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Preparation Characterization and Antifungal Activity of Chitosan Thiozole Metal Complex

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ABSTRACT

In this paper we synthesized a natural polymer based metal complex. The complex were characterize using different spectrochemical techniques like FT-IR, Mass spectroscopy and X-ray diffraction etc. The complex was found to be active towards different fungal, MIC80 was determined in vitro in liquid medium by the macro broth dilution method as per the guidelines of CLSI reference document M27-A3 for fungi, document NCCLS/CLSI M11-A6 for gram negative bacteria, and document CLSI M100-S15 for gram positive bacteria. The order of sensitivity to these compounds was C. albicans > E. coli > S. aureus.

Keywords: MIC80, in vitro, X-ray diffraction, gram positive bacteria.

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INTRODUCTION

Fungal infections have affected a large fraction of the human populations during last few years with more adverse effects in immuno-compromised patients [1]. The main causes of the increase in primary and opportunistic fungal infections are the prolonged uses of antibiotics, cancer chemotherapy regimes and the occurrence of HIV infections [2, 3]. Candida albicans has been established as the main agent responsible for most of the candidal diseases. Besides, some other Candida species viz. Candida glabrata and Candida krusei are also posing serious threats [4,5]. Despite of the availability of some new antifungal drugs, the treatment of invasive candidiasis is still a major Challenge owing to the adverse effects and resistance associated with the widespread use of these drugs [6,7]. Fluconazole, traconazole, voriconazole and posaconazole are some of the widely used antifungal drugs for the treatment of systemic fungal infections [8,9].

MATERIALS AND METHOD

Medium molecular weight chitosan powder was obtained from Hi- media chemical company, India. This chitosan powder was further purified by the dissolution of 2.5 g chitosan powder in 1 litre of dilute 0.5 $molL^{-1}$ acetic acid solution. Merceptobenzothiozole was purchased from Merck-India. All other chemicals and solvents were obtained from Merck and used as received. All chemical reactions were carried under aerobic conditions. FT- IR Spectra of polymer supported Metal-complex at various stages of synthesis was recorded using Perkin Elmer Spectrometer. Thermo gravimetric analysis was recorded using EX STAR 6000 whose temperature ranges from $50^{O}C$ to $800^{O}C$ in a nitrogen atmosphere with Alumina as reference standard. Powder X-ray diffraction was accomplished using an x-ray diffractometer (XPERT PRO PAN analytical National physical lab New Delhi) for phase identification. The patterns were run with Cu K α radiation with a secondary mono chromator (χ=0.1545nm) at 40kv and 30mA. The analyses of various liquid products obtained in the catalytic oxidation reactions was carried out by Hewlett–Packard gas chromatography (HP 6890) having FID detector, a capillary column (HP-5), with a programmed oven temperature from 50 to 200 °C and a 0.5cm3min^{-1}low rate of N2 as a carrier gas. Pre-coated aluminium silica gel 60 F_{254} thin layer plates were purchased from E. Merck, Germany. Stock cultures of the microorganisms were maintained on nutrient agar slants and stored at 4°C. *Staphylococcus aureus* MTCC 902, *Escherichia coli* MTCC 443 and *Candida albicans* ATCC 90028 were grown and sub-cultured in Mueller-Hinton broth, Luria-Bertani broth and YPD

media

Respectively at 37 ºC in orbital shaker at 200 rpm (REMI CIS 24 BL). YPD medium consisted of 2% (w/v) glucose, 2% peptone, and 1% yeast extracts (Hi Media, India).

Synthesis of L (Chitosan MBT ligand)

Chitosan (1gm) was dissolved in the dilute acetic acid (2%)then Stirred for 4hr.it was then add to methanol solution of Mercaptobenzothiazole (5mmol) in a single necked flask and Stirred continuously in 15hrs at 40°C. The Resultant yellowish suspension was filtered and was thoroughly washed with methanol and then ether. The purified product was dried under vacuum at 50°C in 1hr

Synthesis of Cu-L (MBT metal complex)

5mmol of mercaptobenzothiozole chitosan was added to 5mmol of $CuCl_2.2H_2O$ in a flask the whole solution was magnetically stirred for 12 hr at 40°C.The resultant light green complex was washed with methanol and then dried under vacuum for 30 min.

Fungal strains

Antimicrobial susceptibility test

The minimal inhibitory concentration (MIC) is the lowest concentration of the test compound that causes inhibition of visible growth (turbidity). MIC_{80} was determined *in vitro* in liquid medium by the macro broth dilution method as per the guidelines of CLSI reference document M27-A3 for fungi, document NCCLS/CLSI M11-A6 for gram negative bacteria, and document CLSI M100-S15 for gram positive bacteria. Fluconazole was included as positive control. In addition to this, a drug-free control was also included

Disk diffusion assays

The assay was performed as discussed previously [10]. Briefly, strains were inoculated into liquid media and grown overnight at 37 °C. Cells were then washed three times with distilled water and approximately 1×10^5cells/ml were inoculated into half-strength molten agar media at 42 ºC and poured into 100 mm diameter petri-plates. After the top layer had solidified; sterile paper discs (4 mm) were impregnated with the test compounds and placed on the agar surface. After incubation at 37ºC for 48h, the size and pattern of the growth inhibition zone around the disc on agar were evaluated.

RESULTS AND DISCUSSION

FT-IR (cm^{-1}) (KBr Pellet), 3300(OH), 1450 and 1360 (OH and NH deformation), 2500-2600 (S-H Stretching), and 1650 (C═N vibration).ESI-MS (m/z): 662.9 ($M+3H^+$), 494.8 ($M-MBT+2H^+$) 261

(M-Chitosan-MBT-Cl_2+$3H^+$) 394.9(M-MBT-Cl_2-$2H_2O$+$5H^+$).Powder XRD: 2θ=24 and Grain size D=7.89 nm.

Antimicrobial susceptibility

The MIC values obtained for all the three microbial species are shown in Table 3. Our results show that both the bacterial strains demonstrate very high MIC values, greater than 1000 mg/ml. The opportunistic fungal pathogen *C. albicans* was more sensitive to these compounds especially CuL (MIC 2.5 mg/ml). The ligand itself showed a high MIC of 50µl/ml in the fungus. The order of sensitivity to these compounds was C. *albicans* > *E. coli* > *S. aureus*.

Table 1: In vitro antimicrobial activity of Ligand (L) and Copper ligand (Cu-L)

Compounds	**MIC**		
	***C. albicans* ATCC 90028**	***S. aureus* MTCC 902**	***E. coli* MTCC 443**
CU (ligand)	50 mg/ml	> 1000mg/ml	> 1000mg/ml
Cu (copper complex)	2.5 mg/ml	> 1000mg/ml	> 1000mg/ml
Fluconazole	4 mg/ml	> 1000mg/ml	> 1000mg/ml

CONCLUSION

Chitosan MBT Cu(II) complex were Synthesized and FT-IR and ESI-MS spectroscopy conforms the mass and formation of Chitosan MBT Metal complex. XRD studies shows the presence of characteristics Peak(24° D=7.8nm). The ligand and its complex displayed good anticandidal activities against *Candida Albicans*. Interestingly, CuL exhibited high activities against Candida albicans much better than fluconazole. The most exciting feature of the reported compound is their fungicidal nature reducing the chances of drug resistance. Overall, it can be concluded from the preliminary biological investigations that the complex have a bright future as anticandidal agents.

REFERENCES

1. J Kriengkauykiat, JI Ito, SS Dadwal, Epidemiology and treatment approaches in management of invasive fungal infections, *Clinical epidemiology* 3,175-91 (2011)
2. GP Bodey, Candidiasis in cancer patients. *American journal of medicine* 77, 13-19 (1984)
3. MH Nguyen, JE Peacock, Jr AJ Morris, DC Tanner, ML Nguyen, DR Snydman, The changing face of candidemia: emergence of non-Candida albicans species and antifungal resistance.*American journal of medicine* 100,617-23, (1996)
4. L Li, H Kashleva, A.Dongari-Bagtzoglou, Cytotoxic and cytokine-inducing properties of Candida glabrata in single and mixed oral infection models. *MicrobialPathogenesis* .42, 138-147(2007)

5. SY Ruan, PR Hsueh, Formos Invasive candidiasis: an overview from Taiwan, *J. Med. Assoc.* 108, 443-451 (2009)
6. P Eggimann, J Garbino, D.Pittet, Epidemiology of Candida species infections in critically ill non-immunosuppressed patients, Lancet *Infection Disease* 3, 685-702 (2003)
7. MM Canuto, FG Rodero, Antifungal drug resistance to azoles and polyenes, *Lancet Infection Disease* 2,550-563, (2002)
8. A.Heras, N. M. Rodriguez, V. M. Ramos, E.Agullo, *N*-methylene phosphonic chitosan: a novel soluble derivative. *Carbohydrate Polymer* 1, 44 (2001)
9. G. Ceyhan, M. Köse, V. McKee, S. Uruş, A. Gölcü, M. Tümer, Tetradentate Schiff base ligands and their complexes: Synthesis, structural characterization, thermal, electrochemical and alkane oxidation, *Spectrochimical Acta A*, 95, 382–398 (2012).
10. C. Jin, Y.J. Jia, B.B. Fan, J.H. Ma, R.F. Li Chin, Chemical Letters 17, 419–422 (2006)