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SCREENING OF SOME SELECTED INDIAN MEDICINAL PLANTS WITH SPECIAL REFERENCE TO ANTICANDIDAL ACTIVITY

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ABSTRACT:
One of the most common and significant opportunistic fungal infections, candidiasis, is brought on by Candida species such as *Candida albicans, Candida glabrata, and Candida krusei*. Typically, amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and echinocandins are used to treat suspected Candida infections. Careful selection of an antifungal medication is required for treatment due to the potential risk of azole-resistant Candida isolate strains emerging. Medicinal herbs have anti-infective properties against bacteria, viruses, and fungi. This study's goal is to assess the in vitro effectiveness of popular Indian medicinal plants, including neem, tulsi, and aloe vera, against isolates of Candida sp. derived from clinical samples. The clinical samples gathered include oral swabs, vaginal swabs, nail scrapings, and skin scrapings. The samples were cultivated in the lab, where Candida sp. was isolated and identified. Testing for antifungal disk diffusion susceptibility was done. The medicinal herbs' aqueous and alcoholic extracts were made, and the Minimum Inhibitory Concentration (MIC) against Candida sp. was calculated. 35 out of 180 Candida isolates were discovered to be drug-resistant, which means that 19% of all isolates were resistant. All of the resistant isolates were just C. tropicalis. Finally, it can be seen that alcoholic aloe vera leaf extracts have greater anticandidal action than aqueous extracts. Neem leaf alcohol extract has a comparable impact on Candida species.

Keywords: Anticandidal, Medicinal Plants, Candida species.

INTRODUCTION:

One of the most common and significant opportunistic fungal infections, candidiasis, is brought on by Candida species such as Candida albicans, Candida glabrata, and Candida krusei. The skin, bronchi, gastrointestinal system, lungs, oral and genital mucosa, skin, and vaginal mucosa are only a few of the bodily regions that are impacted by various types of candidiasis. (Marzieh et al. 2016).

Background of the Study:

Patients in intensive care units (ICUs) are at a significant risk of developing an infection with a Candida species due to the frequent use of broad-spectrum antibiotics, central venous catheters, urine catheters, prosthetic devices, and the increasing frequency of abdominal surgery. (Ravinder et al., 2017). Amphotericin B, flucytosine, and fluconazole are typically used to treat suspected Candida infections. (Claudio et al., 1991) Itraconazole, Canadian

Itraconazole Trial Group, 1996) Echinocandins with voriconazole (Peter et al., 2016). A study by Shyamala and Prashanth (2014) does highlight the potential risk of the establishment of Candida isolates that are resistant to azoles. This study calls for the cautious selection of an antifungal medication for treatment. Medicinal herbs have anti-infective properties against bacteria, viruses, and fungi (Armando et al., 2013). This study's goal is to assess the in vitro effectiveness of popular Indian medicinal plants like tulsi, neem leaves, and aloe vera against isolates of the Candida sp. bacterium that were taken from clinical samples.

Review of Literature:

According to Ying Li et al. (2019), Candida albicans' pathogenicity is related to its capacity to adhere to epithelial cells or mucosa surfaces, establish germ tubes, transform into hyphae, and produce drug-resistant biofilms. In this study, the effects of Kalopanaxsaponin A (KPA) on the pathogenicity of Candida albicans were investigated. KPA's impact on C. albicans pathogenicity was evaluated using the XTT reduction test and fluorescence microscopic examination. The action mechanism was further elucidated using GC/MS and BioTekSynergy2spectrofluorophotometry. The cytotoxicity and therapeutic effectiveness of KPA in vivo were assessed using the Caenorhabditis elegans-Candida albicans infection paradigm. The minimum inhibitory concentration (MIC) of KPA was 816 g/mL for a number of C. albicans genotypes. The generation of biofilm, morphological change, and C. albicans adherence were all significantly impacted by the chemical. Fluorescence microscopy and the GC/MS system's findings point to the possibility that KPA can increase farnesol production by regulating Dpp3 expression and reducing intracellular cAMP levels, which together inhibit biofilm formation and morphological alteration. Particularly with KPA, there was little toxicity and a limited chance of resistance in vivo. Our findings show that KPA is very effective at reducing the pathogenicity of C. albicans.

According to Bora L. (2016), the purpose of this study was to ascertain whether different Pseudomonas aeruginosa strains and herbal remedies demonstrated anticandidal activity in vitro against Candida species. The antifungal effectiveness of Cinnamomum porrectum, Lippianudiflora, Cestrum nocturnum, Trachyspermumammi, and Sidacarpinifolia was examined using methanolic extracts. The medicinal qualities of these plants were compared to those of commercially available antibiotics. The antimicrobial assay was conducted using the agar-well diffusion method and the broth dilution method. T. ammi and C. nocturnum were shown to be more effective than the other plants. Twenty strains of P. aeruginosa were discovered in a range of clinical samples. Sabouraud dextrose agar (SDA) had overall inhibitions of 57 percent, 48 percent, and 37 percent, compared to blood agar's 47 percent, 38 percent, and 36 percent.

MATERIALS AND METHODS:

The patients who had candidiasis symptoms provided the clinical samples. The clinical samples were isolated and identified using standard techniques, and they included oral swabs, vaginal swabs, nail scrapings, and skin scrapings.(Sachin C Deorukhkar and Santosh Saini, 2014)

Antifungal Disk Diffusion Susceptibility Testing was performed using Mueller-Hinton Agar (HimediaM173) + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium – with pH between 7.2 and 7.4. Antimicrobial Disks for fluconazole (FLC 25 mcg/disc), voriconazole (VRC 1 mcg/disc), itraconazole (IT 10 mcg/disc) and amphoterecin B (AP 100 units/disc) were used. In a 35°C fixed in the incubator, the plates were placed upside down. After incubating for 24 hours, each plate was examined. (Deorukhkar S.C et al., 2014)

Extraction Technique of Medicinal Plants:

Before extracting the plant material, a botanist verified its authenticity. Allium sativum bulb, Aloe vera, Azadirachta indica, Ocimum sanctum, and Curcuma longa rhizome were all utilized.

The garlic's translucent covering was taken off. Using a sharp knife and the sharp spines on the leaf margin, the unharmed Aloe vera leaves were sliced from the base. The pulp was taken out of the leaves, and the inside mucus material was collected and processed into a liquid. Neem's young, fresh leaves were gathered. The young leaves of the green tulsi variety were taken. We bought some fresh turmeric rhizomes and cut them into little pieces.

All of the plant components were dried in a hot air oven for 72 hours before being ground into a powder for homogenization. Water and ethanol were used as solvents during the extraction process in the Soxhlet apparatus. It was stored in bottles after being filtered through a fine nylon cloth to remove debris (Stephen Olaribigbe Majekodunmi, 2015).

On RPMI 1640 (HimediaM1972), a pilot study was conducted utilizing the agar-well diffusion method to assess the effectiveness of plant extracts against Candida sp. By examining the existence or absence of a zone of inhibition around the wells, the test's results were interpreted. A zone of inhibition was deemed sensitive if it had a diameter of 12 mm or more and resistant if it had a diameter of less than 12 mm (K. Das et al., 2010).

Broth microdilution test procedure:

As the stock solution, a 10% concentration of an aqueous and alcoholic extract was utilized. 96-well microdilution plates that were disposable and sterile were used for the broth microdilution test. 8 wells received 50 µl of SD (Sabourauds Dextrose) broth. The first batch received 50 µl of the plant extract. 50 µl of the suspension was transferred to the second well after being thoroughly mixed, and so on through the seventh well. 50 µl of the suspension were removed from the seventh well and discarded. A control well without any plant extract is kept at number eight. 50 µl of the inoculum suspension are used to inoculate each well. The lowest concentration of the plant extract, or 0.0781 mg in 100 µl, is found in the seventh well, while the first well has the highest concentration, or 5 mg in 100 µl. Now, just 50 ml of the SD broth and 50 µl of the inoculum suspension are present in the control well. For 48 hours, the microdilution plates were incubated at 37°C, and growth was monitored. Each well's growth is contrasted with the growth of the control well. The last tube in each series of wells that has a clear supernatant was chosen as the MIC (Minimum Inhibitory Concentration) value since it was assumed to be free of any microbial growth.

RESULTS AND DISCUSSION:

Results:

A total of 325 clinical samples, including nail scrapings, skin scrapings, vaginal swabs, and oral swabs, were gathered throughout the study period. 180 Candida isolates were isolated from these samples. A total of 84 C. albicans isolates were collected, and an antifungal disk susceptibility test revealed that every single isolate was susceptible to voriconazole, fluconazole, itraconazole, and amphoterecin B. A total of 92 C. tropicalis isolates were collected, and of these, 35 exhibited drug resistance to voriconazole, fluconazole, and amphoterecin B, while the remaining 57 exhibited drug sensitivity. Itraconazole was effective against every single C. tropicalis isolate. There is evidence that all four C. glabrata isolates are susceptible to the antifungals voriconazole, fluconazole, itraconazole, and amphoterecin B.

The standard deviation and average MIC displayed by plant extracts against several species of Candida were determined using statistical analysis. To ascertain whether there are statistically significant variations in the MIC values of the various plant extract groups, the Kruskal-Wallis H test was utilized. The Mann-Whitney U test was performed to see whether there were any appreciable differences between the two groups. (SPSS V20).

Discussion:

It is significant to note that in this study, 35 out of 180 isolates of Candida were discovered to be drug-resistant, giving the overall percentage of resistant strains to be 19%. Additionally, all of the resistant isolates were just C. tropicalis. According to Yu-Shin et al.'s (2006) comparison of the antifungal susceptibilities of various Candida species, C. glabrata is more

resistant to fluconazole than other Candida species, but in the current study, C. tropicalis was discovered to be more resistant. Aloe vera's aqueous and alcoholic extracts were reported to have mean MICs against Candida albicans of 0.24 and 0.26, respectively. As the MIC of both the aqueous and alcoholic extracts of aloe vera on Candida albicans was 0.50, the analysis did not reveal any appreciable differences from the work by Mbajiuka et al. (2014). Aloe vera extract's mean MICs against Candida tropicalis were 0.23 and 0.22, respectively. The mean MIC values for Aloe vera's aqueous extract and alcoholic extract for C. glabrata were 0.23 and 0.35, respectively. None of these variations may be regarded as important. This study established the aloe vera leaf extract's potent anticandidal properties. On C. albicans, the mean MIC for aqueous neem extract was 0.72 and for alcoholic neem extract was 0.33. Neem aqueous extracts at doses of 0.05-0.15 and neem alcoholic extracts at concentrations of 0.025–0.0375 showed sensitivity in C. albicans Nayak et al.(2011). The figures for C. tropicalis were 1.92 and 0.26, respectively. The mean MIC values reported by C. glabrata are 0.625 and 0.42. The current research could aid in the search for proof of neem leaf extracts' anticandidal properties. The mean MIC of the Tulsi aqueous extract and alcoholic extract against Candida albicans, respectively, was 0.74 and 1.74. The research Mithra et al. (2012) provides more support for the findings. On C. tropicalis, the mean MIC for the aqueous and alcoholic extracts of Tulsi was 0.23 and 0.36, respectively. The mean MIC values for both extracts were 0.93 against C. glabrata. Additionally, tulsi extract has anticandidal properties.

CONCLUSION:

This work emphasizes the possibility of azole-resistant non-Albicans Candida (NAC) strains like C. tropicalis, which are increasingly being identified from clinical specimens, emerging. Finally, it can be seen that alcoholic aloe vera leaf extracts have greater anticandidal action than aqueous extracts. Neem leaf alcohol extract has a comparable impact on Candida species.

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