analyses. The 1:3 ratio showed the lowest activity among the combination groups, indicating that the proportion of each extract substantially influenced the overall antifungal activity. These results highlight the potential of both plants—particularly *I. balsamina*—as promising candidates for the development of natural antifungal agents and justify further investigation through MIC determination, bioassay-guided fractionation, interaction studies, and in vivo evaluation.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

I Nyoman Wiranata: conceptualization, methodology, data collection, analysis, and original draft writing; Marta Setiabudy: supervision, methodology, review, and editing; Ni Wayan Widhidewi: supervision, review, editing, and validation. All authors approved the final manuscript.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors used ChatGPT (OpenAI, GPT-5) to assist in language editing and improving the clarity of the manuscript. The authors critically reviewed and verified all content to ensure accuracy and integrity.

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