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SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL ACTIVITIES OF Mn(II), Co(II), Cu(II), and Zn(II) MIXED-LIGAND COMPLEXES CONTAINING 1,10 - PHENANTHROLINE AND 2,2`-BIPYRIDINE.

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ABSTRACT

Mixed-ligand metal (II) (M = Mn, Co, Cu, Zn) complexes containing 1,10-phenanthroline and 2,2`-bipyridine as ligands have been synthesized and characterized by melting point, elemental analyses, conductance measurements, visible and infrared spectroscopic studies. While the IR spectra indicate that both ligands are coordinated to metal ions, molar conductance values reveal that the complexes are 1:2 electrolytes. All the complexes assume octahedral geometry in which Mn(II) and Zn(II) have two molecules of 2,2`-bipyridine and one molecule of 1,10-phenanthroline and the Co(II) complex has one molecule of 2,2`-bipyridine and two molecules of 1,10-phenanthroline in the coordination sphere. On the other hand the Cu(II) complex has one molecule of each of ligand and two water molecules in the coordination sphere. *In vitro* antifungal screening indicates that the complexes show enhanced antifungal activities against four yeasts compared with the ligands. The complexes also show greater antifungal activities than the reference antibiotic, nystatin. These complexes could therefore be further exploited as possible substitutes to fight against nystatin resistance.

Keywords: 1,10-phenanthroline and 2,2`-bipyridine, Mixed- ligand complexes, antifungal activities, Manganese(II), Cobalt(II), Copper(II) and Zinc(II).

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INTRODUCTION

Metal complexes have attracted considerable attention in modern medicine due to their antibacterial and antifungal properties [1-8]. The abusive use of antimicrobials has resulted in high levels of resistance which constitute a major problem coupled with the emergence of strains resistant to almost all drugs [9, 10]. Methicillin resistant Staphylococcus aureus (MRSA) and now glycopeptides resistant enterococci (GRE) are typical examples [11]. Despite the ready availability of many treatments, the increasing prevalence of multidrug resistant strains of bacteria and fungi has led to an increase in the number of untreatable bacterial and fungal infections [9-11]. The resistance associated with the existing therapies for fungal infections, such as polyene and azole drugs necessitate the search for alternative antifungal agents [12, 13]. Candida species are pervasive pathogens capable of causing systemic infections in critically ill and severely immuno-compromised patients [14, 15]. Causative agents of most fungal infections [16-21], therefore need novel, safe and effective antifungal drugs.

Metal-based drugs may represent a novel group of anti-mycotic agents which could be used as pharmaceuticals due to the possibility of a difference in mode of action [22,23]. It has been shown that some metal complexes of N-N -donor ligand are potent in vitro inhibitors of the growth of candida albicans [22, 23].

In our previous work, we reported on the synthesis, characterization and antibacterial properties of mixed- ligand complexes of 1,10- phenanthroline and 2,2`-bipyridine [24, 25]. To our knowledge little or nothing is reported on antifungal activities of mixed-ligand metal complexes containing the above ligands. Antifungal resistance is a major problem in our country, Cameroon and so this has spurred us to carry out fundamental research work on the biological activity of mixed-ligand metal complexes on some of these resistant fungal strains.

The different fungi species used in this study are: Candida albicans, candida krusei, Cryptococcus neoformans and candida parapsilosis, which are the causative agents of the incidence of most fungal infections in our environment, such as Meningitis [20,21], Vulvovaginitis [18], Candida pneumonia [16,17] and gastrointestinal infections [19].

In this paper, we therefore report on the synthesis, characterization and antifungal activities of mixed-ligand Mn(II), Co(II), Cu (II) and Zn(II) complexes containing 1,10-phenanthroline and 2,2`-bipyridine as ligands.

EXPERIMENTAL

Commercial reagents were used as obtained without further purification. The solvents, ethanol and diethylether were dried and distilled according to standard methods. The fungi species were clinical isolates from the Yaounde central hospital (Cameroon).

Physical measurements

Elemental analysis for carbon, nitrogen and hydrogen were carried out on a Fisons instrument 1108 CHNS-O (France), while Mn(II), Co(II), Cu(II) and Zn(II) were quantitatively estimated by complexiometric titrations [26]. The melting point/decomposition temperatures of the complexes were determined using the LEICA VmHB melting point apparatus (Koffler`s system) in the temperature range 50-260 °C. Conductance measurements were carried out in water using the Tacussel conductimeter, model CD810 at room temperature. Infrared spectra were recorded on a Perkin- Elmer model IR-457 spectrometer and a spectrum 100 FT-IR Perkin-Elmer spectrometer while electronic absorption spectra of the complexes dissolved in ethanol were recorded on a Hitachi U-2000 Spectrophotometer at room temperature.

Synthesis

The complexes were synthesized using a similar procedure as reported by Agwara et al. [24,25] Generally, 1,10- phenanthroline (1 mmol) and 2,2`-bipyridine (2 mmol) were dissolved separately in 15 mL of ethanol. The metal salt (1 mmol) was dissolved in 10 mL of water. The 2,2`-bipyridine solution was added dropwise to the metal salt solution while stirring magnetically at room temperature. The 1,10- phenanthroline solution was then added dropwise to the reaction mixture while stirring at room temperature. The resulting solution was further stirred for one hour and the solution allowed to stand at room temperature for some days to allow for crystallization to occur.

For example, $[Mn(bipy)_2(phen)]Cl_2.2H_2O$ was prepared by adding dropwise a solution of 2,2`-bipyridine (312 g, 2.0 mmol) in 15 mL of ethanol to a solution of $MnCl_2.4H_2O$ (0.127 g, 1 mmol) in 10 mL of water while continuously stirring at ambient temperature. A yellow solution was obtained to which was added dropwise 1,10-phenanthroline (0:180 g; 1 mmol) dissolved in 15 mL of ethanol while still stirring. This mixture was further stirred for one hour and the resulting yellow solution was preserved for five days during which yellow crystals were obtained. These crystals were filtered, washed with diethylether and dried in a desiccator under vacuum. The synthesis of the other complexes were similarly carried out.

Antifungal tests

The antifungal tests were carried out in the Applied Microbiology and Molecular Pharmacology Laboratory of the University of Yaoundé I, Cameroon. Four candida fungi species namely: Candida albicans, Candida parapsilosis, Candida krusei and Cryptococcus neoformans were used in the screening.

Disc diffusion test

This test was performed to screen and select the best active products according to their inhibition zone diameter prior to running on MIC determination. The antimicrobial agent

incorporated in a disc diffuses at the surface of the inoculated medium and creates an inhibition zone diameter that is proportional to the efficacy of the compound being tested.

Culture media (Mueller Hinton and Sabouraud Dextrose Agar) were prepared according to the manufacturer's guideline. Briefly, a mass of the culture medium was weighed and dissolved in distilled water. After boiling, the culture medium was autoclaved at 121°C for 15 minutes, then it was spread at the surface of Petri dishes and left to solidify.
The compounds were weighed (40mg) and dissolved in 1mL of tween 80 (10%) making a concentration of 40mg/mL. Positive control, (4mg/mL) nystatin, was also prepared.

Yeasts, Candida albicans, Candida parapsilosis, Candida krusei and Cryptococcus néoformans were clinical isolates from pathological samples. A colony from a 24 hours fresh culture was dissolved in 1 mL of sterile distilled water, adjusted at 0.5 Mc Farland scale (1-5.10^8cfu/mL) and diluted at 1/1000.

After labeling, prepared inocula were spread at the surface of the solidified culture medium using a sterile cotton swab and kept for pre-diffusion at room temperature for 15 minutes.

Filter paper discs (6 mm) were deposited at the surface of the inoculated Petri dishes, and 5µL of products were added on discs, using a micropipette.

Petri dishes were then incubated at 37°C in 48 hours and the inhibition zone diameters measured.

Each test was performed three times and the mean calculated and expressed in the form of diameters ± SD (Standard deviation). A compound were considered active when the IZ was greater than 6 mm.that presented an inhibition zone diameter superior to 12mm were selected for MIC determination.

Minimum inhibition concentration

The Microbroth dilution method [27] was used to determine the minimum inhibitory concentration (MIC) of the compounds and reference antibiotic (RA) on a given microorganism. MIC is defined as the lowest concentration of an antimicrobial agent that inhibits any observable growth of the microorganism.

The principle is based on a series of 2 fold dilution of a compound carried out in wells of a micro titer plate in the presence of a broth culture medium. After adding the inoculums and incubating, the lowest concentration of the compound that completely inhibits the growth of the microorganism is identified as the MIC.

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The preparation of the culture medium (Mueller Hinton broth), inoculates, and compounds is the same as previously described [27]. Only compounds whose inhibition zone diameter was greater than 12 mm were used for the MIC determination.

The sterile broth medium (80µL) was introduced in each well of the micro titer plate, except wells of the first column where 110µL were introduced. 50µL of a product were introduced in its corresponding well on the first column. A 2 fold series of dilution is done from the second to the 10^{th} line. The 11^{th} line is left as sterility control and the 12^{th} line as positive growth control. The concentrations range from 10mg/mL to $9.7.10^{-3}$mg/mL. After the dilution, 20µL of the inoculums were introduced in each corresponding well and incubation assessed at 37°C in 48 hours.

RESULTS AND DISCUSSION

The physical and analytical data of the complexes are presented in Table 1. All the complexes have melting points greater than 260° C, which are different from the melting points of 1,10-phenanthroline and 2,2`-bipyridine which of 117.3 °C and 71.3 °C respectively [28]. The high melting points of the complexes are an indication that they are probably ionic solids. The elemental analytical results for carbon, hydrogen and nitrogen as well as the estimated metal contents are very close to the calculated values (Table 1).

Table 1: Physical and analytical data of the complexes

Complex	Color	%Yield	Melting Point (°C)	Elemental Analysis %Found (%calculated)			
				%C	%N.	%H	%M
$[Mn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.2H_2O$	Yellow	60	>260	58.91 (58.72)	12.72 (12.85)	3.98 (4.31)	8.41 (8.39)
$[Co(C_{12}H_8N_2)_2(C_{10}H_8N_2)](NO_3)_2.2H_2O$	Reddish-Brown	20	>260	55.40 (55.51)	14.90 (15.23)	3.71 (3.84)	8.35 (8.01)
$[Cu(C_{12}H_8N_2)(C_{10}H_8N_2)(H_2O)_2]Cl_2.2H_2O$	Greenish-Blue	28	>260	48.95 (48.66)	9.93 (10.32)	4.26 (4.46)	12.00 (11.70)
$[Zn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.6H_2O$	Cream-white	47	>260	52.83 (52.14)	11.85 (11.40)	4.55 (4.90)	8.65 (8.87)

The molar conductivity values for the complexes in water (Table 3) suggest a 1: 2 electrolyte type (three ions) [29]] indicating that the counter ions Cl^- and NO_3^- are in the outer coordination sphere.

The relevant IR band frequencies of the ligands and metal complexes are presented in Table 2. Strong and sharp peaks characteristic of phen and bipy rings are observed in the spectra of the complexes, with significant shifts, an indication that both ligands have coordinated [30]. The spectra of the complexes show bands which are due to ring vibrations of the uncoordinated 2,2`-bipyridine at 1621$cm^{-1}$ and shifted to 1587-1591 $cm^{-1}$ in the spectra of the complexes. This shift of 30-33 $cm^{-1}$ to lower frequency is as a result of coordination of 2,2`-

bipyridine to the respective metal centers [31]. Similarly, the C-H and C=C stretching bands of 1,10-phenanthroline undergo a coordination-induced lower frequency shift of 7-12 and 18-26 cm^{-1} respectively [32]. The band observed at1750 cm^{-1} in the spectrum of the Co(II) complex (**2)** indicates that the nitrate ion is not coordinated [33]. This is in agreement with the molar conductance value, which indicates a 1:2 electrolyte. Large bands around 3300-3500 cm^{-1} in all the complexes indicates the presence of crystalline water molecules [34]. In the IR spectrum of the copper complex, the bands observed at 1660 cm^{-1} and 641 cm^{-1} are assigned to $\upsilon(H_2O)$ and υ(Cu-O) respectively [35,36]. The band observed at 446-479 cm^{-1} has been assigned to υ(M-N) [37].

The visible absorption spectrum of the cobalt complex revealed a band at 19763 cm^{-1} region which is typical of a d-d transition [36] and has been assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}$. A similar band at 19700cm^{-1} for Co (II) complexes has been reported for which octahedral geometry was proposed [38]. The copper (II) complex revealed a band at 13966 cm^{-1} which has been assigned to the $^2E_g \rightarrow {}^2T_g$ transition [37]. The above visible bands for which octahedral geometry was proposed are similar to those reported in literature [24,25].

Table 2: Selected IR Absorption bands of 1,10-Phen, 2,2`-bipy and the complexes (cm^{-1})

Compound	υ (C=N), υ(C=C)	γ (C-H)	υ(O-H)	$\upsilon(NO_3^-)$	υ(M-N)	υ(M-O)
1,10-phenanthroline	1438vs	854vs/738vs	-	-	-	
2,2`-Bipyridine	1621vs	757vs	-	-	-	
$[Mn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.2H_2O$	1590s 1438vs	846vs/727vs, 772vs	3433,br	-	467 w	
$[Co(C_{12}H_8N_2)_2(C_{10}H_8N_2)](NO_3)_2.2H_2O$	1588s 1446vs	848vs/726vs 768s	3385br	1750m	446w	
$[Cu(C_{12}H_8N_2)(C_{10}H_8N_2)(H_2O)_2]Cl_2.2H_2O$	1587vs 1438vs	850vs/724vs 772vs	3398s,br	-	479w	641m
$[Zn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.6H_2O$	1591m 1436vs	850vs/726vs, 772vs	3420s, br	-	469w	

br = broad, vs = very strong, s = strong, m = medium, w = weak

Table 3: Molar Conductivity and Electronic Spectral data for the Complexes

Complex	Molar Conductivity (Ω^{-1} cm^2 mol^{-1})	Electrolytic Type	Band Maxima (cm^{-1})	Assignment
$[Mn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.2H_2O$	151	1 : 2		
$[Co(C_{12}H_8N_2)_2(C_{10}H_8N_2)](NO_3)_2.2H_2O$	190	1 : 2	19763	$^4T_{1g}(F) \rightarrow {}^4A_{2g}$
$[Cu(C_{12}H_8N_2)(C_{10}H_8N_2)H_2O]Cl_2.2H_2O$	172	1 : 2	13966	$^2E_g \rightarrow {}^2T_g$
$[Zn(C_{12}H_8N_2)(C_{10}H_8N_2)_2]Cl_2.6H_2O$	160	1 : 2		

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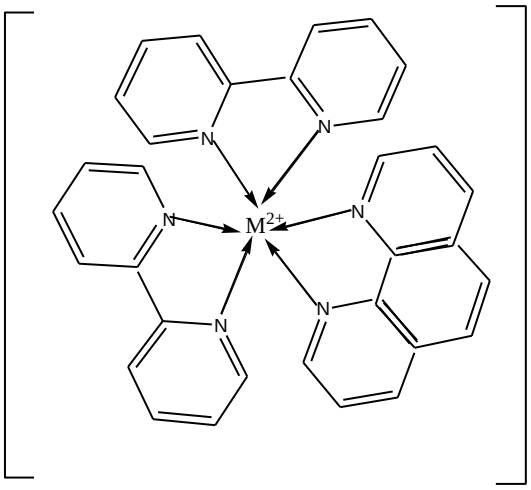
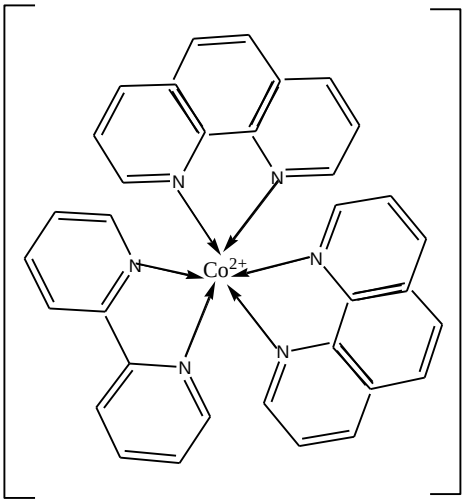


M = Mn, Zn ; X = Cl^-, NO_3^-



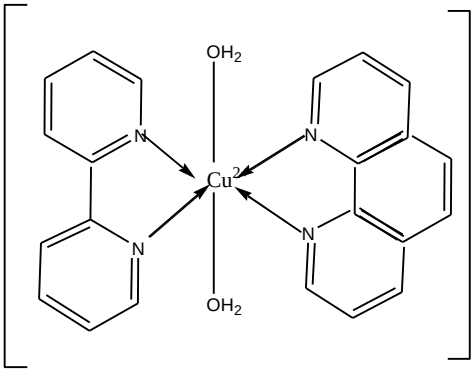


Figure 1: Proposed structures of the Complexes

Antifungal tests

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The ligands, metal salts and metal-mixed ligand complexes were tested for antifungal activity against four yeasts obtained from the Central hospital, Yaoundé, Cameroun. The results of susceptibility of these yeasts towards the compounds, judged by the inhibition zone growth diameter (IZ), are presented in Table 4.

Table 4: Antifungal activity (IZ diameter in mm

Fungi	C. Krusei	C. Albicans	C. Neoformans	C. Parapsilosis
1	37 ± 0.2	31 ± 1.1	35 ± 1	32 ± 0.6
2	35 ± 0	28 ± 0.5	25 ± 0	25 ± 0.9
3	30 ± 1.1	30 ± 1.6	25 ± 1	25 ± 0.9
4	14 ± 0.3	7 ± 0	13 ± 0	15 ± 1.2
5	13 ± 0.1	13 ± 0.6	13 ± 0.2	14 ± 0.7
6	27 ± 0.4	25 ± 0.6	21 ± 0.1	22 ± 0.7
7	14 ± 1.2	13 ± 0.6	0 ± 0	17 ± 1
8	0 ± 0	5 ± 0.1	0 ± 0	0 ± 0
9	8 ± 1.3	0 ± 0	7 ± 1.1	0 ± 0
10	12 ± 1.4	11 ± 1.3	16 ± 0.7	14 ± 0
RA	13 ± 0.8	20 ± 1.1	20 ± 0.7	14 ± 0.7

IZ = inhibition zone; 1 = 1,10-phenanthroline (phen) ; 2 = 2,2`-bipyridine (bipy) ; 3 = $[Mn(bipy)_2(phen)]Cl_2.2H_2O$; 4 = $[Co(bipy)(Phen)_2](NO_3)_2.2H_2O$; 5 = $[Cu(bipy)(phen)(H_2O)_2]Cl_2.2H_2O$; 6 = $[Zn(bipy)_2(phen)]Cl_2.6H_2O$; 7 = $Co(NO_3)_2.6H_2O$, 8 = $ZnCl_2$, 9 = $MnCl_2.4H2O$, 10= $CuCl_22H_2O$, RA = Reference antibiotic(Nystatin).

Table 5: Minimum inhibitory Concentrations

Fungi	C. Krusei	C. Albicans	C. Neoformans	C. Parapsilosis
1	$1.9x10^{-2}$	$4.88x10^{-3}$	$3.9x10^{-2}$	$3.9x10^{-2}$
2	$7.8x10^{-2}$	$7.8x10^{-2}$	$3.9x10^{-2}$	$7.8x10^{-2}$
3	$9.76x10^{-3}$	$4.88x10^{-3}$	$4.88x10^{-3}$	$9.76x10^{-3}$
4	$9.76x10^{-3}$	$4.88x10^{-3}$	$7.8x10^{-2}$	$1.9x10^{-2}$
5	$3.9x10^{-2}$	$4.88x10^{-3}$	$4.88x10^{-3}$	$3.9x10^{-2}$
6	$9.76x10^{-3}$	$4.88x10^{-3}$	$4.88x10^{-3}$	$3.9x10^{-2}$
RA	$3.9x10^{-2}$	$1.9x10^{-2}$	$3.9x10^{-2}$	$3.9x10^{-2}$

IZ = inhibition zone; 1 = 1,10-phenanthroline (phen) ; 2 = 2,2`-bipyridine (bipy) ; 3 = $[Mn(bipy)_2(phen)]Cl_2.2H_2O$; 4 = $[Co(bipy)(Phen)_2](NO_3)_2.2H_2O$; 5 = $[Cu(bipy)(phen)(H_2O)_2]Cl_2.2H_2O$; 6 = $[Zn(bipy)_2(phen)]Cl_2.6H_2O$; RA = Reference antibiotic(Nystatin).

The antifungal activities of the compounds showed 1,10-phenanthroline exhibiting the greatest activity (4/4), with inhibition zone ranging from 31 to 37 mm. 2,2`-bipyridine showed activity (4/4) with inhibition zones within the range of 25-35 mm. The metal complexes showed high activities (4/4) with IZ values within the range 13-30 mm, while the metal salts showed

very low activities (Table 4). The cobalt complex (**4**) however exhibited low activity on C. albicans. The activity of each metal salt is relatively very low compared to that of the corresponding complex. This shows that the bioactivity of the metal ions increases upon coordination [22]. The increase in activity on coordination could be explained by Overtone`s concept and chelation theory [7,39, 40]. According to Overtone`s concept of cell permeability, the lipid membranes that surround the cell favour the passage of only lipid-soluble material and lipid-solubility is an important factor that controls antimicrobial activity. On coordination, the polarity of the metal ion is reduced due to overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with the donor atoms of the ligand and possible π-electron delocalization over the whole chelate ring. Such chelation may increase the lipophilic character of the metal complex, enabling it to permeate the lipid membrane of the fungus and more effective [40]. The antifungal activity of the most active compounds were further studied by the determining the minimum inhibitory concentration (MIC) of the compounds on a given candida specie as shown in Table 5. The MIC of the metal complexes are quite low compared with those of the ligands and the reference antibiotic, nystatin. This implies that the antifungal activity of the ligands increase upon coordination which is in agreement with literature [1,24,25]. The mixed- ligand complexes have an advantage in that the respective bioactivities of the uncoordinated ligands and metal ions are combined thus could make them more potent antifungal agents. The cobalt(II) complex (**4)** however shows activity comparable to that of free 2,2-bipyridine and slightly lower activity compared to free 1,10- phenanthroline on candida neoformans , while the activity of the copper(II) complex (**5)** is equal to that of free 2,2`-bipyridine, and slightly lower than that of free 1,10- phenanthroline on candida krusei and candida parapsilosis.

The activities of the metal complexes are far greater than that of the reference antibiotic, nystatin, with complexes having 10 times greater activity than nystatin (Table 5 and Fig. 2). These complexes could further be studied as substitutes to fight nystatin resistance which could be a potential relief for this worldwide problem. These results show that 1,10-phenanthroline is biologically more active than 2,2`bipyrine though they are both N-N donor ligands. The metal copper(II) complex (**5)** however**,** has activity equal to that of nystatin on C. krusei and C. Parapsilosis. The manganeses (II) complex, $[Mn(bipy)_2(phen)]Cl_2.2H_2O$ (**3**) with a MIC value of (0.00488-0.00976)mg/mL, shows the best activity. This complex could further be studied for the treatment of infections caused by these candida species.

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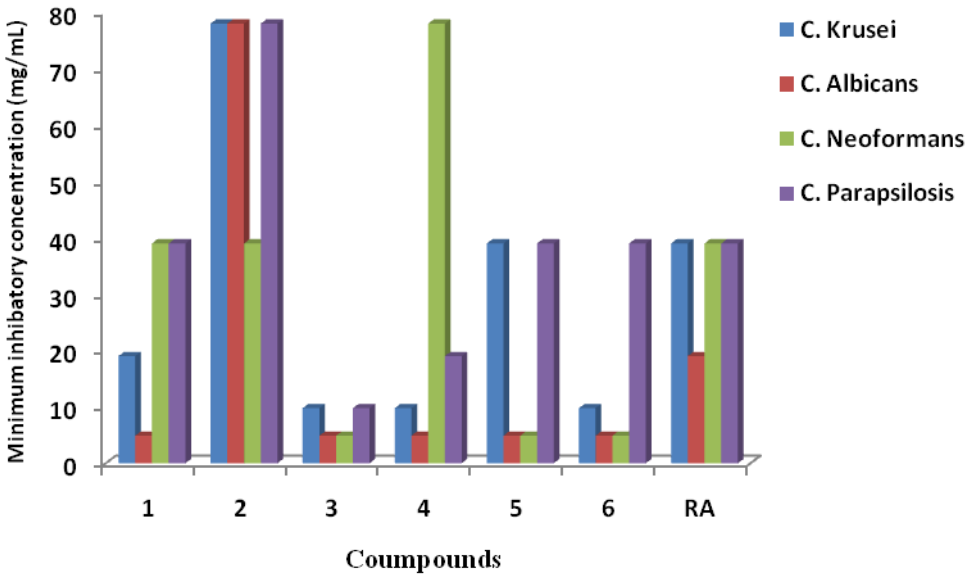

Figure 2: Histogram showing Comparative MIC for the compounds

CONCLUSION

Mixed- ligand metal(II) (M=Mn, Co, Cu, Zn) complexes containing 2,2`bipyridine, and 1,10-phenanthroline as ligands have been synthesized and characterized using elemental analyses, conductance measurements and spectroscopic studies.

All the complexes assume octahedral geometry in which Mn(II) and Zn(II) have two molecules of 2,2`-bipyridine and one molecule of 1,10-phenanthroline and the Co(II) complex has one molecule of 2,2`-bipyridine and two molecules of 1,10-phenanthroline in the coordination sphere. On the other hand the Cu(II) complex has one molecule each of ligands and two water molecules in the coordination sphere. The elemental analytical results for carbon, hydrogen and nitrogen as well as the estimated metal contents are very close to the calculated values. Conductance values indicate that the complexes are 1:2 electrolyte type. Infra red shifts of relevant bands of the ligands in the complexes indicate that both are coordinated to the metal ions. Visible spectra confirm octahedral geometry for the complexes.

In vitro antifungal studies of these complexes against the fungal species: Candida albicans, Candida parapsilosis, Candida krusei and Cryptococcus néoformans were carried out . The minimum inhibitory concentration values (MIC) show that there is increased antifungal activity of the ligands when coordinated to the metal ions. The results also clearly show that the activities of the complexes are greater than that of nystatin, the reference antibiotic.

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