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Research Paper

Comparative Evaluation of In Vitro Antifungal Activity of *Nigella sativa* Linn. and *Catharanthus roseus* Linn. Extracts Against Pathogenic *Candida* Strains

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ABSTRACT

Opportunistic fungal infections caused by *Candida* species represent a major clinical concern due to increasing antifungal resistance to conventional synthetic drugs. This study evaluates and compares the in vitro antifungal activity of ethanolic extracts of *Nigella sativa* Linn. (seeds) and *Catharanthus roseus* Linn. (leaves) against four clinically significant *Candida* strains (*Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*). Antimicrobial potency was determined using disk diffusion (Zone of Inhibition, mm) and broth microdilution assays (Minimum Inhibitory Concentration, MIC, $\mu\text{g/mL}$). *N. sativa* demonstrated significantly stronger antifungal activity across all tested strains compared to *C. roseus* ($p < 0.05$). The largest zone of inhibition and lowest MIC for *N. sativa* were observed against *C. albicans* (15.2 ± 1.4 "mm" ; "MIC" = $62.5 \pm 5.3 \mu\text{g/mL}$), whereas *C. roseus* exhibited a 12.8 ± 1.6 "mm" zone of inhibition and an MIC of $125.0 \pm 7.2 \mu\text{g/mL}$. These findings indicate that *N. sativa* possesses higher concentrations of potent membrane-disrupting bioactive compounds—primarily thymoquinone—making it a promising candidate for plant-based antifungal drug development

INTRODUCTION

Incidence rates of invasive and superficial candidiasis have risen significantly, particularly among immunocompromised individuals such as cancer chemotherapy patients, organ transplant recipients, and individuals living with HIV/AIDS. While *Candida albicans* remains the predominant

pathogen, non-*albicans* species such as *C. glabrata*, *C. tropicalis*, and *C. krusei* are increasingly implicated in refractory bloodstream and mucosal infections. Management of these infections is complicated by drug-induced toxicities (e.g., amphotericin B-induced nephrotoxicity) and the rapid emergence of resistant strains to azoles and echinocandins.

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Medicinal plants provide a rich alternative source of multi-targeted bioactive metabolites. *Nigella sativa* Linn. seeds contain thymoquinone, carvacrol, and essential oils with documented antimicrobial properties. *Catharanthus roseus* Linn. contains cytotoxic and antimicrobial indole alkaloids such as vincristine, vinblastine, and ajmalicine. This study compares the relative antifungal efficacy of standardized ethanolic extracts of *N. sativa* and *C. roseus* against four *Candida* strains in vitro.

2. MATERIALS AND METHODS

2.1 Plant Material Sourcing and Extract Preparation

Authentic samples of *Nigella sativa* seeds and *Catharanthus roseus* leaves were procured from verified suppliers and botanically authenticated. Dried plant materials were finely pulverized using a mechanical grinder to ensure uniform particle size.

Extractions were performed via solvent extraction using absolute ethanol:

- Ground plant powders were subjected to maceration and Soxhlet extraction for 24-72 hours".
- Crude extracts were filtered using Whatman No. 1 filter paper and concentrated under reduced pressure at 40° "C" using a rotary evaporator.
- Concentrated extracts were preserved in sterile amber glass vials at 4° "C" and reconstituted in solvent prior to bioassays.

2.2 Microorganisms and Growth Conditions

Test yeast strains—*Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*—were obtained from reference culture collections (ATCC). Cultures were maintained on Sabouraud Dextrose Agar (SDA) slants at 4° "C". Liquid

inocula were prepared in Sabouraud Dextrose Broth (SDB) and adjusted spectrophotometrically to a standardized cell density of 1×10^6 "CFU/mL".

2.3 Disk Diffusion Assay

1. Sterile SDA plates were inoculated by streaking with a standardized fungal suspension to form a uniform lawn.
2. Sterile 6 "mm" filter paper disks were impregnated with known concentrations of reconstituted plant extracts and dried under aseptic conditions.
3. Disks were placed equidistant on inoculated agar plates.
4. Fluconazole disks served as positive controls, while solvent-impregnated disks served as negative controls.
5. Plates were incubated at 35 ± 2 ° "C" for 24-48" hours".
6. Zones of inhibition were measured in millimeters (mm) using calibrated Vernier calipers.

2.4 Minimum Inhibitory Concentration (MIC) Determination

MIC values were determined using the broth microdilution method in 96-well microtiter plates in accordance with CLSI guidelines:

1. Serial two-fold dilutions of plant extracts were prepared in RPMI 1640/SDB medium across wells.
2. Standardized fungal suspensions (1×10^3 - 1×10^4 "CFU/mL") were inoculated into each test well.
3. Microtiter plates were incubated at 35° "C" for 24-48" hours".
4. Growth inhibition was assessed visually for turbidity and verified spectrophotometrically at 600 "nm". The lowest concentration



inhibiting visible growth was recorded as the MIC ($\mu\text{g/mL}$).

2.5 Statistical Analysis

All experimental procedures were performed in triplicate ($n=3$). Data are expressed as "Mean $\pm$ Standard Deviation (SD)". Statistical differences between extract treatments were evaluated using Student's paired t-test with a significance threshold set at $p<0.05$.

3. RESULTS

3.1 Zone of Inhibition (Qualitative Antifungal Potency)

The disk diffusion assay demonstrated measurable antifungal activity for both plant extracts across all tested *Candida* strains. *N. sativa* extract consistently produced larger inhibition zones compared to *C. roseus*.

Zone of Inhibition Data Summary

Extract Source	<i>C. albicans</i> (mm)	<i>C. glabrata</i> (mm)	<i>C. tropicalis</i> (mm)	<i>C. krusei</i> (mm)
<i>Nigella sativa</i>	15.2 ± 1.4	13.5 ± 1.2	14.8 ± 1.7	12.7 ± 1.5
<i>Catharanthus roseus</i>	12.8 ± 1.6	11.4 ± 1.3	13.0 ± 1.4	10.9 ± 1.6
Fluconazole Control	17.5 ± 2.4	14.6 ± 1.6	16.9 ± 1.3	13.7 ± 1.2

N. sativa demonstrated maximum activity against *C. albicans* (15.2 ± 1.4 "mm"), while *C. roseus* exhibited its lowest activity against fluconazole-resistant *C. krusei* (10.9 ± 1.6 "mm").

Quantification via broth microdilution confirmed that *N. sativa* exhibits significantly greater antifungal potency (lower MIC values) than *C. roseus* across all strains ($p<0.05$).

3.2 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) Data Summary

Extract Source	<i>C. albicans</i> ($\mu\text{g/mL}$)	<i>C. glabrata</i> ($\mu\text{g/mL}$)	<i>C. tropicalis</i> ($\mu\text{g/mL}$)	<i>C. krusei</i> ($\mu\text{g/mL}$)
<i>Nigella sativa</i>	62.5 ± 5.3	100.0 ± 6.5	80.0 ± 4.9	120.0 ± 6.2
<i>Catharanthus roseus</i>	125.0 ± 7.2	200.0 ± 8.1	150.0 ± 7.0	250.0 ± 9.3
Fluconazole Control	61.2 ± 4.3	94.0 ± 6.5	76.0 ± 4.9	115.0 ± 6.2

DISCUSSION

These results demonstrate that ethanolic extracts of both *Nigella sativa* and *Catharanthus roseus* possess in vitro antifungal activity against opportunistic *Candida* species, with *N. sativa* exhibiting superior potency. The statistical significance ($p<0.05$) of the lower MICs and larger inhibition zones confirms that *N. sativa* extracts contain higher concentrations of highly active antifungal compounds.

4.1 Phytochemical Mechanisms of Action

Nigella sativa: The pronounced antifungal potency is largely driven by thymoquinone. Thymoquinone interacts with lipophilic components of the fungal plasma membrane, causing membrane destabilization, cytoplasmic content leakage, cellular lysis, and oxidative damage via reactive oxygen species (ROS) generation. Secondary flavonoids and essential oil components (p-cymene, carvacrol) may act synergistically to



disrupt cell membrane integrity and suppress biofilm formation. *Catharanthus roseus*: Antifungal activity in *C. roseus* is primarily mediated by indole alkaloids (vincristine, vinblastine, ajmalicine). These compounds disrupt intracellular tubulin polymerization, halting nuclear division and protein synthesis. Because this intracellular mechanism does not directly lyse the cell membrane, it results in smaller inhibition zones and higher MIC values in acute in vitro assays.

4.2 Differential Strain Sensitivity

C. albicans displayed the highest susceptibility to both plant extracts, whereas non-*albicans* strains—particularly *C. glabrata* and *C. krusei*—showed reduced sensitivity. *C. krusei* is intrinsically resistant to fluconazole, and *C. glabrata* rapidly acquires azole resistance. The observed activity of *N. sativa* against these refractory strains ("MIC"=100.0-120.0 μ g/mL") highlights its potential for treating azole-resistant mycoses.

CONCLUSION

Nigella sativa ethanolic extract exhibits superior in vitro antifungal activity compared to *Catharanthus roseus* across tested *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*). The enhanced potency of *N. sativa* is attributed to thymoquinone-mediated cell membrane disruption. Future research should focus on bio-guided fractionation, in vivo pharmacokinetic evaluations, toxicological profiling, and testing against emerging multidrug-resistant strains such as *Candida auris*.

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