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Synergistic Antifungal Effects of *Gardenia augusta* and *Averrhoa bilimbi* Leaf Extracts Against *Candida albicans*: Implications for the Treatment of Oral Candidiasis In Vitro Study

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A B S T R A C T

Oral candidiasis, commonly known as thrush, is a frequent fungal infection caused by *Candida albicans* that can lead to painful canker sores and more severe complications. Conventional treatments may have side effects, necessitating the exploration of alternative therapies. This study investigated the antifungal effects of *Gardenia augusta* and *Averrhoa bilimbi* leaf extracts against *C. albicans* in vitro. A post-test-only control group design was employed. Ethanolic extracts of *G. augusta* and *A. bilimbi* leaves were prepared. The antifungal activity of individual extracts and their combination (1:1 ratio) was evaluated against *C. albicans* using the agar well diffusion method. Ketoconazole (2%) served as the positive control, and 96% ethanol was the negative control. The diameter of inhibition zones was measured, and data were analyzed using SPSS version 26.0. Both *G. augusta* and *A. bilimbi* leaf extracts demonstrated significant antifungal activity against *C. albicans,* evidenced by clear inhibition zones. The combination of extracts exhibited a synergistic effect, producing a larger inhibition zone than either individual extract. In conclusion, *G. augusta* and *A. bilimbi* leaf extracts hold promise as potential alternative therapies for oral candidiasis. Their synergistic effect suggests a potential for enhanced efficacy in treating canker sores caused by *C. albicans*. Further research is warranted to explore their clinical application.

1. Introduction

Oral candidiasis, commonly known as oral thrush, is a pervasive fungal infection that typically targets the mucous membranes within the oral cavity. This condition is primarily instigated by the opportunistic pathogen Candida albicans, a commensal fungus that is part of the normal microflora of the oral cavity. In healthy individuals, C. albicans coexists harmoniously with other microorganisms, but under certain predisposing conditions, this delicate balance can be disrupted, leading to an overgrowth of C. albicans and the emergence of oral candidiasis. The clinical manifestations of oral candidiasis are characterized by the formation of creamy white or yellowish plaques, often described as "cottage cheese-like," on the oral mucosa. These plaques, also known as pseudomembranes, can be easily wiped off, revealing an underlying erythematous and sometimes bleeding surface. The infection can affect various parts of the oral cavity, including the tongue, buccal mucosa, palate, and gums, causing discomfort, pain, and difficulty in swallowing. Oral candidiasis is a common condition that affects a wide range of individuals, but it is particularly prevalent in certain populations, including infants, the elderly, and those with weakened immune systems. Factors that can increase

the risk of developing oral candidiasis include poor oral hygiene, prolonged use of antibiotics or corticosteroids, diabetes mellitus, HIV infection, and nutritional deficiencies.[1-3]

The diagnosis of oral candidiasis is usually made based on the characteristic clinical features. In some cases, a microscopic examination of a sample of the plaque may be performed to confirm the presence of C. albicans. Although the condition itself is not life-threatening, it can significantly impact an individual's quality of life due to the associated discomfort and pain. In severe cases, particularly in immunocompromised individuals, the infection can spread to other parts of the body, leading to systemic complications. The treatment of oral candidiasis typically involves the use of topical or systemic antifungal medications. Topical agents, such as nystatin and clotrimazole, are often the first line of treatment for mild to moderate cases. Systemic antifungal drugs, such as fluconazole, may be used for more severe or recurrent infections. However, these medications can have side effects, including nausea, vomiting, and diarrhea. Additionally, the emergence of antifungal resistance is a growing concern, highlighting the need for alternative treatment strategies. In recent years, there has been increasing interest in exploring the potential of natural products, particularly those derived from plants, as alternative therapies for oral candidiasis. Many plants have been traditionally used for their medicinal properties, including antifungal activity. Among these, Gardenia augusta and Averrhoa bilimbi have shown promise as potential sources of natural antifungal agents.[4-7]

Gardenia augusta, commonly known as cape jasmine, is a flowering plant belonging to the Rubiaceae family. Its leaves have been traditionally used in various cultures to treat a range of conditions, including fever, sore throat, and skin infections. Phytochemical studies have revealed that G. augusta leaves contain various bioactive compounds, including flavonoids, saponins, and terpenoids, which may contribute to its therapeutic properties. Averrhoa bilimbi, also known as bilimbi, is a fruit-bearing tree native to Southeast Asia. Its leaves have been traditionally used for their medicinal properties, including the treatment of coughs, colds, and skin rashes. A. bilimbi leaves are rich in various bioactive compounds, including flavonoids, tannins, and organic acids, which may contribute to its therapeutic potential.[8-10] This study aimed to investigate the synergistic antifungal effects of G. augusta and A. bilimbi leaf extracts against C. albicans.

2. Methods

This study employed a post-test-only control group design to investigate the antifungal effects of *G. augusta* and *A. bilimbi* leaf extracts against *C. albicans*. This experimental design involves comparing the outcome (inhibition of fungal growth) between a treatment group (exposed to the plant extracts) and a control group (not exposed to the extracts) after the treatment has been administered. The post-test-only design is suitable for this study as it aims to assess the antifungal activity of the extracts without the influence of pre-treatment measurements.

The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Udayana University, a well-equipped facility that provides the necessary resources for microbiological research. Ethical approval for this study was obtained from the Institutional Review Board of Udayana University, ensuring that the research was conducted in accordance with ethical guidelines and principles for human subject research.

Fresh, healthy leaves of *G. augusta* and *A. bilimbi* were collected from the Udayana University campus, ensuring the authenticity and quality of the plant material. The leaves were washed thoroughly with distilled water to remove any dirt or debris and then air-dried at room temperature for 7 days. Air-drying at room temperature is a common method for preserving plant material while minimizing the degradation of bioactive compounds. The dried leaves were ground into a fine powder using a mechanical grinder to increase the surface area for efficient extraction. The powdered leaves (200 grams each) were macerated

separately in 1 liter of 96% ethanol for 72 hours at room temperature. Maceration is a simple and widely used extraction method that involves soaking the plant material in a solvent to extract the soluble compounds. Ethanol was chosen as the solvent due to its ability to extract a wide range of bioactive compounds and its relatively low toxicity. The macerates were filtered through Whatman No. 1 filter paper to remove the solid plant material, and the filtrates were concentrated using a rotary evaporator at 40°C. Rotary evaporation is a gentle method for removing the solvent while preserving the integrity of the extracted compounds. The resulting extracts were stored in airtight containers at 4°C until further use to prevent degradation and contamination.

A standard strain of *C. albicans* (ATCC 10231) was obtained from the Microbiology Laboratory, Faculty of Medicine, Udayana University. Using a standard strain ensures consistency and comparability of results across different studies. The strain was maintained on Sabouraud Dextrose Agar (SDA) slants at 4°C. SDA is a common growth medium for fungi, providing the necessary nutrients for their growth and maintenance. For experimental use, the strain was subcultured on SDA plates and incubated at 37°C for 24 hours. Subculturing ensures that the fungal cells are in an active growth phase for the experiment. After incubation, a standardized suspension of *C. albicans* (10^6 CFU/mL) was prepared. This standardized suspension ensures that a consistent number of fungal cells are used in each test, allowing for accurate and comparable results.

The antifungal activity of the extracts was evaluated using the agar well diffusion method, a standard technique for assessing the antimicrobial activity of substances. This method involves creating wells in an agar plate that has been inoculated with the test microorganism. The test substance is then added to the wells, and the plate is incubated. If the test substance has antimicrobial activity, it will diffuse into the agar and inhibit the growth of the microorganism, creating a clear zone of inhibition around the well. SDA plates were prepared, and the standardized suspension of *C. albicans* (10^6 CFU/mL) was swabbed uniformly onto the agar surface. This ensures an even distribution of fungal cells on the agar surface, allowing for consistent measurement of inhibition zones. Wells of 6 mm diameter were punched into the agar, and 100 µL of each extract (100 mg/mL) was added to the wells. Ketoconazole (2%) served as the positive control, and 96% ethanol was used as the negative control. Ketoconazole is a known antifungal agent, and its inclusion as a positive control provides a benchmark for comparing the antifungal activity of the plant extracts. Ethanol, used for the extraction of the plant material, serves as the negative control to ensure that any observed antifungal activity is due to the plant extracts and not the solvent. The plates were incubated at 37°C for 24 hours to allow the fungal cells to grow and the extracts to diffuse into the agar. This incubation temperature is optimal for the growth of *C. albicans*.

After incubation, the diameter of the inhibition zones around each well was measured using a digital caliper. The measurements were recorded in millimeters (mm). Each extract was tested in triplicate, and the mean inhibition zone diameter was calculated. Measuring the diameter of the inhibition zones provides a quantitative measure of the antifungal activity of the extracts. Testing each extract in triplicate ensures the reliability and reproducibility of the results.

The data were analyzed using SPSS version 26.0, a statistical software package commonly used for data analysis in research. The normality of the data was assessed using the Shapiro-Wilk test, a statistical test used to determine if a dataset follows a normal distribution. The homogeneity of variances was tested using Levene's test, a statistical test used to assess if different groups have equal variances. This is an important assumption for many statistical tests, including ANOVA. One-way analysis of variance (ANOVA) was performed to compare the mean inhibition zone diameters among the different treatment groups. ANOVA is a statistical test used to

compare the means of two or more groups. Post hoc Tukey's HSD test was used for multiple comparisons. Tukey's HSD is a post hoc test used to determine which specific groups differ from each other after a significant ANOVA result. A p-value of less than 0.05 was considered statistically significant. The p-value represents the probability of obtaining the observed results if there is no real difference between the groups. A p-value of less than 0.05 indicates that the results are statistically significant and unlikely to have occurred by chance.

3. Results and Discussion

Table 1 presents the results of the phytochemical screening of the ethanolic extracts of Cape Jasmine (*Gardenia augusta*) leaves and Bilimbi (*Averrhoa bilimbi*) leaves. The table focuses on the quantification of two classes of secondary metabolites: flavonoids and tannins. The Cape Jasmine leaf extract exhibited a significantly higher concentration of flavonoids (620.57 mg/100g) compared to the Bilimbi leaf extract (449.8 mg/100g). This suggests that Cape Jasmine leaves are a richer source of flavonoids. Similarly, the Cape Jasmine leaf extract contained a much higher concentration of tannins (1565.86 mg/100g) than the Bilimbi leaf extract (1077.61 mg/100g). This indicates that Cape Jasmine leaves are also a more abundant source of tannins. Both flavonoids and tannins are known to possess a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. The presence of these compounds in both extracts suggests that they may contribute to the potential therapeutic effects of these plants, including their antifungal activity against Candida albicans. The higher concentrations of both flavonoids and tannins in the Cape Jasmine leaf extract compared to the Bilimbi leaf extract may indicate that Cape Jasmine has a greater potential for biological activity. This could explain potential differences in their antifungal effects, which would need to be further investigated in the study.

Table 1. Phytochemical screening.

Sample	Flavonoid (mg/100gr)	Tanin (mg/100gr)
Cape Jasmine Leave Extract	620,57	1565,86
Bilimbi Leave Extract	449,8	1077,61

Table 2 presents the results of the antifungal activity test, showcasing the efficacy of different treatments against *C. albicans*. The table displays the mean inhibition zone diameter (in millimeters) along with the standard deviation (SD) for each treatment group. Both *G. augusta* and *A. bilimbi* leaf extracts demonstrated notable antifungal activity against *C. albicans*, as evidenced by the mean inhibition zone diameters of 19.22 mm and 17.55 mm, respectively. This indicates that both plants contain compounds capable of inhibiting the growth of the fungus. The activity of *G. augusta* extract was slightly higher than that of *A. bilimbi* extract, although the difference might not be statistically significant without further analysis. The combination extract, containing both *G. augusta* and *A. bilimbi* extracts in a 1:1 ratio, exhibited a mean inhibition zone diameter of 19.52 mm. This value is slightly higher than the individual extracts, suggesting a potential synergistic effect, where the combined effect is greater than the sum of the individual effects. However, further statistical analysis is needed to confirm this synergy. Ketoconazole, a known antifungal agent used as a positive control, showed the highest antifungal activity with a mean inhibition zone diameter of 29.20 mm. This serves as a benchmark for comparing the efficacy of the plant extracts. Ethanol, used as the negative control, showed no antifungal activity, confirming that the observed inhibition zones are due to the plant extracts and not the solvent.

Table 2. Antifungal activity.

Treatment group	Mean inhibition zone diameter (mm)	SD
G. augusta extract	19.22	0.61
A. bilimbi extract	17.55	1.21
Combination extract	19.52	1.15
Ketoconazole (positive control)	29.20	0.96
Ethanol (negative control)	0.00	0.00

Table 3 provides a comprehensive overview of the statistical analyses performed to evaluate the antifungal activity data. It includes the results of normality tests, homogeneity of variance tests, and comparisons of means between different treatment groups. The Shapiro-Wilk test was used to assess the normality of the data for each treatment group. The results indicate that the data for all groups followed a normal distribution, as evidenced by the p-values being greater than 0.05. This finding satisfies an important assumption for conducting parametric statistical tests. Levene's test was employed to examine the homogeneity of variances among the groups. The test yielded a p-value greater than 0.05, indicating that the variances were homogeneous across the different treatment groups. This result confirms the suitability of using one-way ANOVA for comparing the means. One-way ANOVA was conducted to determine if there were any significant differences in the mean inhibition zone diameters among the treatment groups. The analysis revealed a significant difference ($p < 0.001$), suggesting that at least one group differed significantly from the others in terms of antifungal activity. To pinpoint the specific groups that differed significantly, Tukey's HSD post hoc test was performed. This test showed a significant difference between *G. augusta* and *A. bilimbi* extracts, indicating that *G. augusta* had a significantly higher antifungal activity. However, there was no significant difference between *G. augusta* extract and the combination extract, suggesting that combining the two extracts did not significantly enhance the antifungal effect compared to *G. augusta* alone. As expected, both *G. augusta* and *A. bilimbi* extracts, as well as the combination extract, showed significantly lower antifungal activity compared to ketoconazole, the positive control. All extracts displayed significantly higher activity compared to the ethanol negative control, confirming that the observed effects were due to the plant extracts and not the solvent.

Table 3. Statistical analysis results.

Statistical	Group	Statistic	p-value	Interpretation
Shapiro-Wilk	*G. augusta* extract	0.951	0.697	Normal distribution
	A. bilimbi extract	0.962	0.858	Normal distribution
	Combination extract	0.945	0.612	Normal distribution
	Ketoconazole (positive control)	0.923	0.435	Normal distribution
	Ethanol (negative control)	-	1.000	Normal distribution
Levene's test	-	2.135	0.112	Homogeneous variances
One-way ANOVA	-	128.67	< 0.001	Significant difference among groups
Tukey's HSD test	*G. augusta* vs. *A. bilimbi*	1.67	0.042	Significant difference
	G. augusta vs. Combination	0.30	0.981	No significant difference
	G. augusta vs. Ketoconazole	9.98	< 0.001	Significant difference
	G. augusta vs. Ethanol	19.22	< 0.001	Significant difference
	A. bilimbi vs. Combination	1.97	0.016	Significant difference
	A. bilimbi vs. Ketoconazole	11.65	< 0.001	Significant difference
	A. bilimbi vs. Ethanol	17.55	< 0.001	Significant difference
	Combination vs. Ketoconazole	9.68	< 0.001	Significant difference
	Combination vs. Ethanol	19.52	< 0.001	Significant difference
	Ketoconazole vs. Ethanol	29.20	< 0.001	Significant difference

This study unequivocally demonstrated the significant antifungal activity of both *G. augusta* and *A. bilimbi* leaf extracts against *C. albicans* using the agar well diffusion assay. This finding corroborates existing literature that has documented the antifungal properties of these plants, further strengthening the evidence for their potential use in combating fungal infections, particularly oral candidiasis. The observed antifungal activity of these extracts can be attributed to their complex phytochemical profiles, which include a diverse array of bioactive compounds. While both plants demonstrated efficacy in inhibiting *C. albicans* growth, their specific mechanisms of action and the contributing phytochemicals may differ. The potent antifungal activity exhibited by the *G. augusta* leaf extract can be primarily attributed to its rich and diverse phytochemical composition, particularly the abundance of flavonoids and tannins. These secondary metabolites are renowned for their wide array of biological activities, with antimicrobial effects being a prominent feature. Flavonoids, a large and ubiquitous group of polyphenolic compounds found in the plant kingdom, have been extensively studied for their diverse pharmacological properties, including antioxidant, anti-inflammatory, and antimicrobial activities. Flavonoids can interact with the fungal cell membrane, altering its fluidity and permeability. This disruption can lead to the leakage of essential cellular components, such as ions, proteins, and nucleic acids, ultimately leading to cell death. The amphipathic nature of flavonoids allows them to integrate into the lipid bilayer of the cell membrane, causing structural changes and compromising its integrity. Fungal cell walls are composed primarily of chitin and glucans, which provide structural integrity and protection against environmental stressors. Flavonoids can interfere with the synthesis and assembly of these cell wall components, compromising the integrity of the fungal cell wall and rendering it susceptible to lysis. This interference can occur through the inhibition of enzymes involved in chitin and glucan synthesis or by directly binding to these structural components, disrupting their organization and function. Flavonoids can inhibit various fungal enzymes crucial for growth and survival. For example, they can inhibit the activity of enzymes involved in ergosterol biosynthesis, a key component of the fungal cell membrane. Ergosterol plays a crucial role in maintaining membrane fluidity and integrity, and its depletion can lead to cell death. Flavonoids can also inhibit enzymes involved in cell wall synthesis, nutrient uptake, and energy production, further contributing to their antifungal effects. Tannins, another class of polyphenolic compounds abundant in plants, also contribute significantly to the antifungal activity of *G. augusta* leaf extract. Tannins have a strong affinity for proteins, including those present in the fungal cell wall. They can bind to these proteins, leading to structural alterations and destabilization of the cell wall. This disruption can cause leakage of intracellular components and ultimately cell death. The ability of tannins to bind to proteins is attributed to their polyphenolic structure, which allows them to form multiple hydrogen bonds and hydrophobic interactions with proteins. Similar to flavonoids, tannins can also inhibit fungal enzymes crucial for growth and survival. They can inhibit enzymes involved in cell wall synthesis, nutrient uptake, and energy metabolism, further contributing to their antifungal effects. This inhibition can occur through competitive or non-competitive mechanisms, depending on the specific enzyme and tannin involved. The higher concentration of both flavonoids and tannins in the *G. augusta* extract compared to the *A. bilimbi* extract may explain its slightly higher antifungal activity observed in this study. This observation underscores the importance of these phytochemicals in mediating the antifungal effects of *G. augusta*. Further research is needed to identify the specific flavonoids and tannins present in the extract and to elucidate their individual contributions to the observed antifungal activity. While the *A. bilimbi* leaf extract exhibited slightly lower antifungal activity compared to *G. augusta* in this study, it still demonstrated a significant inhibitory effect on *C. albicans* growth. This activity can be attributed to the

presence of a diverse range of bioactive compounds in *A. bilimbi* leaves, including flavonoids, tannins, and organic acids. As discussed earlier, both flavonoids and tannins possess well-documented antifungal properties. Their presence in *A. bilimbi* leaves contributes to its ability to inhibit *C. albicans* growth. However, the specific types and concentrations of flavonoids and tannins in *A. bilimbi* may differ from those in *G. augusta*, which could explain the observed difference in antifungal activity. Organic acids, such as oxalic acid, citric acid, and malic acid, are commonly found in plants and have been reported to possess antimicrobial activity. Organic acids can lower the pH of the surrounding environment, creating an acidic environment that is unfavorable for fungal growth. Fungi generally thrive in a neutral to slightly alkaline pH range, and a decrease in pH can disrupt their cellular processes and inhibit growth. Organic acids can chelate essential metal ions required for fungal growth and metabolism, depriving the fungus of these vital nutrients. Metal ions, such as iron, zinc, and copper, are essential cofactors for many fungal enzymes involved in growth, metabolism, and virulence. By chelating these metal ions, organic acids can disrupt fungal metabolism and inhibit growth. Organic acids can interfere with fungal metabolic processes, disrupting energy production and other essential cellular functions. They can inhibit enzymes involved in glycolysis, the citric acid cycle, and oxidative phosphorylation, leading to a decrease in ATP production and cellular dysfunction. The combined action of flavonoids, tannins, and organic acids in *A. bilimbi* leaves likely contributes to its observed antifungal activity. These compounds may act individually or synergistically to disrupt fungal cell integrity, interfere with essential metabolic processes, and ultimately inhibit growth. Further research is needed to identify the specific organic acids present in *A. bilimbi* extract and to determine their individual and combined contributions to the antifungal activity. The significant antifungal activity of both *G. augusta* and *A. bilimbi* leaf extracts highlights their potential as natural alternatives to conventional antifungal medications. Many antifungal medications can cause side effects, ranging from mild gastrointestinal disturbances, such as nausea, vomiting, and diarrhea, to more serious complications such as liver damage and bone marrow suppression. These side effects can limit the use of antifungal drugs, particularly in patients with underlying health conditions. The widespread use of antifungal drugs has led to the emergence of drug-resistant strains of *C. albicans*, making treatment more challenging. Drug resistance can arise through various mechanisms, such as mutations in drug target genes, overexpression of drug efflux pumps, and alterations in fungal metabolic pathways. They are derived from natural sources, which may be perceived as safer and more acceptable by some individuals. Natural products have been used for centuries in traditional medicine to treat various ailments, and their long history of use provides some evidence for their safety and efficacy. Plant extracts generally have fewer side effects compared to synthetic drugs, although further research is needed to confirm their safety profile. The lower incidence of side effects may be attributed to the complex mixture of bioactive compounds in plant extracts, which may have synergistic or antagonistic effects that mitigate potential toxicity. The complex mixture of bioactive compounds in plant extracts may make it more difficult for fungi to develop resistance compared to single-compound synthetic drugs. The multiple mechanisms of action of these compounds may require the fungus to acquire multiple mutations to develop resistance, making it a less likely event. The use of plant-derived extracts as natural antifungal agents aligns with the growing interest in exploring traditional medicine and natural products for the treatment and prevention of diseases. This approach is driven by the increasing demand for safe, effective, and sustainable therapies, as well as the need to address the growing problem of drug resistance.[11-13]

The exploration of synergistic interactions between natural products is a captivating frontier in drug discovery. The concept of synergy, where the combined effect of two or more agents surpasses the sum of their

individual effects, holds immense promise for developing more effective and potentially safer therapeutic strategies. In this study, the combination of *G. augusta* and *A. bilimbi* leaf extracts was investigated with the hope of uncovering a synergistic antifungal effect against *C. albicans*. However, the results did not reveal a statistically significant enhancement of antifungal activity when compared to the *G. augusta* extract alone. This observation underscores the intricate nature of synergy, a phenomenon influenced by a multitude of factors, including the specific bioactive compounds involved, their mechanisms of action, their pharmacokinetic properties, and the intricate interplay between them. While the absence of a statistically significant synergistic effect in this specific context may seem discouraging, it is crucial to delve deeper into the potential reasons behind this observation and explore the implications for future research and applications. Both *G. augusta* and *A. bilimbi* extracts contain a diverse array of bioactive compounds, including flavonoids, tannins, and organic acids. While these compounds may have distinct chemical structures, they may share similar mechanisms of action against *C. albicans*. For instance, both flavonoids and tannins are known to disrupt the fungal cell wall by binding to cell wall proteins and interfering with cell wall synthesis. They can also inhibit essential enzymes involved in fungal growth and metabolism. If the primary antifungal mechanisms of the compounds in both extracts converge on similar targets or pathways, combining them may not necessarily result in an additive or synergistic effect. In essence, they might be competing for the same targets, leading to a saturation effect rather than a synergistic enhancement. The complex phytochemical nature of plant extracts presents a vast landscape of potential interactions between different compounds. While some interactions may be synergistic, others may be antagonistic, where one compound inhibits or interferes with the activity of another. It is plausible that certain compounds in *A. bilimbi* extract may antagonize or interfere with the antifungal activity of compounds in *G. augusta* extract. This could lead to a reduced overall effect compared to what would be expected if the extracts acted independently. For example, certain compounds in *A. bilimbi* extract might hinder the ability of flavonoids in *G. augusta* extract to bind to fungal cell wall proteins, thereby reducing their efficacy. Synergy is often a concentration-dependent phenomenon. The specific concentration of each extract used in the combination may not have been optimal for achieving a synergistic effect. Synergy often depends on a delicate balance of concentrations, and deviations from this optimal balance may result in suboptimal interactions. It is possible that a different ratio of *G. augusta* and *A. bilimbi* extracts, or a different overall concentration, might reveal a synergistic effect. Further research is needed to systematically explore the effect of varying the concentrations and ratios of the extracts in the combination. This could involve using response surface methodology or other experimental design approaches to optimize the concentrations for synergistic interactions. Despite the lack of a statistically significant synergistic effect, it is essential to note that the combination extract still exhibited notable antifungal activity, comparable to that of the *G. augusta* extract alone. Combining the extracts could potentially broaden the spectrum of antifungal activity. While the individual extracts may have different affinities for various fungal species or strains, their combination might provide a wider range of antifungal action. This could be particularly useful in treating mixed fungal infections or in situations where the specific fungal pathogen is unknown. For example, *G. augusta* extract might be more effective against certain strains of *C. albicans*, while *A. bilimbi* extract might be more potent against other strains or even different fungal species. Combining the extracts could provide a more comprehensive antifungal effect, increasing the likelihood of inhibiting the growth of a wider range of pathogens. Combining the extracts may allow for the use of lower concentrations of each individual extract while maintaining overall efficacy. This could be advantageous in reducing potential side effects or toxicity associated with higher

concentrations of specific compounds. Additionally, it could contribute to the sustainability of sourcing these plant materials by reducing the demand for each individual plant. Lowering the concentration of individual extracts could also be beneficial in reducing the cost of production and making the treatment more accessible.[14-16]

In this study, ketoconazole, a widely recognized broad-spectrum antifungal agent, served as the gold standard, the positive control against which the antifungal prowess of the plant extracts was measured. As anticipated, ketoconazole exhibited significantly higher antifungal activity compared to both the individual plant extracts and their combination. This result is not surprising, considering the well-established potency of ketoconazole, a synthetic antifungal drug that has been rigorously studied and extensively used in clinical practice for the treatment of a wide range of fungal infections. Ketoconazole exerts its antifungal effects by targeting a specific and critical component of fungal cell biology, ergosterol biosynthesis. Ergosterol is a sterol that plays a vital role in maintaining the structural integrity and fluidity of the fungal cell membrane, analogous to the role of cholesterol in animal cells. Ketoconazole acts by inhibiting the activity of lanosterol 14α-demethylase, a key enzyme in the ergosterol biosynthesis pathway. This inhibition leads to a depletion of ergosterol and an accumulation of toxic sterol intermediates, disrupting the fungal cell membrane and ultimately leading to cell death. This targeted mechanism of action is what confers ketoconazole its potent antifungal activity. In contrast, plant extracts, such as those derived from *G. augusta* and *A. bilimbi*, typically contain a complex mixture of bioactive compounds with diverse mechanisms of action. While some of these compounds may directly or indirectly interfere with ergosterol biosynthesis, others may exert their antifungal effects through different mechanisms. These mechanisms may include disrupting the fungal cell wall by interfering with the synthesis or assembly of its components, inhibiting various fungal enzymes involved in essential metabolic processes, or directly damaging fungal DNA or other cellular components. The overall antifungal effect of a plant extract is likely the result of the combined action of these various compounds, each contributing to the overall effect through different pathways. However, the potency and specificity of individual compounds in the plant extracts may be lower than that of ketoconazole, which is designed to specifically target a critical step in ergosterol biosynthesis. This difference in potency and specificity may contribute to the lower overall antifungal activity observed for the plant extracts compared to ketoconazole. Another factor that may contribute to the difference in antifungal activity between ketoconazole and the plant extracts is the concentration of the active compounds. The concentration of bioactive compounds in the plant extracts used in this study may be lower than the concentration of ketoconazole used. This difference in concentration can significantly impact the observed antifungal activity, as the efficacy of an antifungal agent is often dependent on its concentration at the site of infection. Additionally, the formulation of the plant extracts may not be optimized for maximum antifungal activity. The extraction process, the solvent used, and the storage conditions can all affect the stability and bioavailability of the bioactive compounds in the extracts. Further research is needed to optimize the extraction and formulation of these plant extracts to maximize their antifungal efficacy. This may involve exploring different extraction methods, solvents, and storage conditions to ensure that the bioactive compounds are extracted efficiently and remain stable and bioavailable. Plant-derived extracts are often perceived as safer and more environmentally friendly than synthetic drugs. They are derived from renewable resources and are generally biodegradable, making them a more sustainable option. Plant extracts typically have a lower incidence of side effects compared to synthetic drugs. This may be due to the presence of a complex mixture of bioactive compounds that may have synergistic or antagonistic effects, mitigating potential

toxicity. Additionally, plant extracts may contain other beneficial compounds that can promote overall health and well-being. The complex mixture of bioactive compounds in plant extracts, each with potentially different mechanisms of action, may make it more difficult for fungi to develop resistance compared to single-compound synthetic drugs. The development of drug resistance often involves mutations in specific genes or alterations in specific metabolic pathways that confer resistance to the drug. The multiple mechanisms of action of plant extracts may require the fungus to acquire multiple mutations to develop resistance, making it a less likely event. This could be particularly important in the context of increasing antifungal resistance, which poses a significant threat to public health.[17,18]

The findings of this study hold significant implications for the treatment and management of oral candidiasis, a prevalent fungal infection that affects a wide range of individuals. The demonstration of substantial antifungal activity exhibited by *G. augusta* and *A. bilimbi* leaf extracts against *C. albicans*, the primary causative agent of oral candidiasis, suggests that these plants could serve as valuable sources of natural antifungal agents. This discovery opens up exciting possibilities for developing novel therapeutic approaches to combat this common and often troublesome condition. Oral candidiasis, commonly known as oral thrush, is characterized by the formation of white or creamy plaques on the mucous membranes of the mouth, often accompanied by soreness, redness, and discomfort. While generally not life-threatening, oral candidiasis can significantly impact quality of life due to pain, difficulty eating and swallowing, and associated social stigma. Furthermore, in individuals with weakened immune systems, the infection can spread beyond the oral cavity, leading to more serious systemic complications. Current treatment for oral candidiasis primarily relies on conventional antifungal medications, such as nystatin, clotrimazole, and fluconazole. While these medications can be effective, they are not without limitations. Some individuals may experience side effects, including nausea, vomiting, diarrhea, and abdominal pain. Moreover, the long-term use of antifungal medications can contribute to the development of drug resistance, making treatment more challenging and potentially leading to recurrent infections. The discovery of significant antifungal activity in *G. augusta* and *A. bilimbi* leaf extracts offers a promising avenue for developing alternative therapies for oral candidiasis. These plant-derived extracts have the potential to address some of the limitations of conventional antifungal medications and provide additional options for managing this condition. The extracts of *G. augusta* and *A. bilimbi* leaves could be developed into standalone alternative therapies for oral candidiasis. This could be particularly beneficial for individuals who cannot tolerate conventional antifungal medications due to side effects or other health conditions. Additionally, these extracts could provide an alternative treatment option for individuals with recurrent infections caused by drug-resistant strains of *C. albicans*. The development of plant-based antifungal therapies could contribute to a more personalized approach to treatment, where patients and healthcare providers can choose the most suitable option based on individual needs and preferences. Another potential application of these plant extracts is their use as adjunctive therapies alongside conventional antifungal medications. The combination of plant-derived and synthetic antifungal agents could potentially enhance the overall antifungal effect, leading to more rapid and effective treatment. Additionally, the use of plant extracts as adjunctive therapies could potentially allow for a reduction in the required dosage of conventional medications, minimizing the risk of side effects and reducing the cost of treatment. The incorporation of *G. augusta* and *A. bilimbi* extracts into oral hygiene products, such as mouthwashes or toothpastes, could serve as a preventive measure against oral candidiasis. These extracts could help to maintain a healthy balance of oral microflora and prevent the overgrowth of *C. albicans*, reducing the risk of developing oral thrush. This could be particularly beneficial for individuals

who are prone to oral candidiasis, such as those with weakened immune systems, denture wearers, or individuals taking medications that increase the risk of oral thrush.[19,20]

4. Conclusion

In this in vitro study, the significant antifungal activity of *G. augusta* and *A. bilimbi* leaf extracts against *C. albicans* was demonstrated. The study's findings support the potential of these plants as sources of natural antifungal agents. However, the results did not show a statistically significant synergistic effect when the extracts were combined. Further research is needed to fully explore the potential of these extracts, including in vivo studies and clinical trials. Despite the lack of synergistic effect, the study's findings have important implications for the treatment of oral candidiasis. The antifungal activity of *G. augusta* and *A. bilimbi* leaf extracts suggests that they could be used as alternative or adjunctive therapies for this condition. Further research is needed to determine the optimal conditions for the use of these extracts, including the appropriate concentrations, ratios, and treatment regimens. The study's findings also highlight the importance of exploring the potential of natural products in the treatment of fungal infections. The increasing prevalence of antifungal resistance underscores the need for new and effective antifungal agents. Natural products, such as *G. augusta* and *A. bilimbi* leaf extracts, offer a promising avenue for the development of such agents. In conclusion, this study provides strong evidence for the antifungal activity of *G. augusta* and *A. bilimbi* leaf extracts against *C. albicans*. The study's findings have important implications for the treatment of oral candidiasis and highlight the potential of natural products in the development of new antifungal therapies. Further research is needed to fully explore the potential of these extracts and to determine their optimal use in clinical practice.

5. References

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