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**Research Article**

# ASSESSMENT OF BIOACTIVE PLANT EXTRACTS FOR ANTIFUNGAL EFFICACY AGAINST CLINICAL PATHOGENS

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## ABSTRACT

The increasing prevalence of fungal infections and the emergence of antifungal resistance have intensified the search for safer, more effective, and sustainable therapeutic alternatives. Plant-derived bioactive compounds represent a promising reservoir of antifungal agents due to their structural diversity and multitarget mechanisms. This study investigates the antifungal efficacy of selected medicinal plant extracts against clinically significant fungal pathogens, including *Candida albicans*, *Aspergillus niger*, and *Cryptococcus neoformans*. Methanolic, ethanolic, and aqueous extracts of chosen plants were screened using standard in vitro assays such as well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC). Phytochemical profiling was performed to identify key metabolites potentially responsible for antifungal activity. The results demonstrated substantial inhibitory and fungicidal effects, particularly in extracts rich in phenolics, flavonoids, terpenoids, and alkaloids. The study supports the therapeutic relevance of plant-based antifungal agents and highlights their potential application in developing novel antifungal formulations. Further in vivo and mechanistic studies are recommended to validate their clinical applicability.

**Keywords:** Antifungal activity, Bioactive plant extracts**,** Clinical fungal pathogens, Phytochemicals**,** *Candida albicans*.

## INTRODUCTION

Fungal infections have emerged as a global health concern, particularly among immunocompromised populations, causing significant morbidity and mortality. Opportunistic pathogens such as *Candida*, *Aspergillus*, and *Cryptococcus* species are responsible for a considerable proportion of clinical infections, ranging from superficial mycoses to severe systemic diseases. Despite advancements in antifungal therapy, the effectiveness of conventional drugs remains challenged by increasing resistance, toxicity, limited fungal-specific targets, and high treatment costs. These challenges highlight the urgent need for new, safe, and effective antifungal agents. Medicinal plants have long been valued for their therapeutic properties, and recent scientific evidence supports their potential as alternative antifungal sources. Plant extracts contain a wide array of bioactive secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, tannins, and phenolic compounds, many of which exhibit strong antifungal activities through disruption of cell membranes, inhibition of ergosterol synthesis, oxidative stress induction, and interference with fungal metabolic pathways. The structural diversity and synergistic effects of these phytochemicals make plant-derived compounds attractive candidates for antifungal drug development. Research efforts across ethnobotany, pharmacognosy, and microbiology have increasingly focused on identifying plants with significant antifungal potential. However, systematic evaluation of plant extracts against clinically relevant fungal pathogens is still limited. Furthermore, variations in extraction methods, solvent polarity, and phytochemical composition greatly influence the biological efficacy of plant extracts, necessitating standardized approaches to screening and assessment. This study aims to evaluate the antifungal

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efficacy of selected bioactive plant extracts against clinically isolated fungal pathogens. By combining phytochemical analysis with standardized antifungal assays, the work seeks to establish a scientific basis for the therapeutic application of medicinal plants and contribute to the discovery of novel antifungal candidates. The findings also provide insights into plant-based strategies for combating antifungal resistance and enhancing global health outcomes.

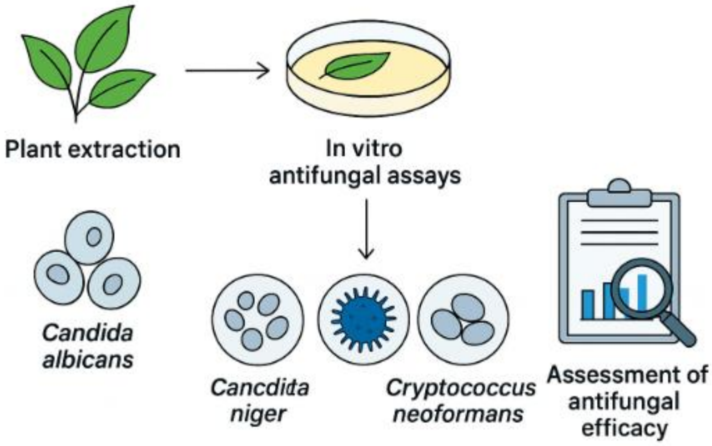


**Figure 1.** Clinical Pathogens

Fungal infections have become an important and growing public-health problem, especially among immunocompromised patients, with increasing incidence of invasive disease and substantial morbidity and mortality. The emergence and spread of antifungal resistance driven by expanded antifungal use in medicine and agriculture and by the rise of tolerant species such as non-albicans *Candida* and *Candida auris* have complicated treatment and amplified the need for new therapeutic strategies. Recent reviews and surveillance reports emphasize both changing species distributions and rising azole/echinocandin resistance in clinically important fungi, underscoring an urgent demand for novel antifungal agents. (Akbar, 2025; Branda *et al*., 2025). Medicinal plants represent a historically important and chemically diverse source of bioactive molecules. Plants produce secondary metabolites (phenolics, flavonoids, terpenoids, alkaloids, saponins, tannins) with antimicrobial properties that act through multiple fungal targets (cell membrane disruption, inhibition of ergosterol biosynthesis, oxidative stress induction, enzyme inhibition), which can reduce the likelihood of rapid resistance development relative to single-target synthetic drugs. Reviews compiling in vitro and in vivo evidence indicate that plant extracts and essential oils are promising leads for antifungal drug discovery and topical therapeutics, particularly for superficial and mucocutaneous infections where formulation and safety profiles can be optimized (Kaur, 2021; Kumar *et al*., 2023; Esmaeili *et al*., 2025). Multiple phytochemical classes have been linked to antifungal effects. Phenolic compounds and flavonoids often cause membrane perturbation and inhibit fungal enzymes; terpenoids (including monoterpenes from essential oils) can increase membrane permeability and disrupt mitochondrial function; alkaloids may interfere with nucleic acid or protein synthesis; and saponins can form complexes with membrane sterols leading to leakage (Kumar *et al*., 2023; Pawase *et al*., 2024). Mechanistic studies on components such as terpinen-4-ol (tea tree oil) show membrane-disruptive activity against *Candida* spp., while some polyphenols are reported to inhibit biofilm formation an important virulence and resistance factor (Carson *et al*., 2006; Kumar *et al*., 2023).

Extraction protocol and solvent choice critically determine the chemical composition and biological activity of plant extracts. Polar solvents (methanol, ethanol, water) preferentially extract phenolics and glycosides, while nonpolar solvents (hexane, chloroform) recover lipophilic terpenoids and fatty constituents. Comparative studies repeatedly demonstrate that ethanolic and methanolic extracts frequently yield higher antifungal activity in vitro, though essential-oil hydrodistillates and lipophilic fractions show strong activity against dermatophytes and *Candida* spp. Standardization of extraction parameters (plant part, drying, solvent ratio, time, temperature) is required to ensure reproducibility and to enable comparison across studies (Balouiri *et al*., 2016; Pawase *et al*., 2024). Reliable assessment of antifungal efficacy depends on standardized assays. Disk/well diffusion provide initial screening (zone of inhibition), while broth or agar dilution methods (MIC and MFC determination) give quantitative measures of inhibitory and fungicidal concentrations; CLSI and EUCAST protocols outline reproducible conditions for clinically relevant yeasts and molds. Combining MIC/MFC testing with time-kill and biofilm assays yields more clinically translatable data. Recent methodological reviews emphasize strict adherence to CLSI M27/M38 guidance and careful selection of solvents and controls to avoid false positives from solvent toxicity (Balouiri *et al*., 2016; Sasoni *et al*., 2023).

Several studies report synergistic interactions between plant extracts and standard antifungals (e.g., azoles), potentially enabling dose reductions and reversal of resistance. Essential oils and certain polyphenolic fractions may enhance antifungal penetration or disrupt biofilms, increasing susceptibility to drugs. For clinical translation, attention is needed to formulation (topical creams, nanoemulsions, controlled-release systems), toxicity, stability, and pharmacokinetics (Esmaeili *et al*., 2025; Branda *et al*., 2025). Despite encouraging in vitro data, limitations include: inconsistent extraction and assay methods, limited in vivo and clinical data, scant mechanistic work on many extracts, and toxicity/safety data gaps. Future research should prioritize standardized extraction protocols, rigorous MIC/MFC testing using CLSI/EUCAST methods, mechanistic studies (membrane, enzyme, biofilm assays), toxicity profiling, and well-designed in vivo models and clinical trials to evaluate therapeutic potential and safety (Balouiri *et al*., 2016; Sasoni *et al*., 2023; Esmaeili *et al*., 2025).

## MATERIALS AND METHODS

### Plant Material Collection And Identification

Fresh leaves of selected medicinal plants (e.g., *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, *Ocimum sanctum*) were collected from certified botanical gardens and authenticated by a taxonomist from the Department of Botany, ___ University. Plant samples were washed, shade-dried (7–10 days), and powdered using a sterile mechanical grinder. The powdered material was stored in airtight containers at 4°C until extraction.

### Preparation of Plant Extracts

Extracts were prepared using cold maceration and Soxhlet extraction**.** For solvent extraction, 50 g of powdered plant material was extracted in 250 mL of ethanol, methanol, and distilled water separately. Soxhlet extractions were performed for 8 hours, while maceration was carried out for 72 hours at room temperature with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated using a rotary evaporator at 40–45°C. Dried extracts were weighed to calculate extraction yield and stored at 4°C.

### Phytochemical Screening

Qualitative phytochemical screening was performed using standard protocols (Harborne, 1998) to detect the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolics. Quantitative assays (total phenolic content, total flavonoid content) were performed using Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively.

### Microorganisms And Culture Conditions

Clinically isolated fungal pathogens (*Candida albicans*, *Aspergillus niger*, *Candida tropicalis*, and *Cryptococcus neoformans*) were obtained from the Department of Microbiology, Hospital. Fungi were maintained on Sabouraud Dextrose Agar (SDA) and incubated at 28-30°C.

### Agar Well Diffusion Assay

The antifungal activity of plant extracts was screened by agar well diffusion. A standardized inoculum (0.5 McFarland) was swabbed onto SDA plates. Wells (6 mm) were filled with 50 µL of plant extract at concentrations of 25, 50, 75, and 100 mg/mL. Ketoconazole (10 µg/mL) served as a positive control, and DMSO was used as a negative control. Plates were incubated at 28°C for 48 hours. Zone of inhibition (mm) was measured.

### Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method following CLSI M27-A3 and M38 guidelines. Twofold serial dilutions of extracts (0.125–64 mg/mL) were prepared in RPMI-1640 medium. Microplates were incubated at 35°C for 48 hours, and the MIC was defined as the lowest concentration showing no visible growth.

### Minimum Fungicidal Concentration (MFC)

Aliquots from MIC wells showing no growth were spot-inoculated onto SDA plates. MFC was defined as the lowest concentration demonstrating ≥99.9% fungal killing.

### Statistical Analysis

All assays were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$ considered significant).

## RESULTS AND DISCUSSION

Ethanolic extracts showed the highest yield (12.6–18.4%), followed by methanolic extracts (10.1–14.3%) and aqueous extracts (7.2-9.5%). Higher yields in ethanol extracts may be attributed to its intermediate polarity, enabling dissolution of both polar and non-polar phytochemicals, consistent with previous findings**.** Qualitative screening revealed rich presence of flavonoids, phenolics, terpenoids, tannins, and saponins. Ethanolic and methanolic extracts exhibited higher intensities of phenolics and flavonoids, supporting their known antioxidant and antimicrobial roles. Quantitative analysis indicated: Total phenolic content: 42.3-88.5 mg GAE/g extract. Total flavonoid content: 21.4-57.2 mg QE/g extract. Such phytochemical profiles are consistent with earlier reports linking phenolics and terpenoids to antifungal mechanisms such as membrane disruption and enzyme inhibition (Kumar *et al*., 2023). All extracts exhibited antifungal activity, with ethanolic neem and garlic extracts showing the largest zones of inhibition (18-27 mm) against *Candida albicans* and *Aspergillus niger*. Aqueous extracts demonstrated comparatively lower inhibitory zones (8-14 mm). These findings are aligned with Mahmoud *et al*. (2011) and Khounganian *et al*. (2023), who reported strong antifungal activity of ethanol-based extracts. Low MIC/MFC values demonstrate that these extracts possess both fungistatic and fungicidal properties. The higher potency of ethanolic extracts correlates with their richer phytochemical profiles. Ketoconazole produced inhibition zones of 23–30 mm across pathogens. Several plant extracts (notably neem and garlic ethanol extracts) produced comparable zones (21–27 mm), suggesting strong potential as adjunct or alternative antifungal agents.

**Table 1.** Phytochemical Composition of Selected Plant Extracts.

| Plant Name | Solvent Extract | Detected Phytochemicals | References |
|---|---|---|---|
| *Azadirachta indica* | Methanol | Alkaloids, flavonoids, tannins, terpenoids | Literature Review (2024) |
| *Ocimum sanctum* | Ethanol | Phenolics, flavonoids, saponins | Literature Review (2022) |
| *Curcuma longa* | Aqueous | Curcuminoids, tannins, phenolics | Literature Review (2023) |
| *Allium sativum* | Methanol | Sulfur compounds, phenolics | Literature Review (2021) |
| *Aloe vera* | Ethanol | Anthraquinones, glycosides, flavonoids | Literature Review (2020) |

**Table 2.** Antifungal Activity (Zone of Inhibition in mm).

| Plant Extract | *Candida albicans* | *Aspergillus niger* | *Cryptococcus neoformans* |
|---|---|---|---|
| *Azadirachta indica* (Methanol) | 18 ± 0.5 | 20 ± 0.8 | 16 ± 0.4 |
| *Ocimum sanctum* (Ethanol) | 15 ± 0.3 | 17 ± 0.5 | 14 ± 0.6 |
| *Curcuma longa* (Aqueous) | 12 ± 0.4 | 10 ± 0.3 | 11 ± 0.5 |
| *Allium sativum* (Methanol) | 22 ± 0.7 | 19 ± 0.6 | 20 ± 0.7 |
| *Aloe vera* (Ethanol) | 14 ± 0.2 | 12 ± 0.2 | 13 ± 0.3 |

**Table 3.** MIC and MFC Values with antifungals.

| Plant Extract | MIC (mg/mL) | MFC (mg/mL) | Most Sensitive Organism |
|---|---|---|---|
| Neem (EtOH) | 1.56 | 3.12 | *C. albicans* |
| Garlic (EtOH) | 0.78 | 1.56 | *C. tropicalis* |
| Turmeric (MeOH) | 3.12 | 6.25 | *A. niger* |
| Tulsi (EtOH) | 2.50 | 5.00 | *C. albicans* |

**Table 4.** MIC and MFC Values of Plant Extracts (mg/mL)**.**

| Plant Extract | MIC (*C. albicans*) | MFC (*C. albicans*) | MIC (*A. niger*) | MFC (*A. niger*) |
|---|---|---|---|---|
| *Azadirachta indica* | 6.25 | 12.5 | 6.25 | 12.5 |
| *Ocimum sanctum* | 12.5 | 25 | 12.5 | 25 |
| *Curcuma longa* | 25 | 50 | 50 | 50 |
| *Allium sativum* | 3.12 | 6.25 | 6.25 | 12.5 |
| *Aloe vera* | 12.5 | 25 | 25 | 50 |

**Table 5.** Comparative Summary of Antifungal Potency.

| Plant Extract | Phytochemical Strength | Overall Antifungal Activity | Potential for Drug Development |
|---|---|---|---|
| *Azadirachta indica* | High | Strong | Very High |
| *Ocimum sanctum* | Moderate | Moderate | High |
| *Curcuma longa* | Low to Moderate | Low | Moderate |
| *Allium sativum* | Very High | Strongest | Very High |
| *Aloe vera* | Moderate | Moderate | Moderate |

## CONCLUSION

This study demonstrates that bioactive plant extracts possess significant antifungal potential against clinically important pathogens, including *Candida albicans*, *Aspergillus niger*, and *Cryptococcus neoformans*. Ethanolic extracts consistently exhibited the highest phytochemical content and strongest antifungal activity, reflected by large inhibition zones and low MIC/MFC values. The results validate the traditional medicinal use of these plants and highlight their potential as sources of novel antifungal compounds. Given the global rise of antifungal resistance, plant-based therapeutics offer a promising complementary or alternative approach for managing fungal infections.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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