Review article

Screening, identification and isolation of some fungal species against opportunistic fungal pathogen candida albicans

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ABSTRACT

Candida albicans or Monilia albicans is an opportunistic fungus, it is basically present on the skin and mucous membranes such like vaginal area, mouth, or rectum part. Candida albicans can travel through the blood and affects intestines, throat, and heart valves of healthy as well as immunocompromised individual. When is there is any change in body temperature Candida albicans becomes an infectious agent and it starting grow out of control it also cause life threating infections to patients with weak immunity (AIDS), patients in ICU and undergoing Chemotherapy. Some of the Candida infections could be treated with home remedies, topical creams or with prescription medication such as clotrimazole, miconazole, nystatin, tioconazole, or oral administration of drugs such as fluconazole and amphotericin B. Although all this antifungal drug helps in getting cure the trouble, but the infection can recur. As per the ongoing research on all over the globe, it has been proven that Candida albicans become resistant over most the drug, so it is necessary to find an alternative drug to cure this life-threatening organism. In the present study we evaluated and screened the secondary metabolites of fungi for the control of Candida albicans by evaluating the potential of fungal bioactive compounds, its purification and characterization.

INTRODUCTIION

Many species of *Candida albicans* can inhabit in environment of human body such as in oropharynx, oral cavity, bronchial-secretions, folds of skin, fecal discharge, vagina and urine [(1).] Not every Candida species affect all regions of body but *C. albicans* is root cause for Candidiasis and almost 50 percent of the cases are found affected due to this opportunist. It is living in warm and humid place like oral and vaginal cavity which is considered as the most favorable place for developing the dreadful infection. Diseases like vulvo vaginal candidiasis (VVC), vaginitis are the most common infection which affects women once in their lifetime [(2).]

Current antifungal treatments are mostly dependent to azoles, amphotericin B and echinocandins[(3).] Due to high occurrence of the of Candida albicans in immune compromised patients, there is a high need to develop novel therapeutic against this opportunistic human pathogen as it is becoming resilient over most of conventional drugs[(4).]

Majority of products been traditionally obtained from natural resources like plant, herbs and fungal compounds which was used as therapeutically to overcome with this situation. Many fungal metabolites have been shown important anti-candidal activities, this is justifying even more to do research on traditional medicine focused on anti-candidal characterization of fungi. The biological activity of fungal metabolites from different regions of the world has been studied by several groups of researchers based on tradition. Currently anti-candidal treatments are mostly dependent upon Fluconazole, echinocandins and amphotericin B.

MATERIALS AND METHOD

Identification and Isolation of fungus

Many the fungal species were collected and isolated from Rani Durgawati University campus ground and mycology research lab of Biological Science Department R.D. University Jabalpur, Madhya Pradesh, india, Shri Satyasai University of Technology & Medical Science Sehore, M.P. and MTCC Chandigarh, The

authenticity of collected fungal species were confirmed by viewing under light microscope 10x, 40x, and 100x oil immersion magnification power by field expert Dr. Jamaluddin sir (Writer 'Fungi of India')[5].

Medium

Potato Dextrose Medium and potato dextrose broth for fungal strains, Sabouraud's agar medium and broth medium for test fungi *Candida albicans*, used as standard medium.

SCREENING OF FUNGI FOR ANDICANDIDAL ACTIVITY

Primary and Secondary screening

Many fungal species were isolated and tested against *Candida albicans*, most of them gives little or no inhibition against test fungi, while some of them have showed maximum zone of inhibition and selected for secondary screening for further experiment. Best fungal species from primary screening were selected for secondary screening against test fungi. Finally, fungi which showed maximum zone of inhibition and great potential were selected for further experiments.

Preparation of Cell free culture filtrate

Cell free culture filtrate was prepared aseptically with the help of seven days old plate culture of fungal growth. Disc containing fungal mycelium was cut with sterile cork borer and impregnated in Potato dextrose broth medium then the fungal broth was kept in Orbital shaking incubator (Remi, India) at 27^{o} for 12 days (Pre calculated optimum condition for best results) at 110 rpm. CFCF was obtained by filtering the fungal broth through whatman filter paper [6].

Organic Solvent Extraction

Fungal secondary metabolites were extracted from the 12 days old broth culture of selected fungal strain; Solvents used in extraction were n-Butanol, Xylene, Ethyl acetate, Acetone, Chloroform. Extraction process was initiated in volumetric flask, fungal broth and organic solvents were mixed in proportional quantity, after rigorous shaking two immiscible layers were formed. Organic solvent layer was separated carefully and subjected to vacuum rotary evaporator (temp 40^{o} at 70-80 vacuum pressure), Fungal bioactive compound obtained after evaporation of solvent was purified with column chromatography followed by TLC.[7]

Antimicrobial assay

The *in vitro* anti-candidal activities of the crude extract of fungi and the isolated purified compound was evaluated by Kirby Bauer's Disc diffusion technique [7]. The inoculums of test fungi was prepared 24 hours before performing the experiments on Sabouraud's dextrose medium and stored at optimum temperature 27◦.Inoculums of fungal strain were prepared in potato dextrose broth (Hi-media), while Sabouraud's Agar medium was used in preparation of test fungi inoculums.[8]

Fungal strains were collected from the Mycology research laboratory, Rani Durgawati University Jabalpur, while test Fungi *Candida albicans* was isolated from urine sample of infected patient affected from yeast infection and Standard Test fungi was collected from MTCC Chandigarh. There after Sterilized medium was poured in pre sterilized petri plates and allowed to cool and solidify. Then the broth of test fungi was taken on cotton swab and was spread evenly on plate.(9)

Dried and sterilized pretreated fungal disc (soaked overnight in fungal strain inoculums) of 5 mm diameter (Whatman filter paper), were then loaded carefully by using sterile forceps and micropipette. Discs containing the test strain were placed on Sabouraud's dextrose agar medium plate uniformly.(10)

Antibiotic Flucanozole 25 (Hi-media) discs were used as a standard control and blank discs (soaked with solvents) were used as positive control and DMSO used as negative control. The plates were incubated for 24 hours at 37°C to allow maximum growth of the organisms. The anti-candidal activities of fungal metabolites were measured from the zone of inhibition and all experiments were carried out in triplicate.

THIN LAYER CHROMATOGRAPHY

TLC used to separate the compound which is present in the crude extract, in this method the separation of the compound depends upon the usage of the solvent. The drug with 1 mg/ml of concentration was plotted on the TLC plate and dried. It was then run with different solvent ratios; the spots were identified both under UV light and by the iodine chamber method.

HPLC ANALYSIS

HPLC analysis was performed for confirmation of crude results. Extracts were dissolved in desired volume with mobile phase (1 ml/samples), filtered through a 0.45µm disposable syringe filter into chromatograph. Aliquots of10 µl were injected on HPLC column and analyzed using Shimadzu liquid Chromatograph, which was equipped with an LC-20Ad pump unit, a Rheodine injector, SPD detector, and with LC Solutions. A reverse-phase DC 18 column (150 × 3.9 mm, 5 µm) was used, at room temperature. The mobile phase used was Acetonitrile and Ethyl acetate (75:25 flow rate of 1 ml/min) for 10 min. Absorbance of samples and standard (Citrinin standard used) was detected at 360 nm range. Retention times of each

samples and peak areas were calculated by LC software. By analysis of sample with standard presence of citrinin in the samples identified.

RESULTS AND DISCUSSION

Anti-candidal assay

The present investigation records the antifungal activities of fungal extracts against *Candida albicans*. However, the degree of inhibition varied depending upon the concentration of the crude extracts. Different solvents such as Chloroform, ethyl acetate, *n*.Butanol, Xylene, Methanol and Acetone were used in extraction of fungal active metabolite compounds and their activities were tested against Test fungi *Candida albicans*. Xylene extract of *Penicillium notatum* exhibited maximum zone of inhibition against *C.albicans*, while Ethyl acetate crude extracts of *Trichoderma viridae* showed minimum growth suppression (Table.2). (Fig.14) these results are in accordance with the earlier report [8] *Aspergillus fumigates* and *A.niger* was also found equally effective in controlling the growth of *C.albicans*. The fungal isolates tried against *C.albicans* were found potentially effective controlling its growth and development. [9]

Solvent extraction method

In the present study, the crude extracts of *Rhizopus nigricans, Aspergillus niger, A. stolonifer, A.fumigatus, Penicillium notatum, Ganoderma lucidum* produced inhibitory activity against human pathogen *Candida albicans*, By using solvent solvents extraction protocol, the crude fungal extraction produced yield enough for the experimental study and it is the most commonly used and cheap, simple method of fungal active metabolite separation. (Fig.10)

Column chromatography and TLC

The results of column chromatography (Fig.11)of selected Fungal strain (*Penicillium notatum* which showed maximum potential of inhibition against *Candida albicans*) shown ,total six number of chromatographic bands were eluted out from the column chamber (loaded with 80 -100 mesh silica gel) and activity of each eluted bands were tested by agar well diffusion assay, the active eluted bands were then purified again by running them in column again, by stepwise elution with mobile phase an yellow crude extracted at room temperature which was then filtered repeatedly to obtain pure active compound, final purification of active isolated elutes was done by TLC(fig.13) with silica gel TLC plates, Then the RF values of isolated bands was compared with standard RF value Chart (reference) . The mobile phases used in chromatographic were combination of n.Butanol: Ethanol: Acetone (120:33:57), stationary phases was silica gel, iodine vapor chamber used in formation of TLC band. Rf values were calculated by formula, and total three bands of RF values, 3.2cm, 0.685cm, 0.812cms were measured. Each band of TLC plates was scrapped out and again their activity was tested against *Candida albicans*. Final isolated active compound were identified and characterized by FTIR Spectroscopy and GCMS technique.

HPLC Analysis

The detection of active compound present in the purified compound obtained after TLC was analyzed with High performance liquid chromatography. By complete analysis of test samples presence of Citrinin is detected.(at 0.76nm) The quantification of Citrinin was done with using HPLC, the retention times of the culture extracts of fungal metabolite is compared with standard sample of citrinin(0.86nm). HPLC elution profiles from the broth of *P. notatum* isolates from all the tested plant extracts showed the same retention time as of standard citrinin retention time = 3.8 min. It has been assumed that some inhibitory metabolites are secreted by the fungal strains. Further studies are possible for the study of such metabolites in detail (Graph.2).

CONCLUSION

Candida albicans is normally present 80% in human body in cellular form but in favorable temperature it forms pseudohyphae and causes infection. Its overgrowth causes diseases like vaginities, oral thrush in infants and dreadful diseases in HIV and cancer patients.

The results of our study clearly demonstrated that different extracts of *Rhizopus stolonifer, R.nigricans, Aspergillus niger, A.Fumigatus, Penicillum notatum* exhibit anti-candidal activity which might be helpful in preventing the growth of these organisms causing various diseases and can be used in alternative system of medicine.

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REFERNCES

1. Achkar J M, Fries B C, 2010. Candida infections of the genitourinary tract. Clin. Microbiol. Rev. 23, Pages-253–273.

2. Santos Ramos M A, Calixto G, de Toledo, L G, Bonifácio B V, dos Santos, L C, de Almeida M T, 2015. Liquid crystal precursor mucoadhesive system as a strategy to improve the prophylactic action of Syngonanthus nitens (Bong.) Ruhland against infection by Candida krusei. Int. J. Nanomed. 10, Pages-7455–7466.

3. Pierce C G, Srinivasan A, Uppuluri P, Ramasubramanian A K, López-Ribot J L, 2013. Antifungal therapy with an emphasis on biofilms. Curr. Opin. Pharmacol. 13, Pages-726–730.

4. Ngo H X, Garneau Tsodikova S, Green K D, 2016. A complex game of hide and seek: the search for new antifungals. Med. Chem. Comm. 7, Pages-1285–1306.

5. Agrawal GP, SK Hasija, 1986. Microorganism in the Laboratory guide for Microbiology, Mycology and Plant Pathology. Print House (India) Lucknow.

6. Walker HL, Templetion GE, 1978. Invitro production of phytotoxic metabolites by colletotricum glesosporoides F sp aeschynomene. Plant science Lett, 13. Pages-91-99.

7. Rollins DM, Joseph SW, 2011. Minimal Inhibitory Concentration (MIC). BSCI 424-Pathogenic Microbiology, life, Umd edu, classroom, bsci 2000, 424.

8. Dhanapal V, Samuel T B, Muddukrishniah K, 2018. Antimicrobial activity of leaf of sphaeranthus indicus against some selected human pathogenic bacteria. Journal of medical Pharmaceutical and allied sciences, V 7 - I 2, 754, 2018, Pages-1008-1016. DOI - 10.22270/jmpas.v7i2.755.

9. L Tamilarasi, Ponnulakshmi R, Ezhilarasi S, Bala Subramanian, 2014. Comparative study on the antifungal activity of Aloe Vera leaf and gel in different extracts. International Journal of Pharma and Bio Sciences, 5,2,B; Pages-895 – 901.

10. Rollins DM, Joseph SW, 211. Minimal Inhibitory Concentration (MIC). BSCI 424 Pathogenic Microbiology.