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Research Article

BIOPROSPECTING MANGROVE-DERIVED MARINE FUNGI FOR ANTIFUNGAL AND ANTICANCER SECONDARY METABOLITES TARGETING *CANDIDA* SPP. AND TRIPLE-NEGATIVE BREAST CANCER

S. Priya Grace*, P. Nirmala and S. Sakthimeena

Department of Microbiology, Hindustan College of Arts & Science, Padur, Chennai. Tamil Nadu, India.

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ABSTRACT

Mangrove ecosystems harbor diverse and largely unexplored marine fungi capable of producing unique bioactive compounds with significant therapeutic potential. This study aimed to isolate and evaluate marine fungi from mangrove sediments for antifungal activity against *Candida* species and anticancer efficacy against triple-negative breast cancer (TNBC) cell lines. Fungal isolates were obtained using standard microbiological methods, and secondary metabolites were extracted using organic solvents. Antifungal activity was assessed by agar well diffusion, revealing a maximum inhibition zone of 22 ± 1.3 mm against *Candida albicans*. Cytotoxic potential was evaluated using in vitro assays, with the most active extract showing 78.6 ± 2.1% cell inhibition at 100 µg/mL and an IC_{50} value of 42.5 µg/mL against TNBC cells. These findings demonstrate that mangrove-derived marine fungi possess potent bioactive properties, highlighting their potential as promising sources for the development of novel antifungal and anticancer therapeutics.

Keywords: Marine fungal metabolites, Antifungal activity, *Candida albicans*, Anticancer activity, Triple.

INTRODUCTION

Marine ecosystems have emerged as a prolific source of structurally diverse and pharmacologically active natural products, largely attributed to the immense microbial diversity they harbor. Among these, marine fungi have gained considerable attention due to their ability to produce a wide range of secondary metabolites with significant biomedical applications. In particular, mangrove ecosystems represent a unique ecological niche characterized by fluctuating salinity, tidal influence, and rich organic matter, which promote the growth of distinctive microbial communities. These environmental pressures often lead to the biosynthesis of novel metabolites with potent biological activities, including antimicrobial, antifungal, and anticancer properties (Strobel and Daisy, 2003; Rateb and Ebel, 2011). Fungal-derived secondary metabolites have been widely recognized for their therapeutic potential, especially in combating infectious diseases and cancer. The increasing prevalence of opportunistic fungal infections caused by *Candida* species, particularly *Candida albicans*, poses a significant global health challenge. The emergence of drug-resistant strains further complicates treatment strategies, necessitating the search for new antifungal agents with improved efficacy and safety profiles (Perfect, 2017). Marine fungi isolated from mangrove sediments have shown promising antifungal activity, making them valuable candidates for drug discovery. Simultaneously, cancer remains one of the leading causes of mortality worldwide, with triple-negative breast cancer (TNBC) being one of the most aggressive and therapeutically challenging subtypes. TNBC lacks the expression of estrogen, progesterone, and HER2 receptors, limiting the effectiveness of targeted therapies and increasing reliance on conventional chemotherapy (Bianchini *et al*., 2016). Therefore, the identification of novel anticancer compounds from natural sources is of paramount importance. Marine-derived organisms, including fungi and microalgae, have demonstrated significant cytotoxic and apoptosis-inducing effects against various cancer cell lines, highlighting their potential as alternative therapeutic agents. Previous studies have provided substantial evidence supporting the anticancer potential of marine-derived bioactive

*Corresponding Author: S. Priya Grace, Department of Microbiology, Hindustan College of Arts & Science, Padur, Chennai. Tamil Nadu, India. Email: priyagraces@gmail.com.

compounds. For instance, alcoholic extracts of microalgal biomass have been reported to modulate cytotoxicity, apoptosis, and gene expression in hepatocellular carcinoma cells, indicating their therapeutic relevance (Anuradha *et al*., 2022). Similarly, green synthesized ruthenium nanoparticles derived from marine algae (*Dictyota dichotoma*) exhibited notable in vitro anticancer activity, further emphasizing the role of marine resources in nanomedicine and oncology (Syed Ali *et al*., 2017). Additional investigations have also demonstrated the anticancer efficacy of marine microalgae such as *Chlorella vulgaris*, *Nannochloropsis oculata*, and *Thalassiosira weissflogii*, reinforcing the importance of marine biodiversity in drug discovery (Saranya *et al*., 2020).

Mangrove plants themselves have been explored for their medicinal properties, particularly in cancer research. Methanolic extracts of *Rhizophora mucronata* have been shown to induce apoptosis in breast cancer (MCF-7) cell lines, suggesting their potential role in cancer therapy (Asbin Mary *et al*., 2022). Furthermore, the protective and bioactive effects of mangrove-derived compounds against cellular toxicity have been documented in HepG2 cell lines, highlighting their pharmacological significance (Chitra *et al*., 2019). Plant-based studies such as the evaluation of *Annona reticulata* seed extracts have also demonstrated antitumor activity, further supporting the exploration of natural products for cancer treatment (Ravimanickam *et al*., 2018). In spite of these advances, marine fungi from mangrove sediments remain relatively underexplored compared to other marine organisms. Their unique metabolic pathways and adaptability to extreme environmental conditions make them a promising yet untapped source of novel bioactive compounds. Therefore, systematic investigation of these fungi is essential to identify new antifungal and anticancer agents. In this context, the present study focuses on the isolation and characterization of marine fungi from mangrove sediments and the evaluation of their bioactive potential. Specifically, the study aims to assess antifungal activity against *Candida* species and cytotoxic effects against TNBC cell lines. By integrating microbial bioprospecting with biological screening, this research seeks to contribute to the development of innovative therapeutic agents derived from marine fungal resources.

MATERIALS AND METHODS

Study area and sample collection

Mangrove sediment samples were collected from coastal regions known for rich microbial diversity. Sampling was carried out from intertidal zones using sterile spatulas at a depth of 5–10 cm to ensure the presence of active microbial communities. The collected samples were transferred into sterile containers, properly labeled, and transported to the laboratory under chilled conditions for further analysis. Mangrove sediments are widely recognized as reservoirs of unique marine microorganisms due to their fluctuating environmental conditions (Kathiresan and Bingham, 2001).

Isolation of marine fungi

Marine fungi were isolated using the serial dilution and spread plate technique. Approximately 1 g of sediment sample was suspended in 9 mL of sterile seawater and serially diluted up to 10^{-5}. Aliquots from appropriate dilutions were spread onto Potato Dextrose Agar (PDA) plates supplemented with 2% NaCl and antibacterial agents such as streptomycin (50 µg/mL) to inhibit bacterial growth. The plates were incubated at 28 ± 2°C for 5–7 days. Distinct fungal colonies were sub cultured repeatedly to obtain pure isolates. This method is widely used for isolating marine fungi from environmental samples (Pang *et al*., 2016).

Morphological and microscopic identification

The purified fungal isolates were identified based on macroscopic and microscopic characteristics. Colony morphology including color, texture, margin, and growth pattern was recorded. Microscopic examination was performed using lactophenol cotton blue staining to observe spore structures, hyphae, and conidia under a light microscope. Identification was carried out by comparing observed characteristics with standard fungal identification manuals (Barnett and Hunter, 1998).

Preparation of fungal extracts

Selected fungal isolates were cultured in Potato Dextrose Broth (PDB) prepared with sterile seawater and incubated at 28°C for 10–14 days under shaking conditions (120 rpm). After incubation, the culture broth was filtered to separate the mycelial biomass. The filtrate was subjected to solvent extraction using equal volumes of organic solvents such as ethyl acetate or methanol. The mixture was vigorously shaken and allowed to separate into layers. The organic phase containing secondary metabolites was collected and concentrated using a rotary evaporator. The dried crude extract was stored at 4°C for further biological assays (Rateb and Ebel, 2011).

Test microorganisms for antifungal assay

Clinically relevant *Candida* species, including *Candida albicans*, were used to evaluate antifungal activity. The fungal cultures were maintained on Sabouraud Dextrose Agar (SDA) and subcultured prior to experimentation. The inoculum was standardized to match 0.5 McFarland turbidity, corresponding to approximately 1×10^6 CFU/mL (CLSI, 2012).

Antifungal activity assay

The antifungal activity of fungal extracts was assessed using the agar well diffusion method. Sterile SDA plates were inoculated with standardized *Candida* cultures using a sterile swab. Wells of approximately 6 mm diameter were punched into the agar, and different concentrations of fungal extracts were added. Plates were incubated at 37°C for 24–48 hours. The zone of inhibition around each well was measured in millimeters. Fluconazole was used as a

positive control, while solvent served as a negative control. This method is commonly used for preliminary screening of antifungal compounds (Balouiri *et al*., 2016).

Cell line and culture conditions

Triple-negative breast cancer (TNBC) cell lines were obtained from a recognized cell repository and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin and streptomycin), and maintained at 37°C in a humidified atmosphere with 5% CO_2. Proper aseptic conditions were maintained throughout the study to avoid contamination (Freshney, 2015).

Cytotoxicity assay (MTT assay)

The cytotoxic potential of fungal extracts was evaluated using the MTT assay, a standard method for assessing cell viability. TNBC cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight. Cells were treated with varying concentrations of fungal extracts and incubated for 24–48 hours. Subsequently, MTT reagent (5 mg/mL) was added and incubated for 3–4 hours. The formed formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability percentage was calculated relative to untreated control cells (Mosmann, 1983).

Determination of IC_{50}value

The half-maximal inhibitory concentration (IC_{50}) was determined from dose-response curves generated by plotting extract concentration against percentage cell viability. The concentration required to inhibit 50% of cell growth was calculated using regression analysis. IC_{50} values provide a quantitative measure of the cytotoxic potency of the extracts (Sebaugh, 2011).

Apoptosis analysis

To further understand the mechanism of anticancer activity, apoptosis induction was evaluated using morphological observation and staining techniques. Treated cells were stained with acridine orange/ethidium bromide (AO/EB) and observed under a fluorescence microscope. Apoptotic features such as chromatin condensation, membrane blebbing, and nuclear fragmentation were recorded. Similar approaches have been used to study apoptosis in marine-derived compounds (Anuradha *et al*., 2022).

Statistical analysis

All experiments were performed in triplicates, and results were expressed as mean ± standard deviation. Statistical significance was analyzed using one-way ANOVA followed by appropriate post hoc tests. A p-value of <0.05 was considered statistically significant. Statistical tools such as SPSS or GraphPad Prism were used for data analysis to ensure accuracy and reliability of results (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Mangrove sediment samples yielded a diverse population of marine fungal isolates. A total of 12 distinct fungal colonies were successfully isolated using serial dilution and plating techniques. These isolates exhibited considerable variation in colony morphology, including differences in color (white, green, black, and grey), texture (powdery, velvety, and cottony), and growth patterns. Microscopic examination further revealed structural diversity in terms of hyphal organization, spore arrangement, and conidial morphology. Among the isolates, predominant genera were tentatively identified as *Aspergillus*, *Penicillium*, and *Fusarium* species based on morphological characteristics. These genera are well-known for their ability to produce bioactive secondary metabolites. Out of the 12 isolates, five isolates **(**MF-3, MF-5, MF-7, MF-9, and MF-11**)** were selected for further biological screening based on their rapid growth and distinct morphological features.

Table 1. Morphological characteristics of selected marine fungal isolates

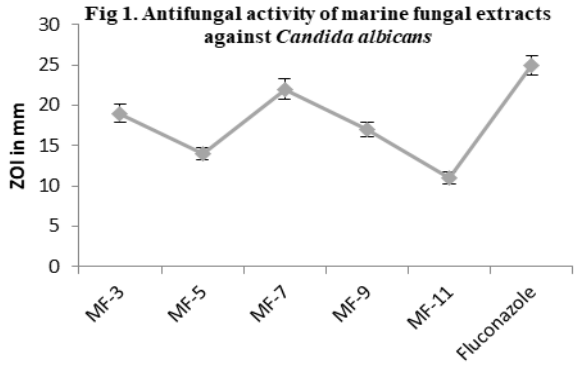
Isolate Code	Colony Color	Texture	Margin Type	Microscopic Features	Identified organism
MF-3	Green	Velvety	Regular	Septate hyphae, conidia	*Aspergillus* sp.
MF-5	White	Cottony	Irregular	Branched hyphae	*Fusarium* sp.
MF-7	Black	Powdery	Regular	Dense conidial heads	*Aspergillus* sp.
MF-9	Grey	Velvety	Smooth	Oval spores	*Penicillium* sp.
MF-11	Yellowish	Smooth	Regular	Septate hyphae	Unidentified

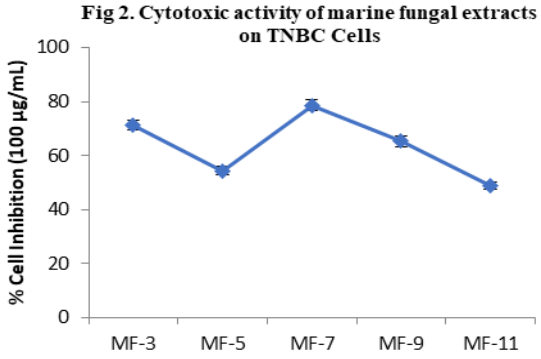
Table 2. Yield of crude extracts from marine fungal isolates.

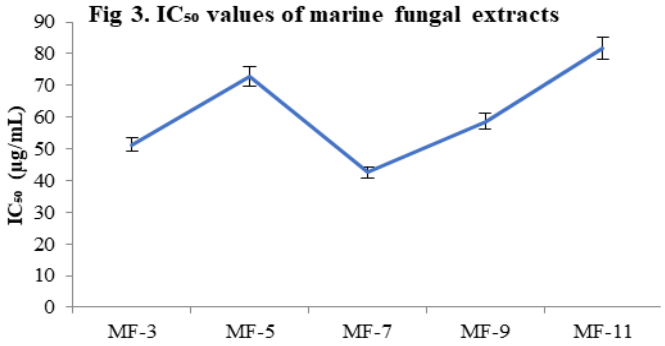
Isolate Code	Extract Yield (g/100 mL)
MF-3	0.76 ± 0.04
MF-5	0.52 ± 0.03
MF-7	0.89 ± 0.05
MF-9	0.68 ± 0.02

MF-11	0.47 ± 0.03

The selected fungal isolates were cultured in liquid medium, and their metabolites were extracted using ethyl acetate. The yield of crude extract varied among isolates, indicating differences in metabolic activity. Among the tested isolates, MF-7 showed the highest extraction yield (0.89 g/100 mL culture), followed by MF-3 (0.76 g/100 mL) and MF-9 (0.68 g/100 mL). Lower yields were observed in MF-5 and MF-11. The antifungal potential of the fungal extracts was evaluated against *Candida albicans* using the agar well diffusion method. All selected isolates exhibited varying degrees of inhibitory activity, indicating the presence of antifungal compounds (Figure 1). Among the tested isolates, MF-7 demonstrated the highest antifungal activity, with a maximum zone of inhibition of 22 ± 1.3 mm, followed by MF-3 (19 ± 1.1 mm) and MF-9 (17 ± 0.9 mm). Moderate activity was observed in MF-5, while MF-11 showed minimal inhibition. The standard antifungal drug fluconazole produced a zone of inhibition of 25 ± 1.2 mm, indicating that some fungal extracts exhibited comparable activity. The anticancer potential of fungal extracts was assessed using the MTT assay on TNBC cell lines. The results revealed a dose-dependent reduction in cell viability for all tested extracts (Fig 2). Among the isolates, MF-7 exhibited the most significant cytotoxic effect.At a concentration of 100 µg/mL, MF-7 showed 78.6 ± 2.1% inhibition of cell viability, followed by MF-3 (71.2 ± 1.8%) and MF-9 (65.4 ± 2.0%). The remaining isolates demonstrated moderate to low cytotoxic activity. The results indicate that the bioactive compounds present in these extracts may interfere with cancer cell proliferation. The IC_{50} values were calculated to determine the potency of the fungal extracts. Lower IC_{50} values indicate higher cytotoxic activity. Among the tested isolates, MF-7 exhibited the lowest IC_{50} value of42.5 µg/mL, indicating strong anticancer potential. MF-3 and MF-9 also showed promising IC_{50} values of 51.3 µg/mL and 58.7 µg/mL**,** respectively.







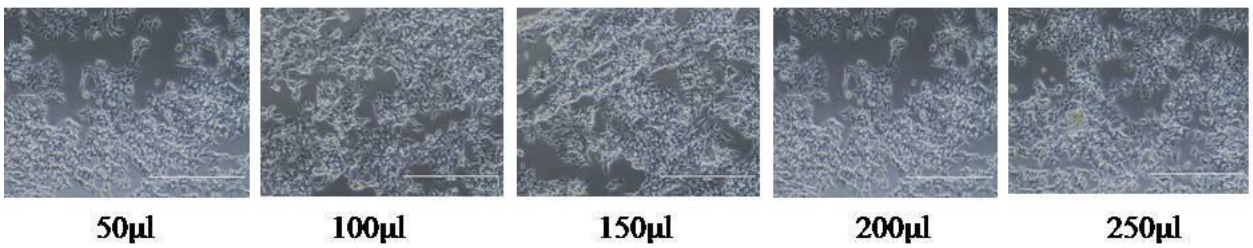


Figure 1. Morphological Changes in TNBC Cells After Treatment.

Microscopic observation of treated TNBC cells revealed characteristic apoptotic changes. Cells treated with MF-7 extract showed cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation, indicating apoptosis induction. In contrast, untreated control cells maintained normal morphology with intact membranes and uniform nuclei. The present study highlights the significant bioactive potential of marine fungi isolated from mangrove sediments, particularly in terms of antifungal and anticancer activities. Mangrove ecosystems are well known for their ecological complexity and environmental stress conditions, which drive microorganisms to produce structurally unique and biologically active secondary metabolites. The diversity of fungal isolates obtained in this study supports earlier findings that mangrove sediments serve as rich reservoirs of metabolically versatile fungi (Kathiresan and Bingham, 2001; Rateb and Ebel, 2011). The observed antifungal activity against *Candida albicans* demonstrates the therapeutic relevance of marine fungal metabolites. Among the isolates, MF-7 exhibited the highest inhibition zone, suggesting the presence of potent antifungal compounds. This aligns with previous studies reporting that marine-derived fungi produce bioactive metabolites capable of disrupting fungal cell membranes and inhibiting growth (Perfect, 2017). The increasing resistance of *Candida* species to conventional antifungal drugs has intensified the need for alternative treatments, and natural products from marine fungi offer a promising solution. The comparable activity of some fungal extracts to standard drugs such as fluconazole further emphasizes their potential clinical significance. In addition to antifungal activity, the study revealed notable cytotoxic effects of fungal extracts against triple-negative breast cancer (TNBC) cell lines. TNBC is a highly aggressive cancer subtype lacking specific therapeutic targets, making the discovery of novel treatment strategies crucial (Bianchini *et al*., 2016). The significant reduction in cell viability and low IC_{50} values observed, particularly for isolate MF-7, indicate strong anticancer potential. These findings are consistent with earlier reports demonstrating the cytotoxic effects of marine-derived bioactive compounds on various cancer cell lines. Marine organisms have been extensively studied for their anticancer properties, and the present results corroborate previous research in this field. For instance, alcoholic extracts of microalgal biomass have been shown to modulate cytotoxicity, apoptosis, and gene expression in hepatocellular carcinoma cells (Anuradha *et al*., 2022). Similarly, marine algae-derived nanoparticles have demonstrated significant anticancer activity through the induction of oxidative stress and apoptosis in cancer cells (Syed Ali *et al*., 2017). The consistency between these findings and the present study reinforces the importance of marine-derived bioresources in cancer therapeutics.

The induction of apoptosis observed in treated TNBC cells further supports the anticancer efficacy of the fungal extracts. Morphological changes such as chromatin condensation, membrane blebbing, and nuclear fragmentation are hallmark features of programmed cell death. These observations are in agreement with studies on mangrove-derived plant extracts, such as *Rhizophora mucronata*, which have been reported to induce apoptosis in cancer cells (Asbin Mary *et al*., 2022). Additionally, the protective and modulatory effects of mangrove bioactive compounds on cellular systems have been previously documented (Chitra *et al*., 2019), suggesting a broader pharmacological potential. The variation in antifungal and anticancer activities among different fungal isolates may be attributed to differences in their metabolic profiles and the nature of secondary metabolites produced. Environmental factors such as salinity, nutrient availability, and microbial competition in mangrove ecosystems influence metabolite production, leading to chemical diversity (Strobel and Daisy, 2003). This diversity is a key factor in the discovery of novel compounds with unique mechanisms of action. Furthermore, natural product-based research has consistently demonstrated that bioactive compounds from marine and plant sources, including *Annona reticulata*, possess significant antitumor properties (Ravimanickam *et al*., 2018). The present findings add to this growing body of evidence, emphasizing the importance of exploring underutilized ecosystems such as mangroves for drug discovery.

CONCLUSION

The present study demonstrates that mangrove-derived marine fungi are a valuable source of bioactive secondary metabolites with significant antifungal and anticancer potential. The isolated fungal strains exhibited notable inhibitory activity against *Candida albicans*, along with strong cytotoxic effects on triple-negative breast cancer (TNBC) cell lines. Among the tested isolates, certain strains showed superior performance, indicating their potential for further pharmaceutical exploration. The induction of apoptosis and reduction in cell viability highlight the effectiveness of these natural compounds in targeting cancer cells. These findings reinforce the importance of mangrove ecosystems as promising reservoirs for novel drug discovery. However, further research focusing on purification, structural characterization, and molecular mechanisms of action is essential to validate their therapeutic applications. Overall, this study contributes to the growing interest in marine biotechnology and supports the development of eco-friendly and effective antifungal and anticancer agents derived from natural sources.

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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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