

Original Research Article

Anti-Fungal Activities of *Azadirachta indica* Seed Extract against *Candida* Species

Arnab Mukherjee¹, Diksha Neogy¹, Zubia Zain¹, Bhaskar Narayan Chaudhuri², Partha Guchchait², Satadal Das^{2*}¹Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India²Department of Microbiology, Peerless Hospitex Hospital and Research Centre Limited, Kolkata, India

Article History

Received: 09.03.2024

Accepted: 15.04.2024

Published: 18.04.2024

Journal homepage:

<https://www.easpublisher.com>

Quick Response Code



Abstract: At present, multidrug-resistant (MDR) fungal species have become widespread worldwide, which leads to high morbidity and mortality. As of now, only a few antifungal agents are coming up in the pharmaceutical industry. This may lead to a threat to humanity for their survival. In this study, we explored the possible antifungal action of ethanolic extract of *Azadirachta indica*, seeds against MDR and ATCC strains of *Candida* species. The extract showed antifungal activities with MIC varied between 0.76 and 1.56 mg/mL in both MDR and ATCC fungal species. Thus, *Azadirachta indica* ethanolic seed extract is an effective antifungal agent on MDR fungal species and may help save the lives of many critically ill patients.

Keywords: *Azadirachta indica*, Azadirachtin, MDR *Candida* sp., MIC value, Antifungal property.

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Azadirachta indica (neem) is a member of Meliaceae family (Table 1). It is commonly found in arid subtropical and tropical areas of Southeast Asia, Africa and other continents. According to the IUCN red list data book, it belongs to the least concerned group. Studies have revealed that *A. indica* seed contains various active compounds like azadirachtin, nimbin, salannin, meliantriol etc. Dihydroazadirachtin is a reduced form of naturally occurring azadirachtin from the seed kernels of the plant. It is mainly used as a pesticide with anti-feedant and insect growth regulator (IGR) properties. It exhibits low toxicity to mammals, and it is eco-friendly. Ubiquitous microorganisms in soil and aquatic environments rapidly metabolize it [1]. In general the plant has antibacterial [2], anti-cancer [3], antimalarial [4], antiviral [5], anti-oxidant [6], and anti-diabetic properties [9].

The genus *Candida* includes about 200 different fungal species, but only a few species are human pathogens. *Candida albicans* is the most important pathogenic species, which may also reside as a commensal in the gastrointestinal and genito-urinary tract [8]. Other important pathogenic *Candida* species include *C. parapsilosis*, *C. tropicalis*, *C. glabrata* [9]. *Candida* infections can be superficial (affect the skin or

mucous membranes) or invasive. Though superficial infections are easily managed, invasive fungal infections are often life-threatening [10]. The National Nosocomial Infections Surveillance System (NNIS) of USA has declared *Candida* species (9%) as the fourth most common nosocomial bloodstream pathogen after coagulase-negative staphylococcus (CoNS) (31%), *Staphylococcus aureus* (20%) and *Enterococcus* species (10%) [11]. Mortality rates are estimated to be as high as 42.3% [12].

Table 1: Taxonomy of *Azadirachta indica*

Kingdom :	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order :	Sapindales
Family :	Meliaceae
Genus :	<i>Azadirachta</i>
Species :	<i>A. indica</i>
Scientific Name :	<i>Azadirachta indica</i>

MATERIALS AND METHODS

1. Collection of sample- The seeds (Fig 1) were collected from a standard shop having experience on plant based medicines of 150 years (Adi Ambika

*Corresponding Author: Satadal Das

Department of Microbiology, Peerless Hospitex Hospital and Research Centre Limited, Kolkata, India

Ausadhalaya; 2-B, Shyamaprasad Mukherjee road, Jatin Das Park, Kolkata-700025).



Fig 1: *A. indica* seeds

2. Preparation of seed extract stock solution- Seeds were powdered using mortar and pestle. Then 500 mg of the powder was taken in a Falcon tube, 10 ml of 70%

ethanol was poured in it and vortexed, then incubated at room temperature for 48 hours (Fig.2).

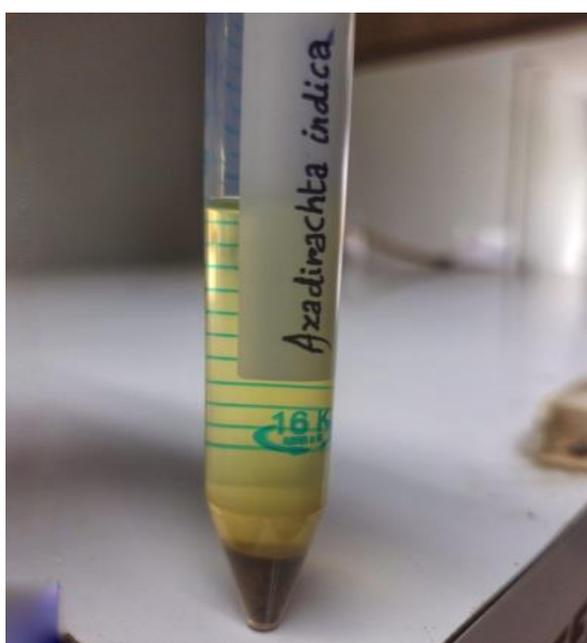


Fig 2: Stock extract of *A. indica* seed in ethanol

3. Preparation of Mueller-Hinton broth- 100 ml of MH broth was prepared by dissolving 2.1 gm of MH powder (HiMedia, India) with distilled water. Then the broth was sterilized by autoclaving.

4. Fungal species- International strains of *Candida* – *C. albicans* (ATCC 10231) and *C. parapsilosis* (ATCC 22019) were used in this study. *C. tropicalis* (MDR) and *C. albicans* (MDR) strains were isolated from a 46 year old female patient and a 59 year old male patient respectively, both admitted at Peerless hospital. Subcultures of *Candida* species were maintained on MH plates and stored in a refrigerator for experiment.

5. 0.5 McFarland standard preparation- Suspensions were prepared in sterile normal saline. Then this

suspension was standardized at 0.5 McFarland standard with the help of DensiCHEK Plus instrument.

6. Antifungal activity test- Minimum inhibitory concentration (MIC) was determined by broth microdilution test. The 96 well sterile microtiter plate was used to determine the MIC. 100 μ L of MH broth was pipetted in each of the wells. Then 100 μ l of seed extract was added in the first well. Then serial dilution (double dilution) was performed in the horizontal wells. After that, 10 μ L of 0.5 McFarland fungal suspension was pipetted in each well. 100 μ l of ethanol as vehicle control was also added in the first well in separate rows and tested similarly. The optical density (OD value) readings were taken with a Thermo MULTISKAN EX microplate

reader immediately at 620nm which was considered as 'zero hour reading'. The microtiter plate was then incubated overnight at 37°C for 24 h. A second reading was taken of each well and this was considered as '24 hour reading'. Subtractions of the '0 hr reading' absorbance from the '24 hour reading' were made for analysis. The MIC value was determined as the lowest concentration of the seed extract that inhibits the growth of the test fungus compared to the vehicle control.

RESULTS

The MIC values of the *A.indica* seed extract against *Candida albicans* ATCC (Fig 3) was 1.56 mg/ml and that against *Candida albicans* MDR (Fig 4) was 3.12 mg/ml. *Candida parasilopsis* ATCC (Fig 5) and *Candida tropicalis* (Fig 6) gave MIC values of 0.78 mg/ml and 3.12 mg/ml respectively.

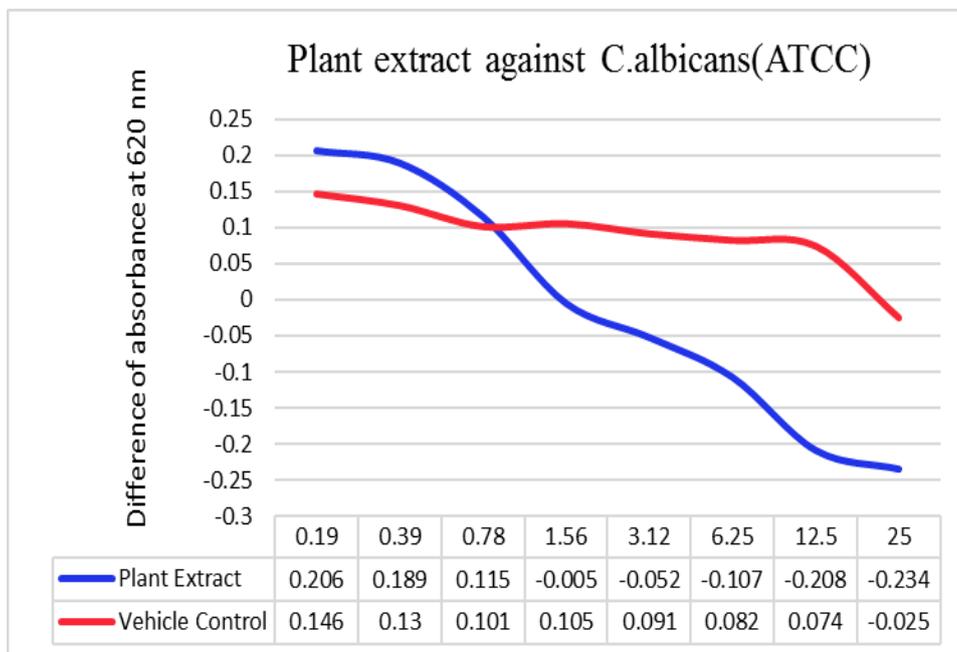


Fig 3: Antifungal activity of the Neem seed extract against *Candida albicans* ATCC. The x-axis represents the concentration of Plant extract in mg/ml

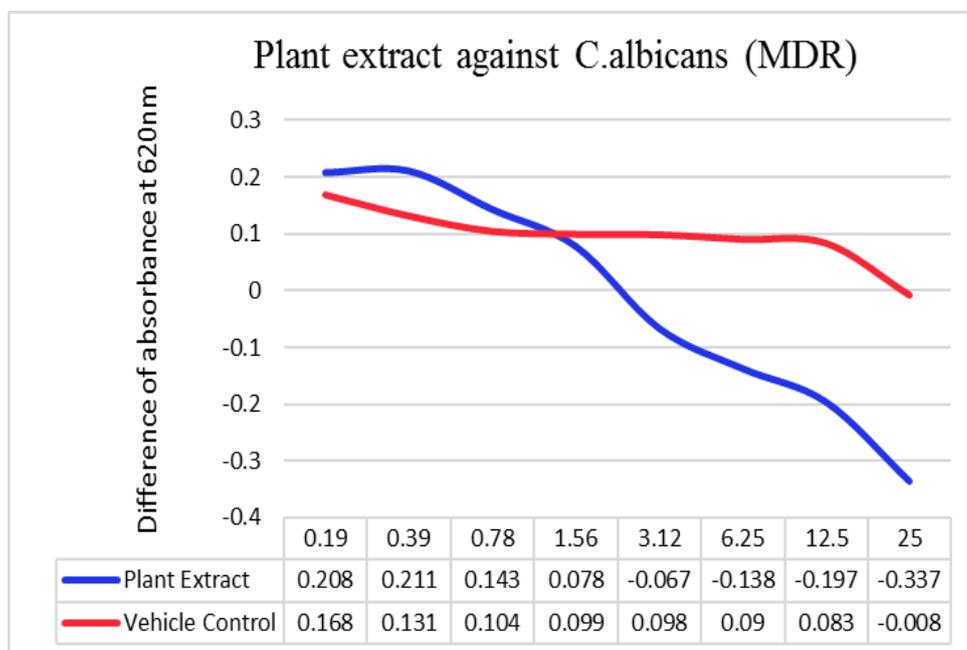


Fig 4: Antifungal activity of the Neem seed extract against *Candida albicans* MDR. The x-axis represents the concentration of Plant extract in mg/ml

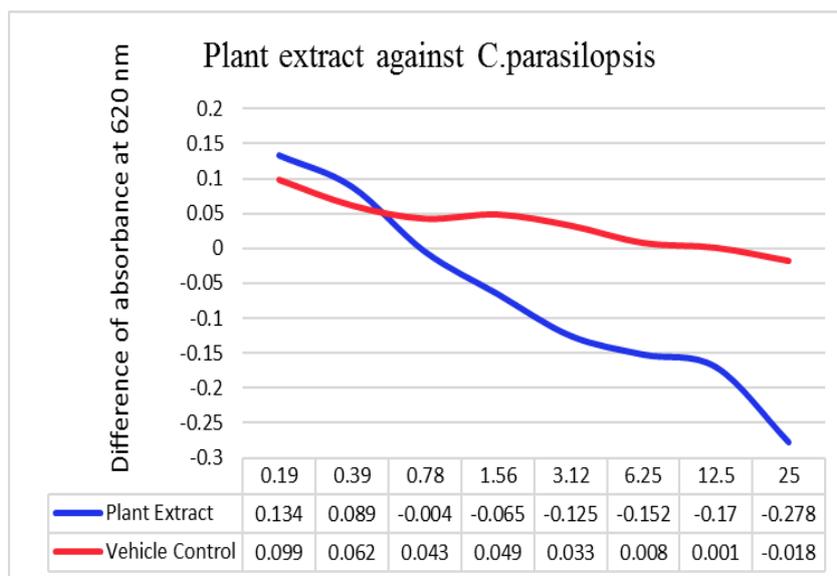


Fig 5: Antifungal activity of the Neem seed extract against *Candida parasilopsis* ATCC. The x-axis represents the concentration of Plant extract in mg/ml

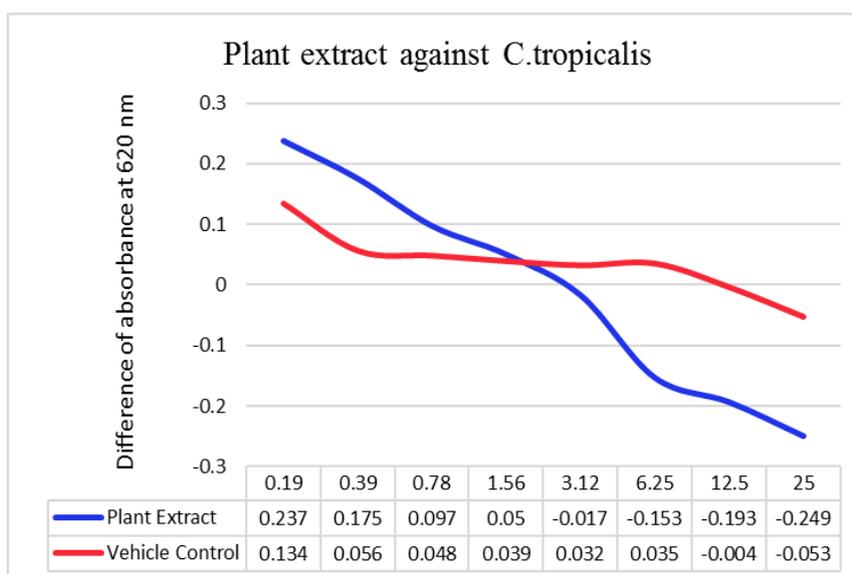


Fig 6: Antifungal activity of the Neem seed extract against *Candida tropicalis*. The x-axis represents the concentration of Plant extract in mg/ml

DISCUSSION

In this study, we investigated the antifungal properties of the ethanolic extract of *Azadirachta indica* seeds against multidrug-resistant (MDR) and American Type Culture Collection (ATCC) strains of *Candida* species. The increasing prevalence of multidrug-resistant fungal species poses a significant threat to public health, as conventional antifungal agents are becoming less effective. Our results demonstrate the antifungal activities of the *Azadirachta indica* seed extract, revealing its potential as an alternative or adjunctive treatment for combating drug-resistant fungal infections.

The MIC values obtained in our study suggest that the ethanolic seed extract of *Azadirachta indica* effectively inhibits the growth of *Candida* species, with

MIC values ranging between 0.76 and 3.12 mg/mL. Notably, the extract exhibited inhibitory activity against both MDR and ATCC strains, indicating its important antifungal potential. These findings align with previous studies highlighting the diverse pharmacological properties of *Azadirachta indica*.

CONCLUSION

Our findings highlight the promising antifungal properties of the ethanolic extract of *Azadirachta indica* seeds against multidrug-resistant *Candida* species. The presence of active components, notably azadirachtin, in the extract is believed to contribute to its antifungal activity. Further research is warranted to explore the mechanisms of action, isolate and characterize the active compounds, and assess the safety and efficacy of the

extract in clinical settings. The potential of *Azadirachta indica* seed extract as a natural antifungal agent holds promise in the fight against drug-resistant fungal infections.

Author's Contribution

AM performed the laboratory work and manuscript writing. SD prepared experimental design and analysed the data. DN and ZZ also performed laboratory work. BNC and PG corrected the manuscript.

Acknowledgement

The authors acknowledge the kind permission given by the Managing Director of Peerless Hospitex Hospital and Research Center Ltd., Kolkata for this study. The authors also thank Dr. Sudeshna Shyam Chowdhury, Head of department, Department of Microbiology, St. Xavier's College; Prof. Debjani Dutta, Faculty of St. Xavier's College and Mr. Arup Kumar Dawn of Peerless Hospitex Hospital and Research Center Ltd., Kolkata for their help and support.

Funding Details: There is no source of funding.

Declarations/Conflict of interest: There is no conflict of interest.

REFERENCES

1. Coloma, A. G., Reina, M., Diaz, C. E., & Fraga, B. M. (2010). Natural product-based biopesticides for insect. In comprehensive natural products II. *Chem. Biol*, 3, 237-268.
2. Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current science*, 1336-1345.
3. Mahapatra, S., Karnes, R. J., Holmes, M. W., Young, C. Y., Cheville, J. C., Kohli, M., ... & Donkena, K. V. (2011). Novel molecular targets of *Azadirachta indica* associated with inhibition of tumor growth in prostate cancer. *The AAPS journal*, 13, 365-377.
4. Farahna, M., Bedri, S., Khalid, S., Idris, M., Pillai, C. R., & Khalil, E. A. (2010). Anti-plasmodial effects of *Azadirachta indica* in experimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a marker. *North American Journal of Medical Sciences*, 2(11), 518-525.
5. Tiwari, V., Darmani, N. A., Yue, B. Y., & Shukla, D. (2010). In vitro antiviral activity of neem (*Azadirachta indica* L.) bark extract against herpes simplex virus type-1 infection. *Phytotherapy Research*, 24(8), 1132-1140.
6. Combined antioxidant effects of Neem extract, bacteria, red blood cells and Lysozyme: possible relation to periodontal disease. *BMC Complement Altern Med*, 2017, 17, 399.
7. Gupta, S., Kataria, M., Gupta, P. K., Murganandan, S., & Yashroy, R. C. (2004). Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *Journal of Ethnopharmacology*, 90(2-3), 185-189.
8. Achkar, J. M., & Fries, B. C. (2010). Candida infections of the genitourinary tract. *Clinical microbiology reviews*, 23(2), 253-273.
9. López-Martínez, R. (2010). Candidosis, a new challenge. *Clinics in dermatology*, 28(2), 178-184.
10. Morrell, M., Fraser, V. J., & Kollef, M. H. (2005). Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. *Antimicrobial agents and chemotherapy*, 49(9), 3640-3645.
11. Wisplinghoff, H., Bischoff, T., Tallent, S. M., Seifert, H., Wenzel, R. P., & Edmond, M. B. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clinical infectious diseases*, 39(3), 309-317.
12. Cheng, M. F., Yang, Y. L., Yao, T. J., Lin, C. Y., Liu, J. S., Tang, R. B., ... & Lo, H. J. (2005). Risk factors for fatal candidemia caused by *Candida albicans* and non-*albicans* *Candida* species. *BMC Infectious Diseases*, 5, 1-5.

Cite This Article: Arnab Mukherjee, Diksha Neogy, Zubia Zain, Bhaskar Narayan Chaudhuri, Partha Guchchait, Satadal Das (2024). Anti-Fungal Activities of *Azadirachta indica* Seed Extract against *Candida* Species. *EAS J Pharm Pharmacol*, 6(2), 77-81.
