

## ORIGINAL ARTICLE

# FUNGAL INFECTIONS BY *CANDIDA ALBICANS* AND *CRYPTOCOCCUS* SP. IN ALKHARJ GOVERNORATE: EFFECT OF THE METHANOLIC EXTRACT OF THE SAUDI SHRUB *ABUTILON INDICUM* L. ON RECORDED PATHOGENS

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## ABSTRACT

**Background:** The incidents of life-threatening complications in immunocompetent and immunocompromised individuals due to hospital-acquired fungal infections are one of the major threats to patients. As most of the pathogenic microorganisms develop resistance to the drugs in use, the scientific world is in search of new natural molecules with promising action, and thus, the present study searched for such biomolecules against infections by two of the common fungal pathogens causing such infections viz., *Candida* sp. and *Cryptococcus* sp. from a medicinal plant *Abutilon indicum* L.

**Materials & Methods:** The methanolic extracts of *Abutilon indicum* were analyzed for their antifungal potential against *Candida* sp. and *Cryptococcus* sp. by microdilution and agar diffusion methods. The antibiofilm efficiency of the extract was studied using the crystal violet method and the biofilm-eradication potential was quantified using biofilm formation assay. The hemolytic assay, time-kill assay, post-antifungal effect (PAFE), etc. were performed as per standard protocols.

**Results:** The methanolic extract of the medicinal plant *A. indicum* showed remarkable antifungal activities against the selected pathogens and the MIC (minimal inhibitory concentration) was calculated to be 1 mg/ml against both *C. albicans* and *Cryptococcus* sp. The time-kill assay displayed the killing kinetics and it showed 3 hours against *C. albicans* and 4 hours against *Cryptococcus* sp. for complete eradication. The PAFE against both the pathogens was 5 hours for both the test pathogens. The biofilm inhibition of *C. albicans* and *Cryptococcus* sp. was observed till their MIC level and a then gradual increase in biofilm formation was observed. Three various concentrations of *A. indicum* treatment could effectively eradicate the mature *C. albicans* biofilms by 67%, 69%, and 73% respectively. *Cryptococcus* sp. mature biofilms treated with the same concentrations of *A. indicum* showed 73%, 76%, and 81% of biofilm eradication indicating the anti-biofilm effect of *A. indicum*. The hemolytic assay with varying concentrations of *A. indicum* extract revealed that it did not affect RBCs.

**Conclusion:** As the plant extract showed potential antifungal activities including fungal biofilm eradication with no adverse effect on RBCs, the phytochemicals in the plant need to be purified and further investigated for its medical applications.

**KEY WORDS:** Biofilm; Concentration; Fungi; Hemolysis; Pathogens.

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## INTRODUCTION

Across the globe, during the last decade, the occurrences of infections by fungi have become the leading causes of life-threatening complications in immunocompetent and immunocompromised individuals and it has been estimated that fungal infections caused by the two main causative yeasts viz., *Candida* sp. a nosocomial pathogen and *Cryptococcus* sp. an encapsulated yeast<sup>1,2</sup> have been resulting in higher than a million mortalities every year.<sup>3,4,5</sup> These two fungi contribute to about 60% of all opportunistic fungal infections resulting in 150,000

deaths annually.<sup>6,7</sup> In humans, *Candida* species are encountered from the gastrointestinal tract, surgery sites, from vaginal swabs, and infections due to these fungi often lead to longer hospital stays and higher death rates.<sup>8</sup> Like anywhere else in the world, both these fungal pathogens are abundant in Saudi Arabian nature also, and the pathogenic strains of *Cryptococci* have been proven to have isolated from the excreta domestic birds.<sup>1,9</sup> Invasive fungal infections (IFIs) including those caused by the species of *Cryptococcus* and *Candida* have been on the rise in the entire North Africa and Middle East region including in Saudi Arabia.<sup>10,11</sup>

The scientific community is in search of antimicrobial agents from nature as the natural bioactive compounds are less vulnerable to form resistance against drugs.<sup>12,13,14</sup> The increasing number of immunocompromised patients and the use of indwelling medical devices which are prone to fungal biofilm-formation<sup>15,16,17</sup> etc., make fungal infections more common and the two species viz., *Cryptococcus* spp. and *Candida* spp. are in the list of most prevalent pathogens<sup>3,5,18</sup> which contribute to 60% of total opportunistic mycoses resulting 150,000 deaths annually.<sup>6,7</sup> Another difficulty in treating fungal infections is that many of the antifungal drugs (like echinocandins, azole, and polyenes) are used for treating fungal infections<sup>19,20</sup> and exert their activity by targeting various cell components.<sup>21-24</sup> Hence, to overcome drug resistance and limited efficacy associated with the currently used drugs, there is a requirement for developing novel antifungals for the management of *C. albicans* and *Cryptococcus* sp. infections.

In these circumstances, herbal medicines gained much concentration owing to their efficient antimicrobial properties, cost-effectiveness, ease of availability, and limited side effects.<sup>25</sup> As a result, many herbs and their products have been exploited to treat various microbial diseases. In this situation, the present investigation was done to analyze the anti-biofilm and antifungal activities of the methanolic extract of *Abutilon indicum* against *C. albicans* and *Cryptococcus* sp. infections.

## MATERIALS & METHODS

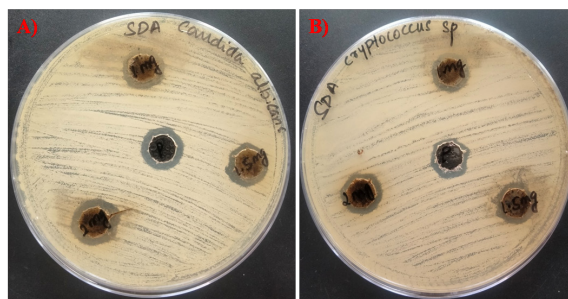
This observational study was conducted at the Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia from April 1, 2024, to May 12, 2024. Sabouraud's Dextrose Agar (SDA, Hi Media) was used to grow both *C. albicans* and *Cryptococcus* sp. For all the experiments, the overnight cultures were used and nystatin was used as positive control. To prepare *A. indicum* crude extract, into a thimble cellulose tube, 20 g of locally purchased *A. indicum* was poured and was kept in the Soxhlet apparatus. Then the solvent methanol was added to the flask

and the reaction was adjusted to 60°C for several hours until a clear solution was obtained and the solvent was evaporated. The attained crude extract was used for all the study.<sup>26</sup>

Using the well-diffusion method, the antifungal activities of the methanol extract of *A. indicum* were analyzed against *C. albicans* and *Cryptococcus* sp.<sup>27</sup> The minimal inhibitory concentration of methanolic extract of *A. indicum* against *C. albicans* and *Cryptococcus* sp. was calculated using the microdilution method. After incubation, using a Spectrophotometer, the plates were read at 600 nm.<sup>28</sup> The biofilm eradication potential of *A. indicum* on *C. albicans* and *Cryptococcus* sp. was investigated by the biofilm formation assay<sup>29</sup> using crystal violet.

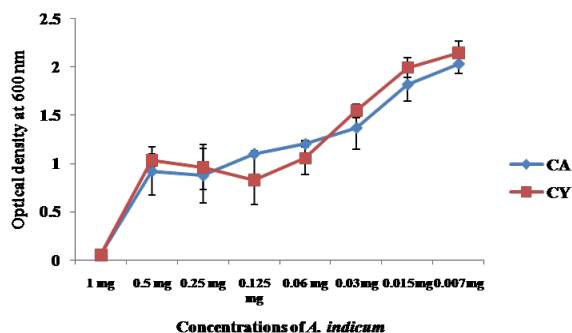
## RESULTS

The antifungal potential of the methanolic extract of *A. indicum* on *C. albicans* and *Cryptococcus* sp. was evaluated and the observations are shown in Figure 1.

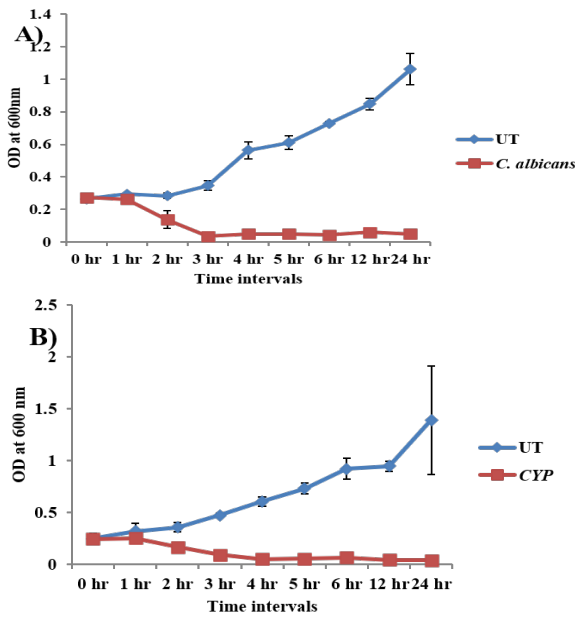


**Figure 1: Zone of growth inhibition of methanolic extract of *A. indicum* (A) *C. albicans* (B) *Cryptococcus* sp.**

The minimum concentrations of *A. indicum* are required to stop the growth of *Cryptococcus* sp. and *C. albicans* and are displayed in the graph in Figure 2.

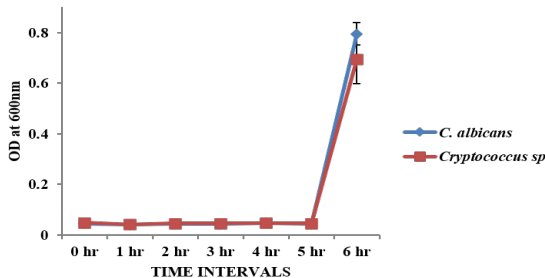


**Figure 2: Least inhibitory concentration of *A. indicum* against *C. albicans* and *Cryptococcus* sp. note: CA- *C. albicans*, CY- *Cryptococcus* sp.** Killing kinetics of *A. indicum* was evaluated against *C. albicans* and *Cryptococcus* sp. and the PAFE effect is shown in figure 3.



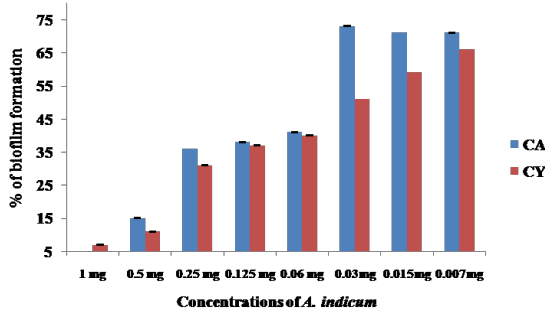
**Figure 3: Killing effect of *A. indicum* against A) *C. albicans* B) *Cryptococcus* sp.**

*A. indicum* PAFE induced against *C. albicans* and *Cryptococcus* sp. for 4 h and subsequent removal of agent is given in figure 4. *A. indicum* had the ability to stop growth till 5h for both the test pathogens.



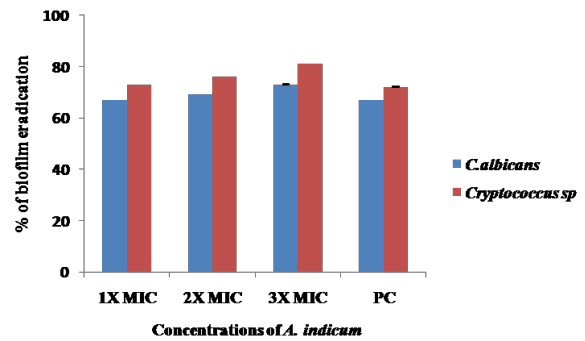
**Figure 4: P Post-antifungal effect of *A. indicum* against *C. albicans* and *Cryptococcus* sp.**

The antibiofilm potential of the methanol extract of *A. indicum* on *C. albicans* and *Cryptococcus* sp. biofilm formation was assessed and the findings are shown in figure 5.



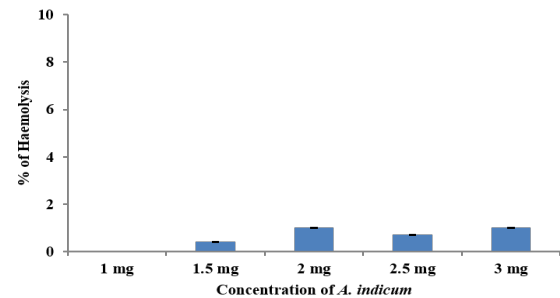
**Figure 5: The ability of *A. indicum* on *C. albicans* and *Cryptococcus* sp. biofilm inhibition. note: CA- *C. albicans*, CY- *Cryptococcus* sp.**

The *C. albicans* and *Cryptococcus* sp. biofilm eradication was quantified and the attained results are displayed in Figure 6.



**Figure 6: Anti-biofilm activity of methanolic extract *A. indicum* against *C. albicans* and *Cryptococcus* sp.**

The hemolytic effect of *A. indicum* was studied and the lysis percentage is mentioned in figure 7.



**Figure 7: *A. indicum* hemolytic effect**

## DISCUSSION

In modern times, the increase in prevalence of mycotic infections indicates the limited efficacy of common antibiotics, and the biofilm-forming ability of the pathogens results in the emergence of drug-resistant organisms underlining the urgent demand for natural antifungal compounds with promising modes of action. The biofilm (structured microbial community) forming ability of opportunistic fungal pathogens is one of the major reasons for the fungi to become resistant to antifungal drugs, which may lead to treatment failure and increasing mortality rates ranging from 35% to 80%<sup>30,31</sup>. The present study analyzed two of the most prevalent fungal pathogens in Alkharj Governorate, Saudi Arabia, and both of them are known to be the plants' materials, used in treating infections caused by various fungal pathogens for long time.<sup>32-35</sup> Another study<sup>36</sup> has also reported a similar conclusion where miltefosine showed sustaining inhibitory potential on *Cryptococcus* sp.

In support of this, a recent study evaluated the *Bauhinia holophylla* extract effect against the planktonic growth of *C. albicans*, and the growth of hyphae which are the essential steps in biofilm formation that

make treatment challenging.<sup>37</sup> Same way, another study evaluated the antimicrobial properties of *Monsonia angustifolia* and *Dodonaea angustifolia* plant extracts against various medically relevant human pathogens including *C. albicans* and *Cryptococcus* sp.<sup>38,39</sup> The antifungal activity may be due to the various phytochemical compounds such as oxygenated monoterpenes, sesquiterpene hydrocarbons, and monoterpenes, representing the potential use of essential oil in treating fungal infections.<sup>40</sup> Another study with methanolic *Guiera senegalensis* extract showed antifungal activities against different pathogenic fungi like *Fusarium oxysporum*, *Aspergillus fumigatus*, *Candida krusei*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *Colletotrichum gloeosporioides*.<sup>41,42</sup> Overall, the antifungal activities of the methanolic extract of *A. indicum* may be the presence of various potential chemical components that support the extract for potential use as an antifungal agent against *C. albicans* and *Cryptococcus* sp. causing fungal infection.

## CONCLUSION

Antifungal and antibiofilm activity of methanolic extract *A. indicum* was investigated against clinically relevant pathogenic fungi such as *Cryptococcus* sp. and *C. albicans*. The extract showed potent antifungal activities with minimal inhibitory concentrations as 1 mg/ml with potential killing and PAFE effect against both microbes tested and also effectively inhibited the biofilm formations of both tested microbes. The *A. indicum* explored antibiofilm activity by eradicating mature biofilms of *C. albicans* and *Cryptococcus* sp. representing the possible use as alternative antifungal agents against fungal infections.

**Limitations and recommendations:** The present study has two major limitations, the phytochemicals responsible for the antifungal activities were not purified and the investigation was conducted *in vitro*. So, the authors recommend that the bioactive compounds need to be further purified to detect them chemically and the study has to proceed *in vivo* to make them ready for clinical trials.

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#### CONFLICT OF INTEREST

Authors declare no conflict of interest.

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#### AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	TBMA, ST
Acquisition, Analysis or Interpretation of Data:	TBMA, ST, MMP
Manuscript Writing & Approval:	TBMA, ST, MMP

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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