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EXPLORATION OF IN-VITRO ANTI-FUNGAL POTENTIAL OF SIDDHA FORMULATION INDIRATHI THRAVAGAM AGAINST CANDIDA ALBICANS USING DISC DIFFUSION ASSAY

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ABSTRACT

Candida species are associated with human beings for quite long time. They are commonly found on the mucosal surfaces of gastrointestinal and genitourinary tracts and skin of humans. However, they become opportunistic pathogens in immunologically weak and immunocompromised patients. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses. The fast and widespread incidents of drug resistant among pathogenic microorganisms is the major limitation factor of using most of the antibiotics. In search of alternate therapy to overcome drug microbial resistance siddha system of medicine offers wide range of potential therapeutic agents which is not much explored globally. The main aim of the present investigation is to evaluate the anti-fungal potential of the siddha formulation Indirathi Thravagam (ID) against *C. albicans* a known opportunistic pathogen by disc diffusion method. Results of the study has revealed that the drug ITD at the concentration of 4000 µg exhibited the maximum zone of inhibition of 10mm when compare to that of the standard fluconazole (20µg) with the maximum zone of 11 mm. It was concluded from the data's obtained from the present investigation that the therapeutic value of siddha formulation depends on the range of phytotherapeutics that present in each bioactive herbal ingredients present in it. Further siddha preparation like ITD with multiple phytoconstituents may tend to act differentially which can even overcome the infections caused by drug resistant pathogens like *C. albicans.*

KEY WORDS: ***C. albicans, Antibiotics, Resistance, Indirathi Thravagam, Anti-fungal, Phytoconstituents***

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1. Introduction

Fungal infections are considered a serious health problem, especially in people with some impairment in the immune system and are a main cause of morbidity and mortality worldwide [1]. In the last two decades, fungal infections have shown a significant increment. This high incidence has been related to factors such as the increase in the number of patients with compromised immune system,

The epidemiology of Candida infections has been in flux in recent decades, most likely due to our own medical practices. Risk factors for infection with Candida are similar to those of fungal infections in general and vary in cause, but are generally due to medical intervention or health status of the patient. Risk factors fall into three distinct categories: factors that promote colonization of Candida, factors that suppress the immune response to Candida, and factors that provide a direct route for Candida infection [2]. Historically, 92–95% of all cases of Candida infection are a result of the five most common species: Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, and Candida krusei.

Because our current antifungal therapies have only modest efficacy with significant toxicities, newer antifungal formulations have been developed that ideally will reduce the occurrence of adverse effects associated with the original formulations [3,4]. Due to the slow pace of novel antifungal drug development, combination therapy has been suggested as an alternative approach to increase fungicidal potency, combat emerging drug resistance, and improve spectrum of activity. Unfortunately, combination antifungal therapy has been shown to improve outcomes in few clinical scenarios [5].

Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine [6]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [7]. Indirathi Thravagam comprises of the decoction of some bioactive therapeutic ingredients vallarai (centella asiatica), Vasambu ilai (Acorus calamus), Vellarugu ,(Enicostemma axillare), Seenthil kodi (Tinospora cordifolia). The main aim of the present investigation is to evaluate the anti-fungal potential of the siddha formulation Indirathi Thravagam (ID) against *C. albicans* a known opportunistic pathogen by disc diffusion method.

2.Materials and Methods

2.1. Disc-diffusion Assay [8]

The anti-fungal activity of the sample IDT was carried out by disc diffusion method. The concentrations of the test compounds were used at the concentration of 500,1000, 2000 and 4000 µg/ml weight e quivalent. Fungal culture was grown in SD broth at 28°C. The stock cultures were maintained at 4°C. After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted fungal strain. Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug fluconazole (20µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37oC for 48-72 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-fungal property.

3.Results

3.1. Effect on ITD on Antimicrobial activity

The siddha formulation ITD exhibited significant zone of inhibition against Candida albicans with the inhibition zone ranges from 7 mm to 10mm at the concentration of 2000 to 4000 µg. The maximum inhibitory zone diameter (IZD) of 10 mm was observed at the concentration of 4000 µg against Candida albicans which is almost similar to that of the standard drug Fluconazole (20µg) which exhibit 11mm. All IZD corresponding to test organisms are tabulated in Table 1 and represented in Figure 1.

Table 1: Zone of Inhibition data of Anti-Fungal activity of the formulation ITD

Sample Code	***Candida albicans***			
Concentration	500 µg	1000 µg	2000 µg	4000 µg
ITD	-	-	7	10
Fluconazole (20µg)	11			

\- = Not active

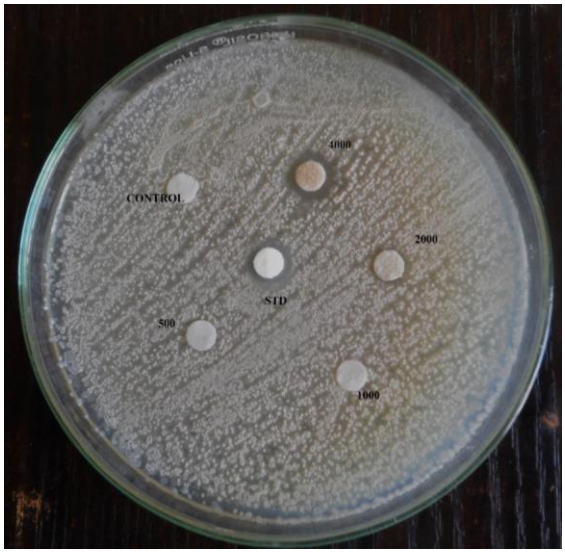

Figure 1: Anti- fungal activity of ITD against *Candida albicans*

4.Discussion

Candida is one of the most common human fungal pathogens [9] and represents the most important cause of opportunistic mycoses worldwide [10]. Candida is known as a major cause of healthcare-related infections among both immunosuppressed and immunocompetent hosts [11]. It is capable of causing both local and hematogenously disseminated infections [12]. The frequency of healthcare-related candidemia increased dramatically over the last decades and it is now considered as one of the most common bloodstream infections in the intensive care units (ICU) [13]. Despite the increase in Candida infections due to non-albicans species, C. albicans remains the main causative agent of candidemia worldwide.

Approximately 1.2 billion individuals worldwide suffer from fungal infections, and the occurrence of these infections has significantly increased in recent years due to a rise in the number of immunocompromised patients, such as patients with AIDS or those with cancer, organ transplant, or autoimmune disease who require immunosuppressive therapy [14,15].Unlike superficial infections that cause local, benign, or self-limiting diseases, invasive fungal infections (IFIs) are deep-seated and include bloodstream and systemic infections as well as infection of specific organs.

The limited effective life span of current antibiotics, the lack of compliance of patients, the unmonitored use in agriculture, and the slow rate in releasing new antimicrobial agents have led to an alarming increase in antimicrobial resistance. Multidrug-resistant (MDR) microorganisms cause almost 50% of the worldwide hospital-acquired infections. Antibiotics that show low efficacy in treating human and animal diseases through antibiotic resistance must be replaced with new drugs to combat the burden of these pathogens [16]. Hence, medicinal plants are expected to be the best source of obtaining a variety of drugs [17].

The World Health Organization estimates that 4 billion people (80% of the World's population) use herbal medicines in some aspects of primary healthcare and there is a growing tendency to "Go Natural" [18]. Infectious diseases are threatening millions of people around the world and the recent upsurge in widespread antibiotic resistance among pathogens [19,20] and the undesirable side effects associated with constant use of synthetic drugs have stimulated the need for alternative therapeutics [21]. The siddha formulation ITD exhibited significant zone of inhibition against Candida albicans with the inhibition zone ranges from 7 mm to 10mm at the concentration of 2000 to 4000 µg. The maximum inhibitory zone diameter (IZD) of 10 mm was observed at the concentration of 4000 µg against Candida albicans which is almost similar to that of the standard drug Fluconazole (20µg) which exhibit 11mm.

5.Conclusion

The alarming increase in the rate of infection by antibiotic-resistant microorganisms has urged scientists to search for formulation which have potential antimicrobial activity. Siddha system of medicine has ability to not only halt the progression of the infection but also prevent the reoccurrence. It was concluded from the data's obtained from the present investigation that the therapeutic value of siddha formulation Indirathi thravagam depends on the range of phytotherapeutics that present in each herbal ingredients present in it. Further the inhibitory zone offered by Indirathi thravagam is almost equal to that of the standard drug. Hence it was concluded that formulations Indirathi thravagam may be considered

as drug of choice in overcome the infections caused by drug resistant pathogens like C. albicans at clinical level for better therapeutic efficacy.

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