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Antifungal activity of aqueous and ethanolic garlic extracts against *Candida albicans*

TYRESE NASHAWN SUCHIT[1], SABRINA DOOKIE[1,♥], ABDULLAH ADIL ANSARI[1], CHARLAN ABRAMS[2]

[1]Department of Biology, Faculty of Natural Sciences, University of Guyana. Turkeyen Campus, Georgetown, Guyana.
Tel.: +592-222-5444 ext 4494, ♥email: sabrina.dookie@uog.edu.gy
[2]College of Medical Sciences, University of Guyana. Turkeyen Campus, Georgetown, Guyana

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Abstract. *Suchit TN, Dookie S, Ansari AA, Abrams C. 2025. Antifungal activity of aqueous and ethanolic garlic extracts against* Candida albicans*. Asian J Trop Biotechnol 22: 94-101*. The growing incidence of antifungal resistance threatens the effectiveness of conventional applications and treatments. This study evaluated the antifungal activity of aqueous and ethanolic *Allium sativum* (garlic) extracts as potential natural alternatives against the common fungus *Candida albicans.* Disk diffusion assays and Minimum Inhibitory Concentration (MIC) tests were used to determine the antifungal efficacy of the extracts at four concentrations (100%, 75%, 50% and 25%) and five serial dilutions (50%, 25%, 12.5%, 6.25% and 3.125%), respectively. Our results revealed that both extracts showed concentration-dependent inhibition from 25% upwards. However, ethanolic extracts produced larger zones of inhibition (30.0±0.0 mm) than the aqueous extracts (27.0±1.0 mm). When compared to both garlic extracts, the control Fluconazole exhibited minimal activity (3.0±0.5 mm), suggesting possible resistance of the tested *Candida* strain or disk preparation limitations. Statistical analyses (one-way ANOVA, Tukey HSD, and Welch t-test) further confirmed significant differences between treatments ($p<0.001$). Our results indicate that garlic extracts are promising antifungal agents and broader exploration of garlic-derived treatments through in vivo research could contribute to developing natural, affordable, sustainable antifungal therapies for both healthcare systems and communities affected by drug-resistant fungal infections.

Keywords: *Allium sativum*, antifungal activity, antifungal resistance, antifungal susceptibility, *Candida albicans*

INTRODUCTION

Fungal infections caused by *Candida albicans* present significant concerns in both medical and public health contexts. *C. albicans* is an opportunistic fungal pathogen that forms part of the normal human microbiota, typically inhabiting areas such as the oral cavity, gastrointestinal tract, and genital regions (Mayer et al. 2013). However, under conditions that compromise immunity or disrupt microbial balance, this fungus can proliferate and cause infections ranging from superficial mucosal infections to life-threatening systemic candidiasis (Talapko et al. 2021). The clinical burden of candidiasis has grown steadily in recent decades, particularly in immunocompromised populations such as individuals with HIV/AIDS, transplant recipients, and cancer patients undergoing chemotherapy. These infections can be challenging to treat, and their ongoing resistance highlights the urgent need for new and accessible antifungal strategies (Gupta and Birdi 2017). Current treatment approaches rely heavily on manufactured antifungal agents such as azoles, echinocandins, and polyenes. Among these, Fluconazole remains one of the most widely prescribed antifungal drugs due to its relatively low toxicity, oral availability, and effectiveness against a range of *Candida* species (Hasan et al. 2023). However, the rising issue of antifungal resistance has reduced the long-term reliability of these drugs.

There is a growing interest in exploring natural products as potential alternative or complementary antifungal agents. Garlic (*Allium sativum*) has a long history of use in traditional medicine, valued for its antimicrobial, antiviral, and antifungal properties (Agustantina and Soekartono 2021). Its bioactive compounds, particularly allicin, have been reported to exert inhibitory effects on a wide range of pathogens, including fungi. Allicin is produced enzymatically when garlic is crushed or chopped, and its antimicrobial action has been actively linked to the disruption of microbial enzymes and cell growth inhibition (Leontiev et al. 2018; Magryś et al. 2021). The choice of solvent plays a key role in the extraction of bioactive compounds from natural products. Different solvents vary in polarity, which influences the types of compounds extracted and their potential biological activity (Vidaković et al. 2024). For example, Lee et al. (2024) reported that water and ethanol are two of the most commonly used solvents for plant extractions, with water typically yielding polar compounds and ethanol extracting a broader range of both polar and non-polar compounds. Comparing aqueous and ethanol garlic extracts, therefore, allows for a thorough understanding of how extraction methods influence the antifungal activity of a particular extract. This provides valuable insights into which preparation methods may be most suitable for developing practical antifungal treatments from garlic.

Despite growing literature on the medicinal effects of natural products such as garlic, few studies have compared aqueous and ethanolic extractions using parallel disk diffusion and Minimum Inhibitory Concentration (MIC)

assays on *C. albicans* isolates. This highlights a significant knowledge gap between traditional usage and evidence-based selections for natural antifungal applications, especially in Guyana. Within this contention, our study aims to (i) investigate the antifungal effects of ethanolic and aqueous garlic extracts against *C. albicans* using disk diffusion assays at four concentrations [500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%)], (ii) compare the antifungal effects of both ethanolic and aqueous garlic extracts against *C. albicans* using MIC assays (broth macrodilution) under five serial dilutions [250 mg/mL (50%), 125 mg/mL (25%), 62.5 mg/mL (12.5%), 31.25 mg/mL (6.25%), and 15.62 mg/mL (3.125%)]; and (iii) evaluate the effectiveness of both garlic extracts against the commercial antifungal drug Fluconazole under standard laboratory conditions. Our study contributes to the broader search for accessible, plant-based antifungal agents that could complement or provide alternatives to conventional drugs. Beyond the immediate scientific findings from the local perspective (Guyana), our research holds potential implications for global health, particularly in developing countries where reliance on affordable and naturally derived remedies may become increasingly important in addressing the challenges of antifungal resistance.

MATERIALS AND METHODS

Collection and maintenance of fungal culture

A pure culture of *C. albicans* (ATCC 10453) was obtained from the Georgetown Public Hospital (Georgetown, Guyana). To ensure viability and freshness, this sample was subcultured and maintained on sterile Potato Dextrose Agar (PDA) at 4°C.

Preparation of ethanolic and aqueous garlic extracts

The method for the preparation of the garlic extracts was adapted from Allen et al. (2023). Fresh, dry garlic bulbs (*A. sativum*) were obtained from a supermarket in Georgetown (Guyana) and cleaned thoroughly. The garlic cloves selected were free from blemishes, discoloration, and damage caused by disease, physical or chemical conditions present in the environment. The cloves were kept in a sterile environment at room temperature and were weighed to prepare the stock extracts at a standard concentration of 500 mg/mL (w/v). This was done using 50 g of peeled garlic cloves, which were homogenized with 100 mL of solvent to produce the initial stock solution (50 g/100 mL = 500 mg/mL). Two solvents, ethanol and distilled water, were selected based on their differential polarity and their efficiency at extracting bioactive compounds. Ethanol is known to extract a broad range of phytochemicals, including organosulfur compounds, while water is a traditional solvent for aqueous extracts and is known to mimic physiological conditions (Chemat et al. 2019; Plaskova and Mlcek 2023). The extracts were then stored in separate sterile labelled amber bottles and placed in a refrigerator at 4°C for 24 hrs to allow proper extraction of the bioactive compounds. After 24 hrs, the homogenate was filtered through Whatman No. 1 filter paper to remove particulate matter. This 500 mg/mL stock solution was subsequently diluted with the respective solvent to prepare working concentrations of 75% (375 mg/mL), 50% (250 mg/mL), and 25% (125 mg/mL) for antimicrobial testing.

Preparation of culture medium

Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB)

A total of 65 g of Sabouraud Dextrose Agar (SDA) was added to 1000 mL of distilled water. The mixture was then heated until the agar was dissolved. It was then autoclaved at 121°C at 12 psi for 15 mins, after which it was cooled to 55°C before being poured into the petri plates using aseptic techniques. 30 g of Sabouraud Dextrose Broth (SDB) was weighed and added to 1000 mL of distilled water. The mixture was heated until the powder was dissolved. One mL of broth was poured into 30 test tubes, which were then autoclaved at 121°C at 12 PSI for 15 minutes (Allen et al. 2023; Alvarez et al. 2024).

Broth macrodilution assay: Minimum Inhibitory Concentration (MIC)

Seven sterile labelled test tubes containing 1 mL SDB were arranged for the dilution series, and two of which were controls. In the first tube for the dilution series, 1 mL of broth was mixed with 1 mL of the appropriate garlic extract. A two-fold serial dilution was then performed by transferring 1 mL from the first tube into the second, mixing well, and repeating this step successively down the line until the fifth tube, after which 1 mL was subsequently discarded. Tubes were prepared in triplicate for both extracts to ensure uniformity across both test sets (Trivedi et al. 2015).

A 0.5 McFarland turbidity was used to standardise the *C. albicans* inoculum and was adjusted to a concentration of approximately 1.0×10^6 cells/mL. Colonies of *C. albicans* were transferred into a test tube containing SDB using a sterilized inoculating loop, and the suspension was mixed. 1 mL of the inoculated broth was then added to each of the fifteen tubes. The final volume present in each tube was 2 mL (1 mL broth + extract and 1 ml inoculum containing), with the final inoculum concentration being 5×10^5 cells/mL (Berkow et al. 2020) (Table 1).

The following controls were established for the MIC: (i) Sterility control (1 mL broth only, no inoculum): confirmed medium sterility. (ii) Growth control (1 mL broth + 1 mL inoculum, no extract): confirmed whether the organism grew under test conditions. (ii) Positive control (1 mL broth +1mL inoculum + 1 mL Fluconazole (16.7 mg/mL)): this was used to confirm the sensitivity of the assay.

Preparation of Fluconazole powder

A half grams of Fluconazole powder [Fungicip – 150 (Cipla), Canex -150] was dissolved in 10 mL of sterile distilled water to create the Fluconazole suspension (50 mg/mL) used for this control. Once all tubes were prepared, they were placed in an incubator set at 37°C. All tubes were observed after 24 hrs and the final reading was

taken after 48 hrs (Trivedi et al. 2015; Allen et al. 2023). All assays were conducted in triplicate, with the MIC defined as the lowest concentration at which no visible growth of *C. albicans* was observed following 48 hrs of incubation at 37°C. The results were recorded as either 1 (positive growth in tube – turbid/cloudy) or 0 (negative growth in tube – clear tube).

Disk diffusion assay (Kirby-Bauer Test)

Sterile filter paper disks were autoclaved at 121°C for 15 mins at 12 psi. After cooling, each disk was treated with 10 µL of garlic extract in four different concentrations of each garlic extract: 500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%). Each concentration of the extract was made up to a volume of 10 mL. The dilutions were prepared by diluting the garlic extract with corresponding volumes of distilled water using the $C_1V_1=C_2V_2$ formula (Table 2). In addition to the experimental disks, both positive and negative control disks were also prepared. The negative control disk was soaked with sterile distilled water and ethanol to confirm that the solvent had no inhibitory effects on its own. The positive control was prepared using disks (6 mm, ~ 25 µg per volume) soaked in a standard Fluconazole suspension.

C. albicans suspensions were standardized using a 0.5 MacFarland Standard and adjusted to a concentration of approximately 1.0×10^6 cells/mL. The fungal suspension was then evenly spread over the entire surface of the SDA plates using a swabbing technique. Once inoculated, the plates were left for about 5 to 10 mins to allow the fungal suspension to settle before applying the treatment disks (Shetty et al. 2013; Serrano et al. 2020). Using sterile forceps, the disks were gently placed onto the surface of the inoculated agar plates, approximately 20 mm apart to avoid any overlapping zones of inhibition. Each disk was lightly pressed down to ensure good contact with the agar surface. After placing all disks, the plates were left at room temperature for 15-30 mins to allow solvent evaporation and the antifungal disks to hydrate. They were subsequently incubated at 37°C. Plates were observed after 24 hrs and initial readings were noted. The final readings were subsequently recorded after 48 hrs. Following the incubation period, the plates were carefully examined for zones of inhibition. A ruler was then used to measure the diameter of each zone in millimeters (mm). In cases where the zone was irregularly shaped, the average of three measurements taken at different points was taken to provide more accurate readings (Shetty et al. 2013; Allen et al. 2023).

Data analysis

All experimental data were analyzed using Microsoft Excel and RStudio Software (Version 2025.09.2) at a significance level of $p<0.05$. After conducting a Shapiro-Wilks test for normality ($p>0.05$), the parametric datasets were subjected to a one-way Analysis of Variance (ANOVA) to determine whether there were significant differences in the zones of inhibition (ZOI) between the two types of garlic extracts during the disk diffusion assays. Furthermore, where significant differences were detected, a Tukey's Honest Significant Difference (HSD) test was applied to identify the specific concentration pairs that differed. Datasets obtained from the MIC test were subjected to Pearson's Chi-squared test to examine whether the distribution of growth and no growth outcomes varied significantly across concentrations. To compare the antifungal activity of the two types of garlic extracts (at 100% concentration) against Fluconazole, a Welch two-sample t-test was conducted, accounting for any unequal variances between the tested groups.

Table 2. Garlic extract disk dilution series for the disk diffusion assay

Extract solution (disk)	Total dilution factor (cumulative)	Concentration (mg/mL)	Concentration (%)
Stock	NA	500	100
1	1.33	375	75
2	2	250	50
3	4	125	25

Table 1. Serial dilution for garlic extract (aqueous and ethanol) concentrations for the determination of Minimum Inhibitory Concentration (MIC) against *C. albicans*

Tube #	Total dilution factor (Cumulative)	Concentration before inoculation (mg/mL)	Concentration before inoculation (%)	Final concentration after inoculation (mg/mL)
Stock	NA	500	100	NA
1	2	250	50	125
2	4	125	25	62.5
3	6	62.5	12.5	31.25
4	8	31.25	6.25	15.62
5	10	15.62	3.12	7.81

RESULTS AND DISCUSSION

Aqueous garlic extract

The recorded ZOIs produced by the aqueous garlic extract at four different concentrations highlighted that at the lowest concentration tested (25%), no measurable inhibition zone was observed across all replicates, resulting in a mean value of 0.0±0.0 mm, which can be observed in Figure 1. At 50% concentration, inhibition was consistently observed, with replicate values ranging between 19 and 25 mm and a calculated mean value of 21.0±3.0 mm. At 75%, the extract produced inhibition zones that averaged 26.0±1.5 mm, with replicate values clustered closely between 25 and 28 mm. The highest concentration tested, 100%, produced inhibition zones ranging between 26 and 28 mm, with a mean value of 27.0±1.0 mm. Overall, the results displayed in Figure 2 indicate increasing zone diameters from 50% upwards, with significant results at both 75% and 100% concentrations.

The one-way ANOVA indicated statistically significant differences among the four concentrations tested [$F(3,8)=119.2$, $p<0.001$]. Our results indicated that the variance in zone diameters may be attributed to concentration differences rather than random variation among replicates. Post hoc analysis using Tukey's HSD test further revealed that the mean zones at 50% differed significantly from 25% ($p<0.001$), while 75% and 100% also differed significantly from 25% ($p<0.001$). Additionally, 100% differed significantly from 50% ($p<0.05$). The comparison between 75% and 50% approached significance but did not surpass the threshold ($p=0.061$) (Table 3).

Ethanolic garlic extract

The measured ZOI obtained from the ethanolic garlic extract against *C. albicans* at four concentrations highlighted that at 25%, inhibition was minimal, with replicates producing a mean value of 4.3±7.5 mm. At 50%, replicate values ranged from 19 to 22 mm, with a mean value of 20.6±1.5 mm, as observed in Figure 3. At 75%, inhibition zones resulted in a mean zone of 28.0±1.0 mm. However, Figure 4 highlighted that the 100% ethanol extract produced the highest and most uniform inhibition, with all three replicates showing 30 mm zones, corresponding to a mean value of 30.0±0.0 mm.

Table 3. Summary of one-way ANOVA and Tukey's Honest Significant Difference test of the aqueous extract against *C. albicans* at 500mg/mL (100%), 375 mg/mL (75%), 250mg/mL (50%), and 125mg/mL (25%) concentrations

Comparison	Mean Diff (mm)	p-value
50% vs 25%	21.0	0.000006*
75% vs 25%	26.0	0.000001*
100% vs 25%	27.0	0.000001*
100% vs 50%	6.0	0.026*
75% vs 50%	5.0	0.061
One-way ANOVA:	$F(3,8) = 119.2$, $p < 0.001$	

Note: Zones of inhibition are expressed in millimeters (mm). *: Indicates that the p-value is statistically significant

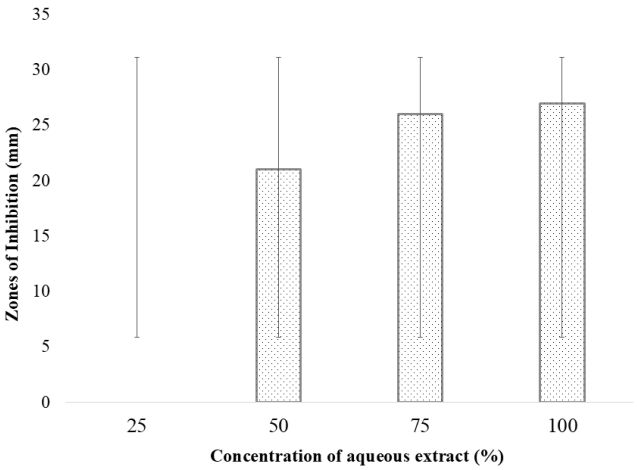

Figure 2. Mean zones of inhibition (mm) for aqueous garlic extract against *C. albicans* at four concentrations: [500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%)]. Error bars represent standard deviation (n=3). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test

Figure 1. Representative image showing the inhibition zones produced by the aqueous garlic extract at four concentrations [500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%)] against *C. albicans*. Note: Each image depicts a single plate corresponding to one concentration tested in triplicate

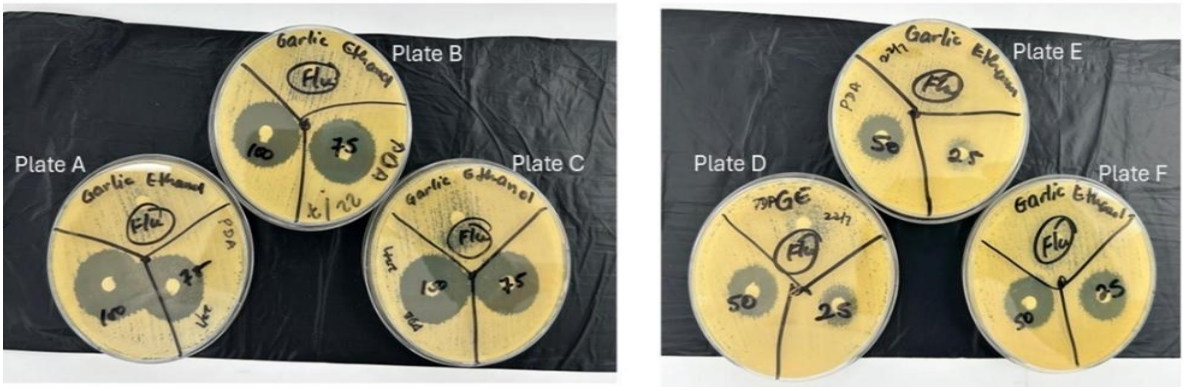

Figure 3. Representative image showing the inhibition zones produced by the ethanolic garlic extract at four concentrations [500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%)] against *C. albicans*. Note: Each image depicts a single plate corresponding to one concentration tested in triplicate

The one-way ANOVA test yielded significant variations in inhibition zones between the four concentrations [$F(3,8)=27.33$, $p<0.001$]. Furthermore, Tukey's HSD test identified that the 50% concentration differed significantly from 25% ($p=0.004$), while both 75% and 100% differed significantly from 25% ($p<0.001$). However, the comparison between 75% and 50% was not statistically significant ($p=0.171$). Our findings indicate that 50% ethanolic extract concentrations, which are ≥50%, consistently produced significantly larger ZOIs compared to the lower concentrations tested (Table 4).

Minimum Inhibitory Concentration (MIC) - aqueous vs. ethanolic garlic extracts

Figure 5 summarizes the results of the Minimum Inhibitory Concentration (MIC) tests performed on both aqueous and ethanol garlic extracts against *C. albicans*. For the aqueous extract, complete inhibition was observed in all three replicates at both 50% and 25% concentrations. At 12.5%, inhibition occurred in one replicate while the other two showed visible growth. At 6.25% and 3.125%, all three replicates displayed growth. For the ethanolic extract, the replicates were inhibited by 50%. At 25%, only one replicate showed no growth, while growth was present in the remaining two. At 12.5%, 6.25%, and 3.125%, growth was observed in all replicates.

Table 4. Summary of One-way ANOVA and Tukey's Honest Significant Difference Tests of the ethanolic extract against *C. albicans* at 500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%) concentrations

Comparison	Mean Diff (mm)	p-value
50% vs 25%	16.3	0.0037*
75% vs 25%	23.7	0.0003*
100% vs 25%	25.7	0.0002*
75% vs 50%	7.3	0.1710
One-Way ANOVA:	$F(3,8) = 27.33$, $p < 0.001$	

Note: Zones of inhibition are expressed in millimeters (mm). *: Indicates that the p-value is statistically significant

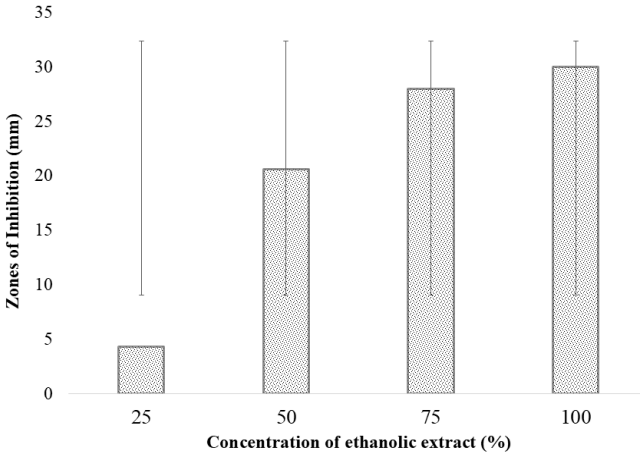

Figure 4. Mean zones of inhibition (mm) for ethanolic garlic extract against *C. albicans* at 500 mg/mL (100%), 375 mg/mL (75%), 250 mg/mL (50%), and 125 mg/mL (25%) concentrations. Error bars represent standard deviation (n=3). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test

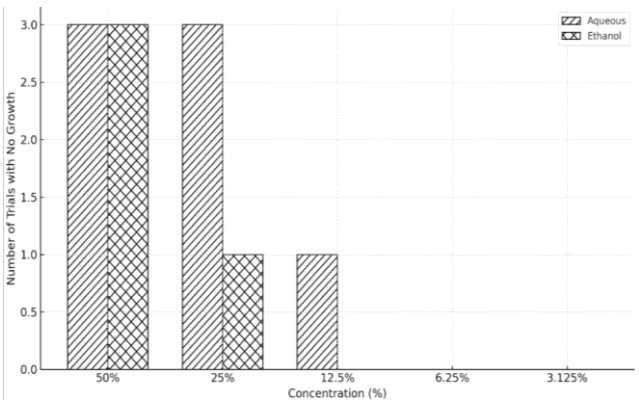

Figure 5. Minimum Inhibitory Concentration (MIC) profiles of aqueous and ethanolic garlic extracts, growth inhibition across five serial dilutions: [250 mg/mL (50%), 125 mg/mL (25%), 62.5 mg/mL (12.5%), 31.25 mg/mL (6.25%), and 15.62 mg/mL (3.125%)]

Table 5. Summary of Welch's t-test: comparison between 100% garlic extracts (aqueous and ethanolic) and Fluconazole against *C. albicans*

Treatment	Mean zone of inhibition (mm ± SD)
100% aqueous garlic extract	27.0±1.0
100% ethanolic garlic extract	30.0±0.0
Fluconazole (observed)	3.0±0.5
Welch's t-test	t(2) = 15.59, p = 0.002***

Note: ***: Indicates that the observed p-value is statistically significant. The observed values for Fluconazole were unexpectedly low, potentially reflecting an issue with disk preparation or strain resistance

Pearson's Chi-squared test for the aqueous garlic extract indicated that the distribution of growth and no-growth outcomes varied significantly across the different concentrations tested (df=4, chi-squared value=12.32, p=0.015). The statistical outcome supports the observed differences in replicate counts, particularly between the higher concentrations where no growth was observed consistently, and the lower concentrations where growth was present. Similarly, analysis of the ethanolic extract was similar to the aqueous extract (df=4, chi-squared value=11.59, p=0.021). This confirmed that the differences in growth outcomes among concentrations were not due to chance. Higher concentrations were associated with a greater frequency of no-growth replicates, while at lower concentrations, growth was observed in all cases.

Garlic extracts vs. Fluconazole: Which one is more effective against *Candida albicans*?

Table 5 compares the inhibition zones produced by 100% aqueous and ethanolic garlic extracts and Fluconazole against *C. albicans*. The aqueous garlic extract produced inhibition zones with a mean value of 27.0±1.0 mm, while the ethanolic garlic extract gave uniform zones of 30.0±0.0 mm across all replicates. However, the Fluconazole disks produced very small ZOIs, consistently measuring <5 mm (mean value: 3.0±0.5 mm), despite multiple trials with different commercial brands. Furthermore, Welch's two-sample *t*-test revealed a statistically significant difference between the treatments ($t(2)=15.59$, df=2, $p=0.002$), indicating that both garlic extracts produced significantly larger zones of inhibition than Fluconazole (95% confidence interval for the mean difference was $[7.31, \infty]$).

Discussion

Disk diffusion assays

The results of our study demonstrated clear, concentration-dependent antifungal activity of *A. sativum* extracts against *C. albicans*, with the ethanolic preparation yielding the largest, most uniform zones of inhibition at higher concentrations when compared to the aqueous extracts. Our disk diffusion and MIC assays provided compelling evidence that garlic contains bioactive constituents capable of suppressing *C. albicans* growth, but the magnitude of this effect is dependent on the extraction method and on the type of assay. Differences in the ZOIs produced by the ethanolic extract relative to the aqueous extract likely reflect differences in solvent polarity and the solubility profile of garlic's bioactive compounds. Ethanol, being less polar than water, can extract a wider spectrum of phytochemicals and can recover higher amounts of organosulfur compounds and secondary metabolites, which have been linked to membrane disruption, enzyme inhibition, and oxidative stress in fungal cells, ultimately enhancing agar-based ZOIs (Leontiev et al. 2018; Bar et al. 2022; Vidaković et al. 2024). Water, on the other hand, extracts primarily polar compounds, which may limit the availability of some bioactive components, resulting in slightly reduced activity compared to ethanol.

Broth macrodilution assay: Minimum Inhibitory Concentration (MIC)

Disk diffusion ZOIs usually correspond with MIC (indirect measure), where larger ZOIs produced usually indicate lower MIC (more potency). This usually allows for similar interpretations of breakpoints when both assays are compared, even though MICs are more sensitive and precise. The MIC reported for the broth macrodilution assay showed a broadly similar threshold for broth inhibition in both extracts at higher concentrations, suggesting that when mass transfer constraints are reduced (as in liquid media such as broth), the difference between solvents is less pronounced (Wadhwani et al. 2009). This provides some explanation for the similar performance exhibited by both extracts in the MIC assay, even though their results varied in the agar-based disk diffusion assay. Our findings agree with research conducted by Katiraee et al. (2017), who found similar MIC values when testing garlic against *C. albicans,* emphasizing the role of extract concentration and type in broth-based assays. These findings suggest that garlic's bioactive compounds can be more effectively harnessed when extracted with organic solvents, which may have implications for the formulation of herbal antifungal treatments and further research into optimizing extraction techniques (Bar et al. 2022).

Garlic vs. Fluconazole: Effectiveness, limitations, and possible explanations

Although Fluconazole is a well-established antifungal agent with documented activity against *C. albicans,* the disks prepared for our study produced small measurable ZOIs, even when two different commercial brands were tested on separate occasions. There was evidence of slowed growth on many trials, but not complete inhibition of growth that would indicate total susceptibility of *C. albicans* to Fluconazole. Several factors may explain this unexpected outcome; however, we provide two possible explanations for Fluconazole's inactivity: (i) methodological shortcomings arising from preparing drug disks from the powdered form of Fluconazole, and (ii) genuine reduced susceptibility of the clinical *C. albicans* isolates. While procedures were followed to ensure proper solubilization, Fluconazole is known to be relatively water-insoluble, and incomplete dissolution may have reduced its bioavailability in the prepared disks, presenting a significant limitation to our study. Preparing antifungal

disks in-house is inherently less reliable than using validated, quality-controlled commercial disks due to variability in dissolution, uneven impregnation of the disk, incomplete drug diffusion from the disk into agar, and potential loss of potency during handling (Swetha et al. 2023). We suggest that future research should employ standardized, commercially prepared disks which are utilized in formal susceptibility testing following CLSI/EUCAST guidelines, including broth microdilution for Fluconazole MIC determination and molecular resistance assays (Berkow and Lockhart 2017). Additionally, strain-level resistance mechanisms could also explain diminished Fluconazole activity since azole resistance in *C. albicans* can arise from efflux pump overexpression, ERG11 mutations, or altered sterol metabolism, all of which reduce accumulation or binding (Liu et al. 2015; Berkow and Lockhart 2017). However, completely attributing the small zones observed in our study to genetic resistance is uncertain and would require further research utilizing various combinations of validated disks and reference MIC methods.

Broader implications of the study and future research recommendations

Our findings are broadly consistent with the prior studies performed globally, which report the potent antifungal effects of garlic extracts and the notable influence of solvent types and extraction methods (Li et al. 2016; Bar et al. 2022; Khounganian et al. 2023). Studies by Katiraee et al. (2017) and Leontiev et al. (2018) have reported larger inhibition zones and lower MICs for organic solvent extracts relative to aqueous ones, a pattern attributed to enhanced extraction of sulfur-containing volatiles and non-polar secondary metabolites. Additionally, Chikwem et al. (2009), Trivedi et al. (2015) and Esmael et al. (2024) have also provided reports of meaningful results from aqueous extracts, particularly in broth assays or when fresh extracts are used, underscoring the effectiveness of aqueous extracts when concentration and exposure conditions are appropriate. Based on our results, the ethanolic extract performs best on agar, while both extracts display inhibitory thresholds in broth at sufficiently high concentrations. Garlic extracts, particularly ethanolic in nature, may offer natural, low-cost alternative antifungal treatments, especially in areas with limited access to commercial drugs or where antimicrobial resistance is prevalent. Unlike synthetic antifungals, which often target a specific pathway, garlic contains multiple bioactive compounds that act on a range of cellular targets. This multi-target mechanism may reduce the likelihood of resistance developing over time. The consistency of our findings with existing research adds credence to the argument that natural compounds such as garlic are not only traditionally useful but also scientifically valid as an anti-fungal treatment. However, the scope of our study was constrained by its applications in clinical settings, as it was exclusively conducted in vitro. We suggest that in-vivo, toxicity, pharmacokinetic, and resistance-evolution research be performed to assess the clinical applications of garlic and its range of effectiveness against standard antifungal treatments. We also suggest that future studies be conducted locally to identify and describe specific bioactive compounds, such as allicin or total thiosulfinate content, to understand the other potential health benefits of garlic.

In conclusion, our study demonstrated that *A. sativum* (garlic) extracts possess measurable antifungal activity against *C. albicans*, with effectiveness directly proportional to extract concentration. Both aqueous and ethanolic extracts inhibited fungal growth, with the ethanolic extract showing consistently stronger activity. At 100% concentration, the ethanolic extract produced the largest and most uniform zones of inhibition (30.0±0.0 mm), while the aqueous extract yielded slightly smaller but comparable inhibition (27.0±1.0 mm). In contrast, Fluconazole exhibited minimal inhibitory activity under the same conditions, which may be attributed to either disk preparation limitations or reduced susceptibility of the test strain. Statistical analysis reported significant differences among concentrations, as well as between garlic extracts and Fluconazole. Minimum Inhibitory Concentration (MIC) assays further supported these results, with consistent growth suppression at 50% and partial inhibition at 25%, while lower concentrations (≤12.5%) permitted fungal growth. In contrast, Fluconazole disks prepared in-house produced very small inhibition zones (<5 mm), highlighting either methodological constraints in disk preparation or reduced susceptibility of the tested *C. albicans* strain. Broader exploration of garlic-derived treatments could contribute to developing affordable, plant-based antifungal therapies offering a sustainable option for both healthcare systems and communities affected by drug-resistant fungal infections. While there are constraints within in vitro experimental designs, we contend that broad-scale applications can include further research focusing on isolating and characterizing the specific bioactive compounds responsible for antifungal activity, assessing their toxicity and stability, and evaluating clinical effectiveness through in vivo and patient-based studies. While further research is needed to expand testing across multiple strains and refine control methods, our findings provide some evidence that garlic has potential as a natural anti-fungal candidate, with relevance in both clinical and public health contexts.

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