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ANTIFUNGAL ACTIVITY OF MADHUCA LONGIFOLIA ON CANDIDA SPECIES ISOLATES FROM CLINICAL SAMPLES

Medical Microbiology

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ABSTRACT

Background: A number of species of Candida are commensals to human body and may often cause systemic infection in immunosupressed patients, due to their great adaptability to different host niches. Approximately 17 different Candida species are considered to possess aetiological effect in human; however, more than 90% of invasive infections are caused by Candida albicans, Candida parapsilosis, Candida tropicalis and Candida krusei. The progression results in invasive infections, which are a significant cause of morbidity & mortality of candidiasis. Also, increasing resistant fungus against antifungal drugs has compelled the scientists to discover the alternate drugs which may be proved as potentially effective drug against resistant fungus. So, present research work was carried out to check the efficacy of active ingredients of medicinal plant, Madhuca longifolia against Candida species in vitro. **Aim & Objectives:** Present study was undertaken to isolate & identify the different Candida species from clinical samples and to observe the effect of Madhuca longifolia plant extract in various concentration on these isolates. **Material And Methods:** A total of 153 clinical 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 and extraction of active plant ingredients was performed by using standard procedures. The 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 47 (30.7%) Candida species were isolated from 153 clinical samples. C. albicans (n =30), 19.6% observed to be the commonest species followed by C. parapsilosis (n=8), 5.2%. Antibiotic sensitivity test (AST) for various control were assessed and compared the activity of isolated plant extract with antifungal drug, caspofungin. MIC was observed maximum at concentration 1000 µg/ml followed by 750 µg/ml for M. longifolia. **Conclusion:** The isolated active ingredients of M. longifolia 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 concrete basis for effective and economical treatment against fungal infection.

KEYWORDS

Candida, M. longifolia, Caspofungin & HPLC.

INTRODUCTION

Fungal infections caused by the yeasts are among the major causes of morbidity and mortality around the globe. The situation turned critical by the dramatic rise of antimicrobial resistance of the current treatment regimens [1, 2]. The antimicrobial resistant condition is not limited only to a specified geographical area; instead, it affects the entire human race worldwide because of lack of immunity against resistant pathogens [3]. Candida species are among the leading causes of superficial and severe life-threatening systemic infections, especially for people living with immuno-compromised state [4]. 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 [5]. Most cases of candidiasis are caused by Candida albicans (C. albicans). However, there is dramatic increase in the frequency of fungal infections caused by the emerging non-albicans Candida (NAC) species [6]. Candida composed of a heterogeneous group of organisms and more than 17 different species are known to have pathogenic action against human [7]. However, more than 90% of invasive infections are caused by Candida albicans, Candida parapsilosis, Candida tropicalis and Candida krusei.

Currently, numerous antifungal drugs mainly derived from microbial sources are available in the market. However, the development of antimicrobial resistance becomes a real challenge for the existing drugs. Since plant ingredients 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 nominal 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 medication against fungal infection. Madhuca longifolia, a native of Indian subcontinent is a deciduous tree, belonging to the Sapotaceae family and known to have strong antimicrobial properties and hence this research was carried out to study the efficacy of M. longifolia extract against Candida species [8, 9]. Present investigation was undertaken to isolate & identify the different Candida species from clinical samples and to observe the effect of M. longifolia extract in various concentration on these isolates.

MATERIALS AND METHODS

A total of 153 age sex cross matched patients attending Out Patient Department of Dermatology, Gynaecology and Surgery, LNCT Medical College, Bhopal from March 2020 to November 2021. Each patient participating in this study counselled appropriately before collecting the samples. Informed and Written consent was collected 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[9].

The whole plant of M. longifolia with bark, flowers, leaf and fruits were collected and used for the preparation of the extract. The Plant parts were washed properly with sterilized distilled water before being air drying in controlled temperature of not more than 40 degree centigrade for 4-5 days following aseptic conditions. 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 to make stock solution, thereafter various concentrations of 1000µg/ml, 750µg/ml and 500µg/ml respectively so as to find the effective concentration of M. longifolia on various Candida species. Antifungal susceptibility testing was done by Disc diffusion method. The E–test was done for MIC and as control for antifungal susceptibility. Caspofungin drug was used as control to compare the zone of inhibition between plant extract of various concentrations.

Inclusion Criteria:

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

Exclusion Criteria:

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

RESULTS

Out of 153 study subjects, 91 female (59.5%) and 62 were male (40.5%). The age group of study subjects ranged from 15 to 65 years but maximum participants were of reproductive age group ranging from 18-30 years. A total of 47 (30.7%) Candida species were isolated from 153 clinical samples. C.albicans (n=30) observed to be the most common species followed by C. parapsilosis (n=8), C. krusei (n=5) and C. tropicalis (n=4) as shown in Fig.1. Antifungal sensitivity test (AST) for various control were assessed using the antifungal strips of caspofungin in Candida species and compared with different concentrations of M. Longifolia extract. The zone of Minimum Inhibitory Concentration (MIC) was observed maximum at 1000 µg/ml followed by 750 µg/ml and no appreciable outcome at concentration 500 µg/ml for M. longifolia on Candida species. The unit of zone of inhibition was recorded in millimetres. The potent activity of plant extract was determined by the length of diameter of zone of inhibition.

Table-1: Comparison Of Zone Of Inhibition Between Caspofungin And M. longifolia Extract At 1000 µg/ml:

Sl.No.	Candida species	Mean (± SD) Zone of Inhibition by Caspofungin (mm) (control)	Mean (± SD) Zone of Inhibition by M. longifolia extract at 1000µg/ml (mm)	*p value
1	C. albicans (n=30)	15.43 ± 2.06	12.77 ± 4.17	0.002*
2	C. parapsilosis (n=8)	16.4 ± 1.67	13.8 ± 4.49	0.26
3	C. krusei (n=5)	15.25 ± 1.5	12.75 ± 5.8	0.43
4	C. tropicalis (n=4)	14.33 ± 1.15	8.33 ± 4.51	0.08

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

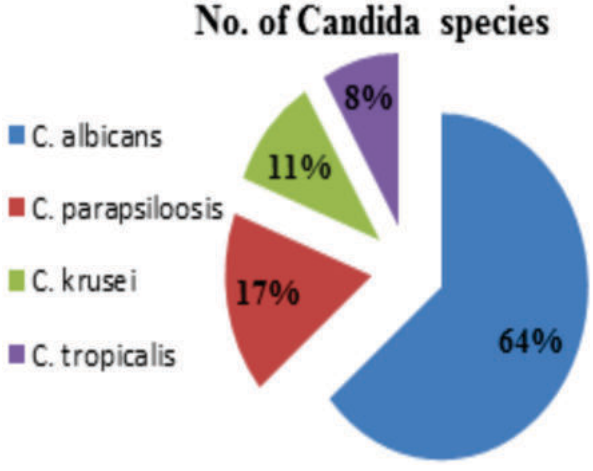

Fig.1:- Total Percentage Of Candida Species Included In Study:

Statistical Analysis:

The variables were noted 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 with test group. 'P' value less than 0.01 was considered significant when compared between size of zone of inhibition of antifungal Caspofungin and M. longifolia extract of concentration 1000µg/ml on Candida species in millimeters.

Though the mean value of diameter of zone of inhibition in case of Caspofungin was more than M. Longifolia still plant extract of M. longifolia showed significant efficacy on inhibition of growth of Candida albicans in vitro. The comparison of Caspofungin and M. longifolia 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 M. longifolia extract at concentration 1000 µg/ml.

DISCUSSION

Present study is peculiar, significant and unique as it highlights the potential anti-fungal activity of M. longifolia extract at various concentrations against opportunistic Candida infection [9]. The antifungal susceptibility test measures and compares colony diameter of individual strains at different drug concentrations on solid medium. In this study the Madhuca plant extract was used in different concentration and compared with Caspofungin as control to understand the potential activity and its efficacy on treatment of Candida infection. The higher concentrations in the purest form of active ingredient of the plant extract have potential inhibitory role on 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 positive correlation between the Caspofungin and M. longifolia plant extract as 'P' value in case of C. parapsilosis (P=0.26), C. krusei (P=0.43) & C. tropicalis (P=0.08) though statistically insignificant but the diameter of zone of inhibition in each case was found coinciding with one another. The M. longifolia extract concentration of 750µg/ml showed limited inhibition in Candida species whereas; the concentration below this didn't show any inhibition. The colony size method was used to read the activity of the plant extracts which is simple, fast and inexpensive without use of any instrumentation [10, 11]. Even without using the microscope, through naked eyes, comparison of the colony sizes could be determined to calculate the MICs for different strains and multiple isolates could be streaked on a single Petri-plate.

CONCLUSION

The positive correlation between the Caspofungin and M. longifolia in present research emphasizes the nature's gift to human being which has crucial role in combating the Candida growth and hence the best medicinal plant species must be preserved and studied intensively with the help of modern techniques so as to get rid of resistant pathogens which have become resistant against many antifungal drugs. Further, the easy availability of plants will decrease the manufacturing cost of the drug and could be made available in large scale. Also, the controlled and sagacious usage of active components of M. longifolia plant extract may have minimum or null side effects to human being but may prove effective against the Candida infection and this could provide support for effective and economical treatment against Candida infection. The limitation of this study was the number of sample size which could be increased for more significant results and secondly, the cost of chemicals used to synthesize the purest active ingredient of M. longifolia.

Also, the output of the pure active ingredient was in small scale through common technique, which made the work quite expensive, cumbersome and time taking with lot of wastage of resources. The best plant species or genetically modified plants could be used for maximum yield of active medicinal components. This study could be beneficial and may provide breakthrough in the age, where most of the fungus have become resistant against the modern antifungal drugs, resulting in treatment failure and death due to Candida infection.

Source of funding

None

Conflict Of Interest Statement

We declare that we have no conflict of interest.

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