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Synthesis, Spectral, Electrochemical and Biological Studies of Nitrogen Donor Macrocyclic Ligand and its Transition Metal Complexes

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Novel complexes of Ni(II), Co(II), and Cu(II) were synthesized with the macrocyclic ligand, i.e., 5,8,13,16-tetraoxo-1,4,9,12-tetraazacyclohexa-decane. The ligand was prepared by the [2+2] condensation of succinic acid and ethylenediamine. Synthesized complexes have been characterized based on elemental analysis, FTIR, ^{1}H NMR, ESI MS, TG/DTA, UV-Vis spectroscopic techniques, conductivity and magnetic measurements. The molar conductance measurements of Cu(II), Co(II) and Ni(II) complexes in DMF correspond to non electrolyte nature. The redox properties of the complexes were extensively investigated by electrochemical method using cyclic voltammetry (CV). Based on these studies, a six coordinate octahedral geometry around the metal ions in the complexes has been proposed. These metal complexes were also tested for their *in vitro* antimicrobial activities against some bacterial and fungal strains to assess their inhibiting potential and the activities shown by these complexes were compared with standard drugs.

Keywords: Macrocycle, Tetradentate, Metal complexes, Antimicrobial activities, Growth curve

1. INTRODUCTION

The dimorphic yeast *Candida* is an obligate associate of warm-blooded animals. *Candida*, a commensal of the skin, gastrointestinal and genitourinary tracts, is responsible for majority of *Candida* bloodstream infections (Candidemia). Clinically, the most significant member of the genus is *Candida albicans* (47.6%) and *Candida tropicalis* (35.4%) followed by *Candida glabrata* [1] which can cause infections (called *Candidiasis* or thrush) in humans and other animals, especially in

immunocompromised patients such as leukemia, acquired immunodeficiency syndrome or patients who undergo cancer therapy, organ transplantation, severe burn cases, pregnancy are particularly susceptible to opportunistic fungal infections [2]. Candidiasis proves to be life threatening mycoses fatal for immuno compromised patients e.g. AIDS and transplantation surgery [3]. The incidence of both superficial and invasive Candidiasis has increased markedly over the last few decades. This diploid opportunistic fungal pathogen *Candida* is increasing importance to modern medicine [4, 5]. Numbers of antifungal agents are available for the treatment of *Candidal* infections [6, 7] majority of them being polyenes such as Amphotericin B and Nystatin or the azoles, such as Itraconazoles and Fluconazole. Currently, uses of standard antifungal therapies are scare due to the high toxicity, low efficacy rates, and drug resistance. Recent studies have indicated C. *albicans* fighting to azoles or heptotoxicity and nephrotoxicity connected to polyene utilize, particularly amphotericin B [8].

Macrocyclic ligands are a emerging class of compounds with changeable chemistry; vide series of molecular topology and, sets of supporter atoms [9, 10]. The macrocyclic systems are of significant interest for not only their pharmacological properties as antibacterial, anticancer, antiviral, antifungal agent [11] but also for their capacity for chemical recognition of anions and metals of biochemical, medical and environmental importance. [12-14]. The chemistry of transition metal ion with macrocyclic ligands has become a rapidly growing area of research, because of their importance in biological processes and constitutes the active site in metalloprotiens and enzymes [15-17]. Copper is an important trace element for life processes and several copper containing proteins have been identified [18, 19]. Biochemistry of nickel is well documented. [20] Nickel and its organometal derivatives shows good antimicrobial properties [21]. It shows toxicity even in low doses in both plants and animals [22]. The cobalt is an essential element for life although it does not participate O_2 metabolism. Some important cobalt-containing metalloproteins are ribonucleotide reductase [23], nitrile hydratase, glucose isomerase [24], where cobalt plays directly or indirectly an important role. Organocobalt complexes have also shown high anti bacterial activity against microbes such as *Staphylococcus aureus* and *Enterococcus faecalis* [25]. It has been well established that many drugs have become resistant to microbes, recognizing the anti microbial properties of macrocyclic transition metal complexes. We therefore have choose to work on the development of new macrocyclic ligand and its metal complexes, which may provide additional options for the treatment of superficial fungal infections, anti microbial in nature even at low doses, and they may help to overcome the limitations of current treatments. In current years, the electrochemical techniques have seen to the advancement in the field of analysis because of their sensitivity, economical and comparatively short analysis time when compared with new techniques. Additional application of electroanalytical techniques includes the determination of reaction mechanisms. Redox properties of a drug can give insights into its metabolic fate or pharmaceutical activity [26-28]. The electron transfer mechanism of the metal complexes is investigated by the aid of cyclic voltammetry. Efforts have been made in the last decades to the design and synthesis of macrocycle or macrocyclic complexes and to study their physico-chemical properties [29]. These investigations emphasized the vast significance of these systems in medicinal chemistry. Several synthetic studies are now days available for the preparation of well organized macromolecular systems which exhibit peculiar physico-chemical properties or have well defined pharmacological properties. [11, 29, 30]. So here, we report synthesis and characterization of a

macrocyclic tetradentate ligand and its transition metal complexes, which can be used as high potential drug. Encouraging results and screening of anticandidial and antibacterial activities by MIC, Resistotyping and growth curve studies have reported here for the first time.

2. EXPERIMENTAL

2.1. Materials and methods

All the synthesis and handling were carried out under an atmosphere of dry and oxygen-free nitrogen, using standard Schlenk techniques. Succinic acid (Merck Ltd, India), and ethylenediamine (Merck Ltd, India) were purchased and used as received. The solvents were purchased from (Merck India Ltd.). Samples for micro analysis were dried in vacuum to constant weight. A Perkin Elmer 2400 CHNSO Elemental Analyser performed elemental analyses. IR spectra were seen as KBr pellets using a Perkin Elmer 1620 FT IR spectrophotometer. Far IR spectra were recorded as CsI pellets in the region 650-100 cm^{-1} using a JASCO FT IR spectrophotometer. ^1H NMR spectra were recorded using a Bruker DPX-300 MHz spectrophotometer operating at room temperature with DMSO d_6 as solvent. The chemical shift (δ) are reported in parts per million (ppm) using tetramethylsilane as internal standard. Positive and negative ESI mass spectra were measured by Bruker (esquire3000_00037) instrument. Thermal analysis (TG/DTA) data were calculated under nitrogen atmosphere using a SII Ex Star 6000 TG/DTA 6300 instrument. Magnetic susceptibility measurements were approved out from a microanalysis laboratory by Gouy method at room temperature. Electronic spectra were recorded on a spectro–UV-Vis Dual Beam 8 auto cell UVS–2700 LABOMED, INC, US spectrophotometer using DMSO as solvent. Electrochemical performance of the metal complexes was measured with CH Instruments, U.S.A (Model 1110A-Electrochemical analyzer, Version 4.01) in HPLC grade DMF containing n-Bu_4NClO_4 as the sustaining electrolyte. The three-electrode system consisted of glassy carbon electrode (3 mm diameter) as a working electrode, a Ag/AgCl (3 M KCl) reference electrode and a platinum wire as auxiliary electrode was used. In order to provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with methanol and double distilled water prior to each electrochemical measurements. The electrode cleaning procedure requires less than 3 min. All the solutions checked by electrochemical techniques were purged for 10 min with water-saturated nitrogen. All measurements were carried out at room temperature (24 ^{0}C). Melting point was recorded on a Metrex melting point apparatus.

2.2. Synthesis of Macrocyclic

Ligand 5,8,13,16-tetraoxo- 1,4,9,12-tetraazacyclohexa-decane

The hot ethanolic solution (25 ml), of succinic acid (8.30 g, 0.05 mol.) and a hot ethanolic solution (25 ml) of ethylenediamine (3.00 g, 0.05 mol) were mixed slowly with constant stirring. This mixture was refluxed at 60-70^{o}C for 7 h in the presence of few drops of concentrated hydrochloric

acid. On keeping it overnight at O ^{0}C, a white cream precipitate was formed, which was filtered, washed with ethanol and dried in vacuo over P_4O_{10} and it was recrystallised from methanol (yield 75%), mp>300 ^{0}C, IR (KBr, cm^{-1}): 3296(N-H), 2995(C-H), 1640(C=O), 1430(C-N), 1033, 858, 751; ^{1}H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.56-12.35 (4H, br N-H), δ 4.25-4.45(8H, C-H_2), δ 2.71- 2.82(8H, OC-N-C-H_2). ESI MS (m/z) 285 [M]$^+$, 286 [M+1]$^+$. Elem anal calcd C 50.72, H 7.03, O 22.52, N 19.71%; found C 50.75, H 7.05, O 22.54, N 19.73%.

2.3. Complex - I

Synthesis of Cobalt (II) complex

To a solution of $CoCl_2.6H_2O$ (0.71 g, 3 mmol) in 20 mL methanol was added drop wise to a methanolic solution (20 ml) of the ligand (1.04 g, 3 mmol) with continuous stirring. The resulting solution was stirred for 7 hours at 30 ^{0}C and the solution was reduced to half of its volume. It was then allowed to stand overnight in a refrigerator. A light pink precipitate separates out, which was secluded by filtration under vacuum. It was washed systematically with hexane and dried in vacuo over fused $CaCl_2$. The compound was recovered in solid state. It was recrystallised from methanol Yield 65% and m.p.>300 ^{0}C. UV-Vis (DMSO) cm^{-1}, 13,650, 15,151, and 25,000, IR (KBr, cm^{-1}): 3281(N-H), 2977(C-H), 1645(C=O), 1416(C-N), 1043, 881, 748.; Far IR (CsI, cm^{-1}) 460 (Co-N), 347 (Co-Cl). ^{1}H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.65-12.46 (4H, N-H), δ 4.28- 4.50(8H, C-H_2), δ 2.75- 2.86 (8H, OC-N-C-H_2). ESI MS (m/z) 415 [M]$^+$, 416 [M+2]$^+$. Molar conductance, Λ_m (Ω^{-1}cm^{-1} mol^{-1}, 10^{-3} DMSO, r.t.): 30. μeff (r.t., BM): 4.93. Elem. anal. calcd C 34.81, H 4.81, O 15.46, N 13.53%; found C 34.84, H 4.85, O 15.48, N 13.55%;

2.4. Complex - II

Synthesis of Nickel (II) complex

$NiCl_2.6H_2O$ was used for the synthesis of Complex (II) following the above procedure. A light green precipitate collected, which was recrystallised from methanol, 62% yield and m.p.>300 ^{0}C; UV-Vis (DMSO) cm^{-1}, 10,362, 15,797, and 21,450, IR (KBr, cm^{-1}): 3279(N-H), 2978(C-H), 1635(C=O), 1410(C-N), 1045, 720; Far IR (CsI, cm^{-1}) 450 (Ni-N), 336 (Ni-Cl). ^{1}H NMR (DMSO d_6, 300K): δ 11.69-12.55(4H, N-H), δ 4.30- 4.53(8H, C-H), δ 2.78- 2.89(8H, OC-N-C-H_2). ESI MS (m/z) 414 [M]$^+$, 415 [M+2]$^+$. Molar conductance, Λ_m (Ω^{-1}cm^{-1} mol^{-1}, 10^{-3} DMSO, r.t.): 36. μeff (r.t., BM): 2.94. Elem. anal. calcd C 34.83, H 4.83, O 15.46, N 13.54%; found C 34.85, H 4.86, O 15.50, N 13.57%;

2.5. Complex - III

Synthesis of Copper (II) complex

For the synthesis of complex (III) following the same procedure $CuCl_2.2H_2O$ was used instead of $CoCl_2.6H_2O$. A Sky blue product was obtained, which was recrystallised from methanol, 67% yield and m.p.>300 ^{0}C; UV-Vis (DMSO) cm^{-1}, 12,987, 114,285 and 23,809, IR (KBr, cm^{-1}): 3285(N-H),

2976(C-H), 1623(C=O), 1412(C-N), 1073, 882, 749; Far IR (CsI, cm^{-1}) 440 (Cu-N), 330 (Cu-Cl). ^1H NMR (300 MHz, δ ppm from TMS in DMSO-d_6, 300 k): δ 11.73-12.63 (4H, N-H), δ 4.32- 4.55(8H, C-H), δ 2.77- 2.90(8H, OC-N-C-H_2). ESI MS (m/z) 421 M^+, 422 $[M+2]^+$. Molar conductance, Λ_m ($\Omega^{-1}cm^{-1}$ mol^{-1}, 10^{-3} DMSO, r.t.): 32. μeff (r.t., BM): 2.02. Elem anal calcd C 34.43, H 4.77, O 15.28, N 13.38%; found C 34.45, H 4.80, O 15.30, N 13.41%;

2.6. Antimicrobial Activity

2.6.1. Growth Conditions

All of the fungal and bacterial species used in this study were obtained from Indian Institute of Integrative Medicines (IIIM) Jammu (India). Stock cultures of *Candida albicans* ATCC 10261, *Candida tropicalis* ATCC 750, *Candida glabrata* ATCC 90030 and *Candida kruesi* ATCC 6258 were maintained on slants of nutrient agar (yeast extract 1%, peptone 2%, D-glucose 2% and agar 2.5%) (HiMedia) at 4 ^{0}C. To initiate growth for experimental purposes, one loop full of cells from an agar culture was inoculated into 25ml of respective nutrient media and incubated at 30-37 ^{0}C for 24 hr i.e. up to stationary phase (primary culture). The cells from primary culture (10^8 cells ml^{-1}) were re-inoculated into 100 ml fresh YEPD medium and grown for 8-10 h i.e., upto mid-log phase (10^6 cells ml^{-1}). Stock cultures of *Pseudomonas* were cultured in King's B media while as that of *E. coli* were cultured in Maconky agar. Fluconazole and Ampicillin were purchased from SIGMA chemicals (USA).

2.6.2. Determination of MIC_{80}

Minimum inhibitory concentration was defined as the lowest concentration of the test molecule that causes inhibition of visible growth of microbial cells. MIC_{80} was determined *in vitro* in liquid medium by serial broth dilution method [31]. Standard drugs Fluconazole and Ampicillin were included as positive controls. The MIC values keep up a correspondence to the most minuscule concentrations that did not allow for the recognition of any visible growth.

2.6.3. Disc Diffusion Assay

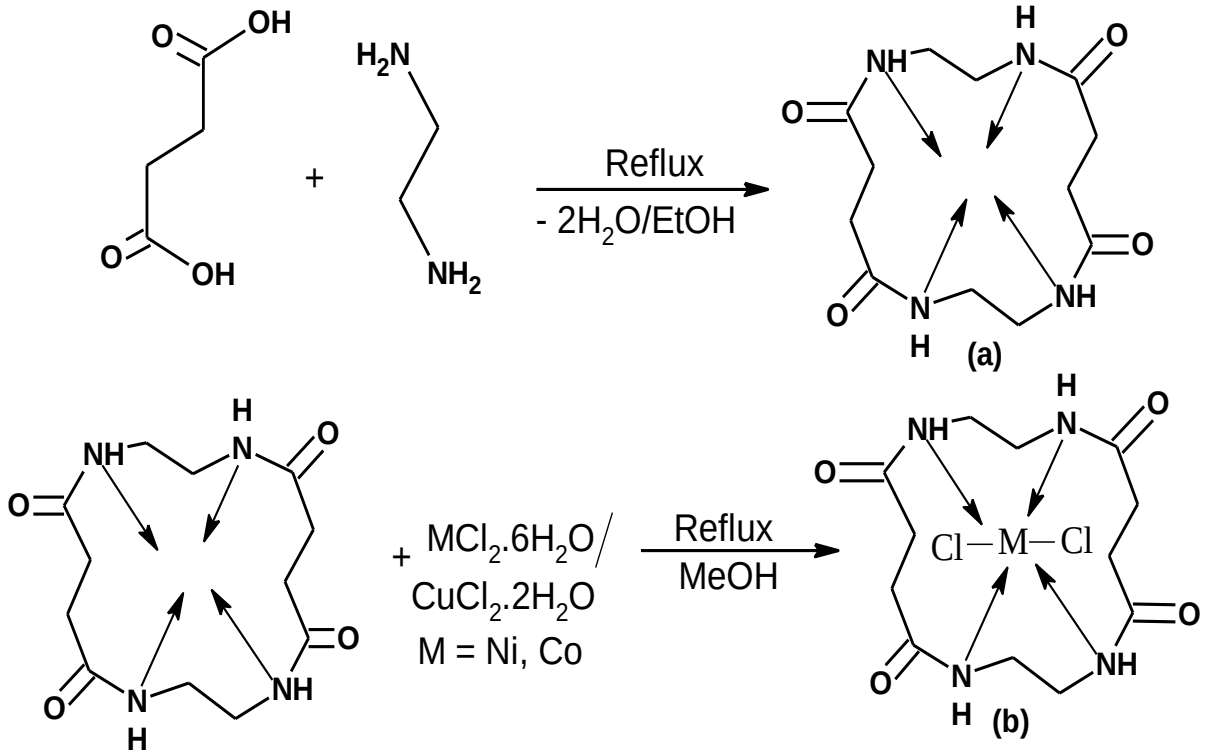
Disc Diffusion Method [32] determined inhibitory activity of ligand, its metal complexes and standard drugs against different microbes. Microbial cells (10^5 cells ml^{-1}) grown in YEPD broth were mixed in molten media with agar (~40 ^{0}C) and poured in a petriplate. Filter discs were kept on solid agar and test compound was spotted on the disc. Different concentrations (10 fold more than MIC) of the test compounds (dissolved in 10% DMSO) were functional on the disc in 10μl volume. The diameter of zone of inhibition was scored after 72h and was compared with that of control. The zone of inhibition has been reported in millimeters.

2.6.4. Growth studies

For growth studies, 10^6 cells/ml (optical density A_{595} = 0.1) culture of microbial cells were inoculated and grown aerobically in YEPD broth for control and along with various concentrations of the test compounds in individual flasks. Growth was calculated turbidometrically at 595 nm using LaboMed Inc. Spectrophotometer (USA). The growth rate of different fungi and bacteria in absence as well as in presence of test compounds was performed for each concentration.

3. RESULTS AND DISCUSSION

The ligand was synthesized by condensing the corresponding acid and the diamine with few drops hydrochloric acid (scheme 1. a). This ligand was then refluxed at room temperature with the metal chlorides to form their corresponding metal complexes (scheme 1. b).



Scheme 1 (a, b): Showing synthesis of macrocyclic ligand and its metal complexes.

(Table 1) showing the physical properties and analytical data of the ligand and its metal complexes sustain their proposed structure. The molar conductivity (Λ_m) of the metal complexes measured in 1×10^{-3} mol L^{-1} DMSO at room temperature show low values indicate that they are non-electrolyte species [33].

Table 1. Analytical data and Physical properties of the complexes

Compound	M.p. (^{0}C)	Color	Yield (%)	Exp. (Calc) (%) C	H	O	N	Λ ($\Omega^{-1}cm^2$ mol^{-1})
Ligand, S	>300	Colorless	75	50.75 (50.72)	7.05 (7.03)	22.54 (22.52)	19.73 (19.71)	---
$[CoSCl_2]$	> 300	Pink	65	34.84 (34.81)	4.85 (4.81)	15.48 (15.46)	13.55 (15.53)	30
$[NiSCl_2]$	>300	Light green	67	34.85 (34.83)	4.86 (4.83)	15.50 (15.46)	13.57 (13.54)	36
$[CuSCl_2]$	>300	Sky blue	62	34.45 (34.43)	4.80 (4.77)	15.30 (15.28)	13.41 (13.38)	32

S = $[(C_{12}H_{20}O_4N_4)]$

3.1. Infrared Red Spectra

Macrocyclic Ligand

IR spectra firmly support the formation of these compounds. Ligand does not show any evidence of the band corresponding for the free primary diamine and hydroxyl group [34]. In the IR spectrum of macrocyclic ligand absences of a broad absorption band characteristic for hydroxyl group of COOH in succinic acid, indicates that the OH group of succinic acid was detached from the COOH group to form a bond between carboxyl carbon atom and amino group nitrogen of ethylenediamine, also suggest complete condensation of reactants and elimination of water molecule. This has been confirmed by the appearance of a strong signal at 1430 cm^{-1} which may be attributed to the C-N bond [34, 35]. A sharp medium intensity band observed at 3296 cm^{-1} may be assigned to υ(N–H) of the secondary amino group [36].The ligand also shows a signals for the C=O at 1640 cm^{-1} and C-H at 2995 cm^{-1} vibrations. The low frequency of C=O group as compared to acetone (1715 cm^{-1}) is attrigrated to resonance with lone pair of the nitrogen (Table 2).

Table 2. IR Spectral data of the ligand and its metal complexes (Cm^{-1})

Compound	ν C – H	ν N – H	ν C = O	ν C – N	ν M – N	ν M – Cl
Ligand, S	2995	3296	1640	1430	…..	……
$[CoSCl_2]$	2977	3281	1645	1416	460	347
$[NiSCl_2]$	2978	3279	1635	1410	450	336
$[CuSCl_2]$	2976	3285	1623	1412	440	330

S = $[(C_{12}H_{20}O_4N_4)]$

Complexes

Based on elemental analysis the complexes were assigned to possess the composition listed in Table 1. The molar conductance measurements of the complexes in DMF correspond to nonelectrolytic nature. Thus, the complexes may be formulated as $[M(S)Cl_2]$ where M= Ni(II), Co(II),

and Cu(II) and S is [($C_{12}H_{20}O_4$ N_4)]. The shifting in the band of υ(C-N) towards the lower wave number in the metal complexes signifying that the coordination takes place through the nitrogen of the υ(C-NH) group. This indicates the flow of electron density towards the metal atom through the C-N group. This has been finally established through far IR spectra by the appearance of new signals seen at 460, 450, 440 cm^{-1} in the spectra of metal complexes which gives us clear proof for the presence of metal–nitrogen bond in Co(II), Ni(II), and Cu(II) complexes respectively [37-40]. Other vibrating signals are seen at 347, 336 and 330 cm^{-1} in the spectra of metal complexes give us proof for the presence of metal-chlorine bond in Co(II), Ni(II) and Cu(II) complex respectively [38-41].

3.2. ^1H NMR Spectra

^1H NMR spectrum of the ligand shows a sharp signal in the range 11.56- 12.35 ppm, which is, attributed to amide CO–NH, (4H) [42, 43] and does not show any signal corresponding to primary amine. A signal appearing in the range 2.71- 2.82 ppm has been ascribed to methylene protons OC–N-CH_2, (8H), while as C-H_2 (8H) protons appear in the range 4.25- 4.45 ppm. The NMR spectrum of the ligand is consistent with the single species present in the solution, since only one set of signals is observed in the ligand. These proton signals undergo down field shifting in all the metal complexes of the macrocyclic ligand, because of the paramagnetic effect of metal (II) ions and hence support the coordination of the ligand towards the metal ions [44-46] and the macrocyclic nature of the product.

3.3. Electro Spray Ionization Mass Spectra (ESI MS)

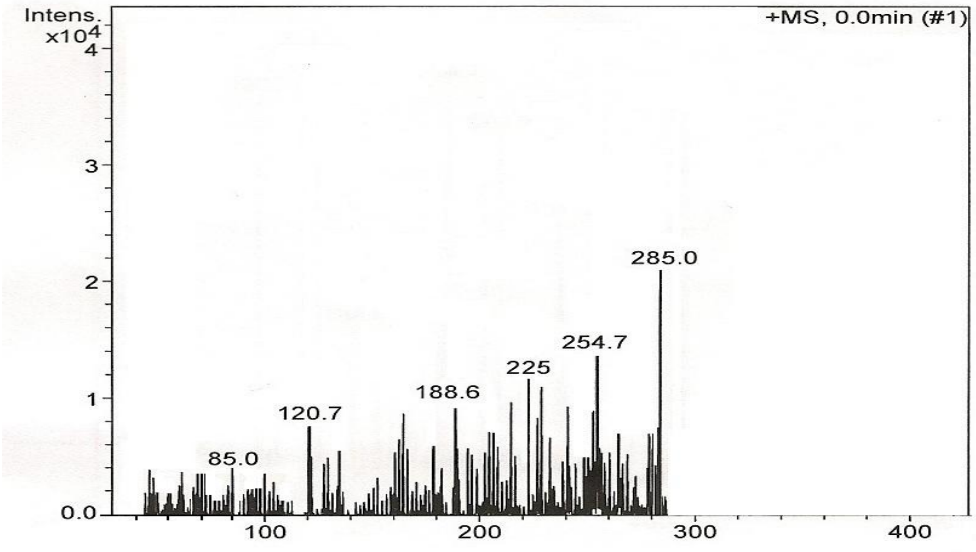


Figure 1. Electro Spray Ionization Mass Spectra (ESI MS) of Ligand.

ESI MS of the ligand and the complexes were calculated in DMSO solution Fig. 1. A positive ion ESI mass spectrum of macrocyclic ligand confirms the proposed formula by showing a peak at m/z 285 corresponding to the moiety [($C_{12}H_{20}O_8N_4$) atomic mass m/z 284.14]. The series of peaks in the

range m/z 85.0, 120.7, 188.6, 225, 254.7 etc, may be assigned to various fragments. These data suggests the 2+2 condensation of succinic acid and ethylenediamine. Their intensity gives an idea of stability of fragments. Correspondingly, positive ion ESI-MS of the cobalt, and copper, negative ion ESI-MS nickel complexes shows a peak at m/z 415, 421, 414 respectively, which is reliable with the molecular ion fragment, and it supports the proposed structure of the complexes. $[M+2]^+$ fragments were observed in all the metal complexes, possibly due to presence of isotopic chlorine in low quantities [47]. In some cases, the molecular ion peak was also associated with the solvent, water molecules and some adduct ions from the mobile phase solution [48-49].

3.4. Electronic Spectra

3.4.1. Cobalt (II) complex

A mononuclear cobalt(II) complex exhibits absorption bands at 13,650 (ν_1), 15,151(ν_2) and 25,000(ν_3) cm^{-1}, which may be consign to $^4T_{1g}$ (F)→ $^4T_{2g}$ (F), $^4T_{1g}$→ $^4A_{2g}$ and $^4T_{1g}$ (F)→ $^4T_{1g}$ (P) transitions respectively [50]. Suggesting an octahedral geometry around a Cobalt(II) ion, in the complexes under study. Furthermore, the magnetic moment measurements recorded at room temperature lie at 4.93 B.M [51]. This value is indicative of an octahedral geometry [52, 53] of these complexes.

3.4.2. Copper (II) complex

Electronic spectrum of the mononuclear copper(II) complex recorded at room temperature, in DMF solution, shows broad band absorption at 12,987, 14, 285 and 23,809cm^{-1}, which may be assign to $^2B_{1g}$→ $^2A_{1g}$, ($d_{x^2-y^2}$ → d_{z^2})(ν_1), $^2B_{1g}$→ $^2B_{2g}$, ($d_{x^2-y^2}$ → d_{zy})(ν_2), and $^2B_{1g}$→ ^2E_g, ($d_{x^2-y^2}$ → $d_{zy,}$ d_{yz})(ν_3) transition and it is in conformity with octahedral geometry [50], an indication of the most probable geometric configuration of the synthesized metal complexes is their magnetic moment values. So, additional confirmed by the magnetic moment measurements at room temperature values lie at 2.02 B.M corresponding to the presence of one unpaired electron and it supports an octahedral geometry [54-56].

3.4.3. Nickel (II) complex

The magnetic moment of the Ni(II) complex at room temperature lie at 2.94 B. M. These values fall in high spin configuration. [53] and confirm the presence of an octahedral environment around the Ni(II) ion. Ni(II) complexes exhibit three absorption bands at 10,362, 15,797, and 21,450 cm^{-1} these bands may be assign to three spin allowed transition: $^3A_{2g}$(F)→ $^3T_{2g}$(F)(υ_1), $^3A_{2g}$(F)→ $^3T_{1g}$(F)(υ2), and $^3A_{2g}$(F)→ $^3T_{1g}$(P)(υ3), respectively [56]. This value is indicative to the octahedral geometry.

3.5. Thermo Gravimetric Analysis (TG/DTA)

Macrocyclic Ligand

TG/DTA of the macrocyclic ligand and its metal complexes was recorded under nitrogen atmosphere at the heating rate of 10 ^{0}C/min, Fig. 2. The macrocyclic ligand is stable upto 215 ^{0}C and shows a continuous weight loss upto 380 ^{0}C, Therefore the whole macrocyclic ligand gets decomposed in a single step. The DTA of the macrocyclic ligand shows two endothermic peaks; one broad endothermic peak at 228 ^{0}C with a shoulder at 210 ^{0}C corresponds to the melting and the first inflexion point. The second inflexion on the DTA curve occurs at 351 ^{0}C, which represents a small weight loss step from 360-380 ^{0}C.

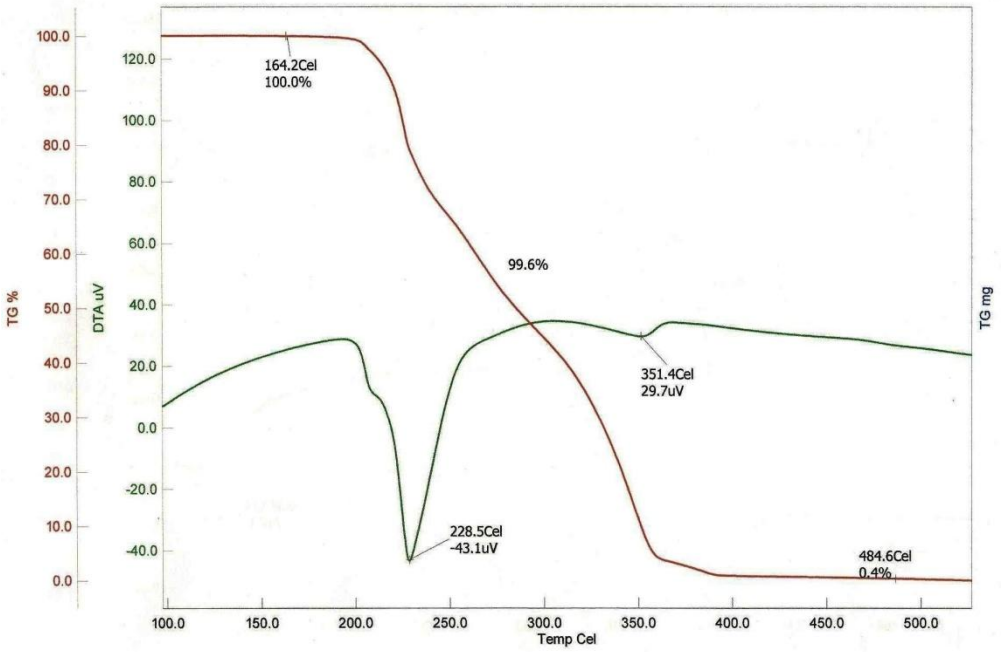


Figure 2. Thermo Gravimetric Analysis (TG/DTA) of the Ligand

Complexes

The thermal gravimetric (TG) analysis was used as a probe to evidence of the coupled water or solvent molecules to be in coordination sphere or in crystalline form [57]. The thermo gram of copper(II), nickel(II) and cobalt(II) complexes are more stable than the macrocyclic ligand and does not decompose upto 255, 253 and 250 ^{0}C respectively. It shows a major step of decomposition from 255-330 ^{0}C which is detected by DTA at 320 ^{0}C, this corresponds to the loss of two succinic acids and two ethylenediamine moieties (observed weight 70.5%, theoretical weight 67.88%). It is very interesting to note that the complexes gains some 5% weight from 335-410 ^{0}C and does not decompose further. This weight gain of macrocyclic complexes may be attributed to the migration of the metal (M_{layer}) to the new vacant sites produced by the partial reduction of M^{2+} to M^{1+} in case of Copper and M^{4+} to M^{2+} in case of cobalt and nickel, then subsequent oxidation of M^{1+}, M^{2+} in copper, cobalt and nickel complexes respectively [58, 59].

3.6. Cyclic voltammetric study (CV)

The cyclic voltammogram of the Cu(II) complex (fig 3a) displays a reduction peak at Epc = -1.6V with an associated oxidation peak at Epa = -0.7V at a scan rate of 50mV/s. The peak separation of this couple (Δ Ep) is 0.9V and increases with scan rate. The Δ Ep is 1.2 and 1.4 at scan rates 100mV/s and 200mV/s respectively. Thus, the analyses of cyclic voltametric responses at different scan rate give the indication for quasi-reversible one electron reduction. The most significant element of the Cu(II) complex is the Cu(II)/Cu(I) couple. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled [60-61].

Co(II) complex exhibits one electron quasi reversible transfer process with a reduction peak at Epc = -1.5V with a corresponding oxidation peak at Epa = -0.6V at a scan rate of 50mV/s (Fig 3b). The peak separation (Δ Ep) of this couple is 0.9V. With the increasing scan rates, Δ Ep value also increases giving further evidence for the quasi-reversible Co(II)/Co(I) couple. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled.

The redox property of the Ni(II) complex was studied in the potential range of +1.8 to -3.0 V. Cyclic voltammogram of Ni(II) complex is shown in Fig 3c. The Ni(II) complex exhibited two quasi-reversible peaks. Cyclic voltammogram displays two reduction peaks, first one at Epc = -0.8 V with an associated oxidation peak at Epa = 0.4 V and second reduction peak at Epc = -1.9 V with an associated oxidation peak at Epa = -0.9 V at a scan rate of 200mV/s. The value of ΔEp is 0.4 and 1 for first and second redox couples respectively and increases with scan rate gives confirmation for quasi-reversible nature associated with one electron reduction [63, 64].

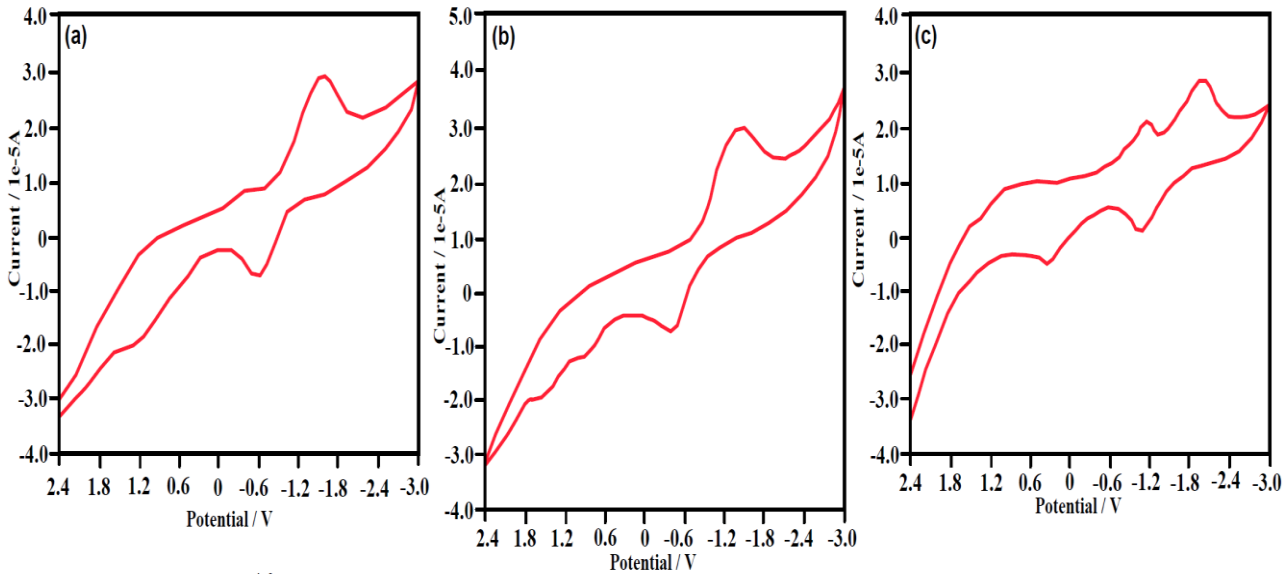


Figure 3. Cyclic voltammogram of Metal Complexes: (a) Cu(II), (b) Co(II) and (c) Ni(II) complexes.

3.7. Antimicrobial Screening

3.7.1. Minimum Inhibitory Concentration (MIC_{80})

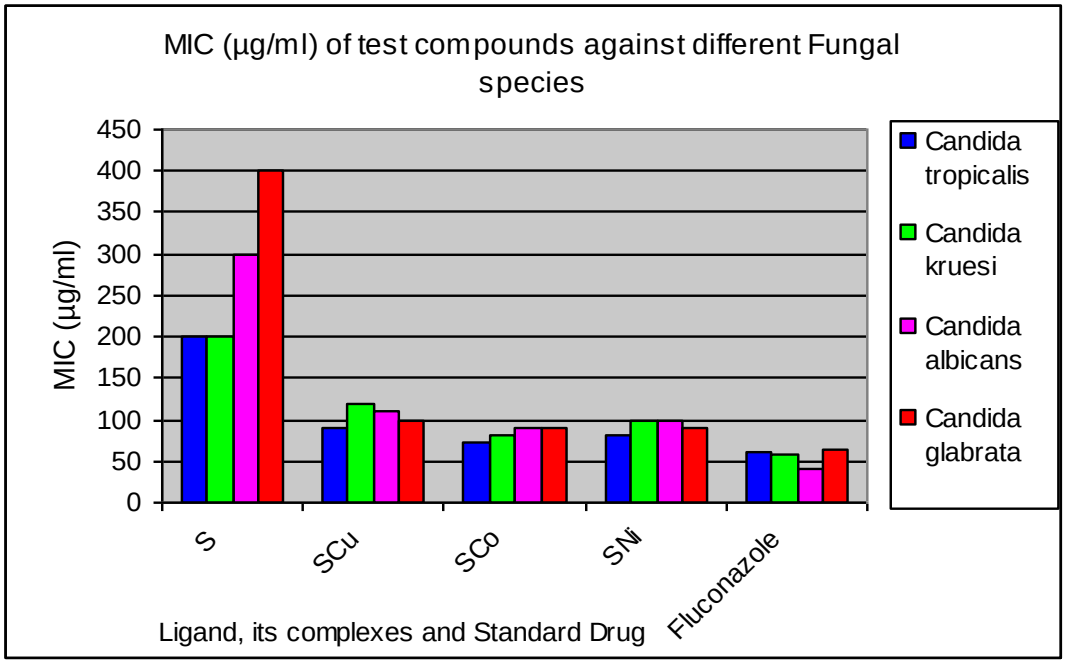


Figure 4. Minimum Inhibitory Concentrations MIC_{80} (µg/ml) of ligand and its metal complexes against different fungal species.

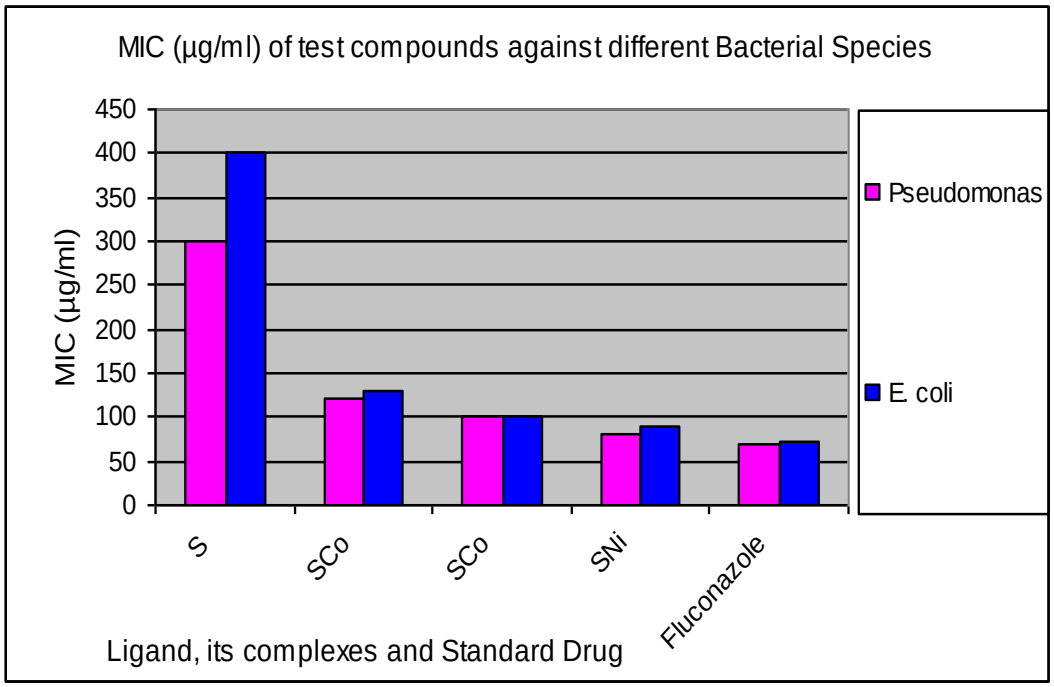


Figure 5. Minimum Inhibitory Concentrations MIC_{80} (µg/ml) of ligand and its metal complexes against different Bacterial species.

Minimum Inhibitory Concentration was defined as the lowest concentration of the ligand and its complexes that causes 80% decrease in absorbance (MIC_{80}) compared with that of the control (no test compound). The MIC_{80} of ligand, its metal complexes and standard drugs were checked against four fungal and two bacterial species using broth dilution method (BDM). In case of *Candida tropicalis*, Co(II) complex has MIC_{80} which is 64% less as compared to parent compound, where as Ni(II) and Cu(II) complexes have MIC_{80} which is 60% and 55% less as compared to the original compound. From the results in (Fig. 4, 5) it was observed that, in fungi generally the MIC_{80} of SCo<SNi<SCu<S, where as in case of bacteria the MIC_{80} of SNi<SCo<SCu<S. It was found that Co(II) complex and standard drugs showed significant and matching inhibitory properties.

3.7.2. Disc Diffusion Assay

Antifungal and antibacterial activities of the compounds on solid media, when compared with standard antifungal and antibacterial drugs (Fluconazole, Ampicillin) showed significant and potential antimicrobial properties. The zone of inhibition is greatly affected by the thickness of the test agar layer. As the thickness increases, the zone of inhibition decreases.

Table 3. Antibacterial activity screening data for the ligand and its metal complexes. Zone of inhibition [a] (mm) µg/ml.

Bacteria	S *	S **	SCo *	SCo **	SNi *	SNi **	SCu *	SCu **	A[b] *	A[b] **
1. *Pseudomonas*	01	03	06	16	09	17	05	12	10	19
2. *E.coli*	01	02	05	13	09	15	05	11	09	17
3. Control (10% DMSO) [c]	-	-	-	-	-	-	-	-	-	-

[a]12-20 mm significant active, 05-12mm moderate active, <05 less active.
[b]Ampicillin (negative control). [c]Solvent control. * 500 µg/ml, ** 1000 µg/ml. S = [($C_{12}H_{20}O_4N_4$)]

This can be attributed to the decrease of concentration of the ligand and its complexes per unit volume of the culture media. Another factor, which influences the inhibition zone, is inoculum size (concentration of the organism per unit volume). The diameter of the inhibition zone decreases with increase in the inoculum size. The chemical composition, temperature of the incubator, and the pH of the medium influence the rate of germination of microorganisms. There is a significant reduction in the growth rate of microorganisms due to unfavorable culture media, low temperature and acidic pH. The activity test was conducted at an optimum temperature of 37 ^{0}C and favorable pH 0.2, both antifungal and antibacterial activities was calculated as a mean of three triplicates. Synthesized compounds were investigated for their antimicrobial activity by agar diffusion method (Table-3). Antibacterial activity (*in vitro*) of the ligand and its complexes were checked against two different bacteria at the concentration range of 500µg/ml and 1000µg/ml (10 fold more than MIC). At higher concentration, the complexes show significant antimicrobial activity against the tested pathogens [65-68]. The degree of inhibition varied with the nature of the compound. Higher concentrations of the ligand and

complexes were used to get visible results. At higher concentration (1000µg/ml) the highest zone of inhibition *i.e.* 17, 16 and 15 mm were measured in *Pseudomonas* and *E.coli* when treated with Ni(II), Co(II) and again with Ni(II) complex respectively.

Antifungal activity was also checked at the same concentrations. At higher concentration (1000µg/ml) the highest inhibitory zone *i.e.* 18, 16 and 16 mm were measured in *Candida tropicalis*, *Candida kruesi* and *Candida tropicalis* when treated against Co(II), Co(II) and Ni(II) complexes (Table-4). The most significant thing seen in this study was that the complexes were more active against fungi than in case of bacteria. The result showed that, in case of solvent control disc no zone of inhibition was observed so as far as our study is concerned 10% DMSO, as a solvent is having no effect on the tested organisms. Hence, we can effectively conclude here that whole of the antimicrobial effect is due to the different concentration of the metal complexes and the ligand used in this study [69, 70]. The antimicrobial activities of the complexes when compared with standard antifungal and antibacterial drugs showed significant and matching biological properties.

Table 4: Antifungal activity screening data for the ligand and its metal complexes. Zone of inhibition [a] (mm) µg/ml

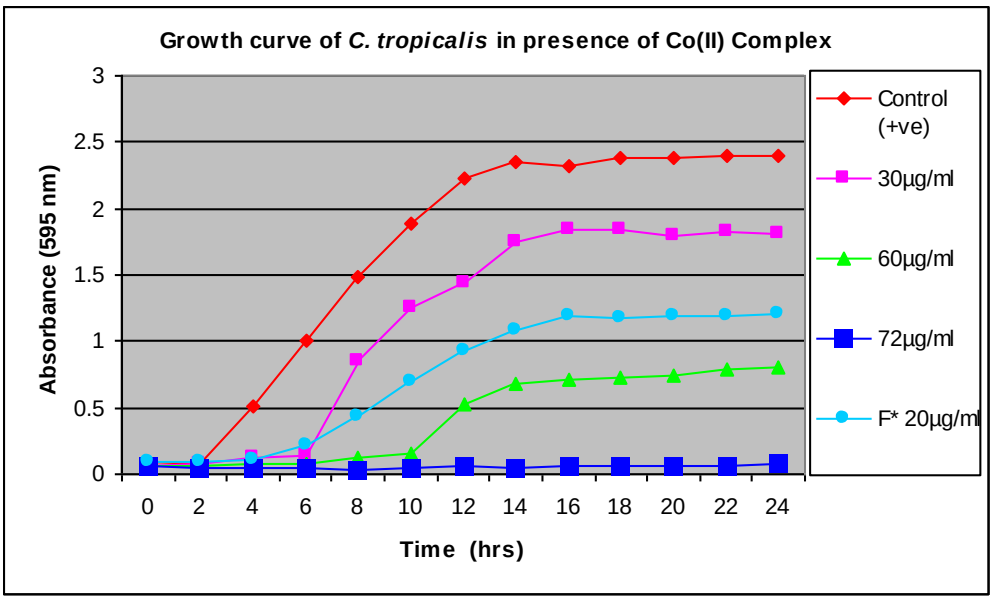
Fungi	S *	S **	SNi *	SNi **	SCo *	SCo **	SCu *	SCu **	F[b] *	F[b] **
1. *C. tropicalis*	02	04	09	16	10	18	08	14	12	20
2. *C. Kruesi*	01	03	07	14	08	16	06	12	11	19
3. *C. albicans*	01	03	05	12	07	13	05	10	10	17
4. *C. glabrata*	01	02	05	11	07	11	05	11	09	15
5. Control (10% DMSO) [c]	-	-	-	-	-	-	-	-	-	-

[a]12-25 mm significant active, 05-12mm moderate active, <05 less active.
[b]Fluconazole (negative control). [c]Solvent control. * 500 µg/ml, ** 1000 µg/ml. S = [($C_{12}H_{20}O_4N_4$)]

3.7.3. Growth curve studies

In growth curve studies the effect of increasing concentrations of the ligand and its complexes on the growth pattern of different fungal and bacterial species have been studied. Control cells showed a normal pattern of growth with lag phase of 4 hrs, active exponential phase of 8-10 hrs before attaining stationary phase. Increase in concentration of test compounds leads to considerable decrease in growth. Co(II) complex when treated against *Candida tropicalis* at concentration of 30µg/ml the growth pattern has changed, the lag phase is extended by 4h, the stationary phase has not reached the same level of cell growth as in case of control and at 60µg/ml the lag phase is further extended by 2h. At concentration of 72µg/ml (MIC_{80} level), there is total inhibition of growth showing a flat line (Fig. 6). (Fluconazole 20µg/ml), showed the lag phase further extended by 4h with respect to control. Significant and prominent effect is observed for all the synthesized complexes. Co(II), Ni(II), Cu(II)

complexes, in concentration dependent manner suppressed growth and delayed exponential phases. At MIC_{80} values complete inhibition of growth was observed.



* Fluconazole (-ve control)

Figure 6. Effect of Co(II) complex in concentration range of 30–72µg/ml was studied on *C. tropicalis* ATCC 750. Growth curve plotted against absorbance at 595 nm and time (hrs) shows complete inhibition of growth at 72µg/ml.

4. CONCLUSION

Ligand and its metal derivatives were successfully synthesized. The structures were confirmed by spectral and elemental analyses. Further their anticandidial and antibacterial activities were evaluated by determining minimum inhibitory concentration (MIC_{80}), disc diffusion assay and growth curve studies. The most important thing noticed in this study was that the complexes were much effective against fungus than in case of bacteria, and all of the fungal species used in this study are responsible for causing candidiasis, a disease that varies from superficial mucosal to life threatening systemic disorders. Present observations may serve as a guide for studying the control release of these complexes that could be a promising future in the field of infectious diseases. One more thing noticed in this study was that the metal complexes have higher activities than that of the parent ligand when treated against the same microorganism. Such increased activity of the metal complexes may be due to chelation of the metal ion in the complexes, which enhances the lipophylic character favoring its permeation through the lipid layer of cell membrane. Other factors are due to presence of favorable structural environment such as aryl binding site with a hydrophobic group, hydrogen bonding domain –NHCO- group, and nucleophiles present in these complexes, stability constant, molar conductivity, solubility and magnetic moment are also responsible for increasing the antimicrobial activity of the complexes. As far as our results are concerned, these metal complexes can thus be explored in future as an option for decreasing pathogenic potential of infecting bacterial and fungal species.

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