IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 11 Ser.8 (November. 2022), PP 05-08
www.iosrjournals.org

Antifungal activity of Datura metel on Candida Species Isolates from Clinical Samples

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Abstract:

Background: *The yeasts are commensal in healthy humans and may cause systemic infection in immuno-compromised patients, due to their great adaptability to different host niches. The genus composed of a heterogeneous group of organisms, and approximately 17 different Candida species are found to be aetiological agents in human; however, more than 90% of invasive infections are caused by Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei. Its progression results in invasive infections, which are a significant cause of morbidity & mortality. Also, increasing resistant to antifungal drugs has paved the path for in vitro laboratory tests of active plant ingredients that may aid the clinician in choosing an appropriate therapy.*

Aim & Objectives: *Present study was undertaken to isolate & identify the different Candida species from clinical samples and to observe the effect of Datura metel plant extract in various concentration on these isolates.*

Material and methods: *A total of 176 samples were collected from patients attending Dermatology, Gynaecology and Surgery departments of LNCT Medical College, Bhopal from March 2020 to November 2021. The Candida species were isolated and identified following standard lab protocols. Plant extraction was performed by using standard procedures and percentage purity of active ingredient was assessed by HPLC method. Antifungal susceptibility testing was done by Disc diffusion method. The E–test was done for MIC and as control for antifungal susceptibility.*

Results: *A total of 51 (28.97%) candida species were isolated from 176 clinical samples. C.albicans (n=34), 19.31% observed to be the commonest species followed by C. parapsilosis (n=8), 4.54%. Antibiotic sensitivity test (AST) for various control were assessed and compared with the purified plant extract. MIC was observed maximum at concentration 1000 µg/ml followed by 750 µg/ml and least at 500 µg/ml for D. metel.*

Conclusion: *The active components of D. metel extracts having minimum or no side effects have shown positive outcome against the candida species and the prudent application of the same could provide the basis for effective and economical treatment against infection.*

Keywords: *Candida albicans, C. Glabrata, Datura metel, AST, CHROM Agar & HPLC.*

Date of Submission: 15-11-2022 Date of Acceptance: 01-12-2022

I. Introduction

Infectious diseases caused by fungi are among the major causes of illness and death worldwide. Those consequences are mainly implicated by the dramatic rise of antimicrobial resistance of the current treatment regimens[1, 2]. The terrifying aspect of antimicrobial resistance is not limited to a specified geographical area; instead, it affects the entire globe because no one is immune to antimicrobial resistance[3]. Globally, the popular fungal resistant pathogenic organisms which are accompanied with increased morbidity and mortality include Candida, Cryptococcus, Pneumocystis and Aspergillus species[4]. Of these, Candida species are among the leading causes of superficial and severe life-threatening systemic infections, especially for people living with immuno-compromised state[5].

Candida species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and genitals, responsible for various clinical manifestations from muco-cutaneous

overgrowth to bloodstream infections[6]. Most cases of oral candidiasis and esophageal candidiasis are caused by Candida albicans (C. albicans). However, there is also a dramatic increase in the frequency of fungal infections caused by the emerging non-albicans Candida (NAC) species. Candida composed of a heterogeneous group of organisms and more than 17 different species are known to be causing human infection; however, more than 90% of invasive infections are caused by Candida albicans, Candida glabrata (C. glabrata), Candida parapsilosis (C. parapsilosis), Candida tropicalis (C. tropicalis) and Candida krusei (C. krusei)[7, 8].

Currently, numerous antifungal drugs mainly derived from microbial sources are available in the market. However, the development of antimicrobial resistance becomes a challenge for the existing drugs. Due to the reason that plant materials are endowed with essential components which render an important scaffold for the development of potential drug candidates, it is relevant to screen plant-based antimicrobials from species that have strong scientific and traditional claims to combat the global concern of antimicrobial resistance.

The commercially available antifungal drugs are cost effective, having minimal or no side effects and increased fungal resistance has led the researchers to explore the natural herbal remedies against fungal infections. Traditionally used medicinal plants are rich source of antimicrobial agents and are readily available in rural areas and these benefits have enlightened the course for in vitro laboratory tests of active plant ingredients that may aid the clinician in choosing an appropriate therapy [9]. Datura metel, a perennial herbaceous plant, belonging to the Solanaceae family known to have strong antimicrobial properties and hence this research was carried out to study the efficacy of Datura metel extract against fungus. Present investigation was undertaken to isolate & identify the different Candida species from clinical samples and to observe the effect of Datura metel extract in various concentration on these isolates.

II. Materials and Methods

A total of 176 age sex cross matched patients attending Out Patient Department of Dermatology, Gynaecology and Surgery, LNCT Medical College, Bhopal from March 2020 to November 2021. The patient participating in this study was counselled individually keeping in priority the language as bar, before collecting the samples. Informed and Written consent was taken from each participant in both Hindi and English language. All samples were collected meticulously under aseptic condition in expert's supervision. The Candida species were isolated from skin, genitals and scars of patients and identified by using standard protocols in microbiology lab. Tests like KOH mount, Gram's staining, Germ tube test, sugar fermentation test and species identification by Hi-chrom media were performed for each isolated sample [8].

Datura plant was collected from botanical garden of LNCT Medical College Bhopal. Whole plant with fruits was used for the preparation of the extract. The Plant parts were cleaned properly before being introduced to air-dry process in controlled temperature of not more than 40 degree centigrade for 4-8 days following aseptic process. The dried plant was crushed into powdered form and introduced in ethanol to extract the active ingredients. Plant identification and extraction of active ingredients was performed in NBRI Lucknow, Ministry of Science & Technology, Government of India undertaken, NABL accredited Laboratory, by following standard protocols. The percentage purity of active ingredient was assessed by HPLC technique. The isolated plant extract in powdered form was dissolved using distilled water into a stock solution, thereafter various concentrations of 1000µg/ml, 750µg/ml and 500µg/ml respectively so as to find out the effective concentration of Datura extract on Candida species. Antifungal susceptibility testing was done by Disc diffusion method. The E–test was done for MIC and as control for antifungal susceptibility. Amphotericin B was used as control to compare the zone of inhibition between plant extract of various concentrations.

Inclusion criteria:

1. Patient diagnosed for fungal disease for the first time.
2. Patient willing to participate in the study.
3. Patient having age not less than 15 years & more than 65 years.

Exclusion criteria:

1. Patient on medication of antifungal drug.
2. Patient taking steroid therapy.
3. Patient not willing to participate in the study.
4. Pregnant women.
5. Patient on medication for critical disease.

III. Results

Out of 176 study subjects, 105 female (59.65%) and 71 were male (40.34%). The study subjects having age group 15 to 65 years were included and the samples were evaluated. The Reproductive age group (18-30 years) participants were maximum among others. A total of 51 (28.97%) Candida species were isolated from

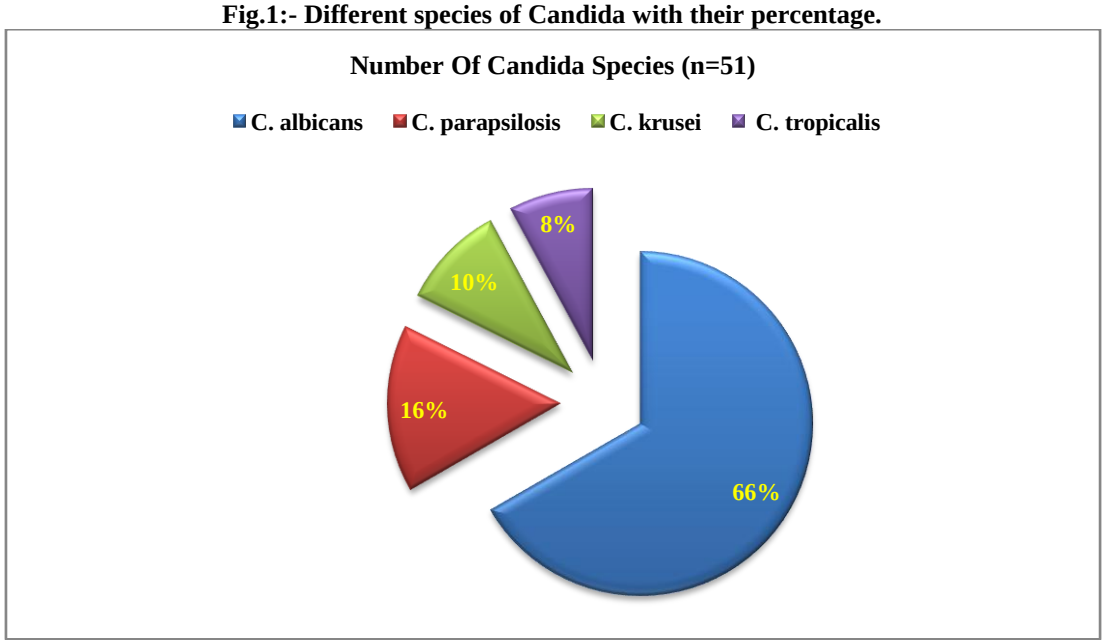
176 clinical samples. C.albicans (n=34, 19.31%) observed to be the most common species followed by C. parapsilosis (n=8, 4.54%), C. krusei (n=5, 2.84%) and C. tropicalis (n=4, 2.27%). Antibiotic sensitivity test (AST) for various control were assessed using the antifungal strips in Candida species and compared with the test (Datura metel plant extract). The Minimum Inhibitory Concentration (MIC) was observed maximum at concentration 1000 µg/ml followed by 750 µg/ml and no appreciable effect of concentration 500 µg/ml for D. Metel on Candida species. The zone of inhibition was measured in millimetres. The potent activity of plant extract was determined by the diameter of zone of inhibition.

Table-1: Comparison of zone of inhibition between Amphotericin B and Datura metel extract at 1000 µg/ml :-

Sl.No.	Candida species	Mean (± SD) Zone of Inhibition by Amphotricin B (mm) (control)	Mean (± SD) Zone of Inhibition by Datura metel extract at 1000µg/ml (mm)	*p value
1	C. albicans **(n=34)**	17.41 ± 1.76	15.85 ± 2.49	0.004
2	C. parapsilosis **(n=8)**	17.38 ± 1.3	15.75 ± 2.12	0.086
3	C. krusei **(n=5)**	18 ± 1	17.2 ± 1.3	0.31
4	C. tropicalis **(n=4)**	16 ± 0.82	15.5 ± 0.58	0.36

*p < 0.01 is significant at 95% confidence interval.

Fig.1:- Different species of Candida with their percentage.



Statistical Analysis: The variables were recorded and calculated in MS Office excel worksheet and statistical analysis was performed using SPSS 21.0 software. The mean value, standard deviation and paired *t* test was performed to compare control and test group. 'p' value less than 0.01 was considered significant when compared between size of zone of inhibition of antifungal Amphotericin B and Datura metel extract of concentration 1000µg/ml in candida albicans.

Though the overall mean value of Amphotericin B as control was higher but Datura extract also showed significant effect on Candida albicans. The comparison of Antifungal and plant extract between C. parapsilosis, C. Krusei & C. Tropicalis was not significant as described in Table-1, but the potent inhibition was observed in all microbial cultures by Datura extract in higher concentrations.

IV. Discussion

Present study is unique, relevant and rare of its own type which highlights the potential anti-fungal activity of D. Metel at various concentration against opportunistic candida infection. The antifungal susceptibility test measures and compares colony sizes of individual strains at different drug concentrations on solid medium. In this study the Datura plant extract was used in different concentration to understand the potential activity and its efficacy in candida species. Higher concentrations of the plant extract have potential to inhibit the microbial activity. Due to availability of limited resources, the expected goal couldn't be achieved but, the result shows distinct and significant antifungal property against the Candida species. Present research shows the correlation between the Amphotericin B and plant extract as positive in a way that 'P' values in case of C. parapsilosis (P=0.086), C. krusei (P=0.31) & C. tropicalis (P=0.36) when compared found to be

insignificant because the zone of inhibition in each case was found similar. Also, the Datura extract concentration of 750µg/ml showed significant inhibition in Candida species. The the colony size method was used to study the activity of the plant extracts which is simple, fast and inexpensive and requires no instrumentation. Even without using the microscope, simple comparisons of the colony sizes could usually determine the MICs for strains and multiple isolates could be streaked on a single Petri-plate [10].

V. Conclusion

The positive outcome of this study emphasizes the nature's gift to human being which has immense utility and hence these medicinal plants must be preserved and studied intensively with the help of modern molecular techniques so as to get rid of resistant pathogenic dermatophytes which have become ineffective against many antifungal drugs. Further, the easy availability of plants will decrease the expenses to manufacture the drug in large scale. Also, the controlled and cognitive usage of active components of Datura plant extracts may have minimum or no side effects to human being but may prove effective against the candida species and this could provide the basis for effective and economical treatment against candida infection. The limitation of this study was the number of sample size and cost to synthesize purest active plant ingredients in small scale which was quite expensive and cumbersome. Also, the best breed of genetically modified plants could be grown for maximum yield of active medicinal components. This study could be beneficial in the day to come as it provides the breakthrough in treatment of candidiasis where the modern drug remains ineffective against resistant species.

Source of funding
None

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgment
The authors thank to Prof. S.K. Barik, Director, CSIR-NBRI, Lucknow for his guidance in selection of best plant species and Dr Abhishek, senior scientist, CSIR-NBRI Lucknow for his contribution in identification and extraction of active ingredients of D. Metel.

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Geeta Yadav, et. al. "Antifungal activity of Datura metel on Candida Species Isolates from Clinical Samples." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(11), 2022, pp. 05-08.