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Der Chemica Sinica, 2010, 1 (3): 124-137

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Synthesis, spectroscopic and cytotoxic studies of biologically active new Co (II), Ni (II), Cu (II) and Mn (II) complexes of Schiff base hydrazones

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ABSTRACT

The synthesis, structure, spectral and biological studies of Cu (II), Ni (II), Co (II) and Mn (II) complexes of unsymmetrical Schiff base hydrazones ligand are described. Ligands are synthesized starting from cinnamaldehyde hydrazone with substituted salicylaldehyde. It is evident from the IR data that in all the complexes, only one part of the ligands is coordinated to the metal ion resulting mononuclear complexes. The ligand coordinates through the nitrogen atoms (N-N) of the hydrazone moiety and oxygen atom after deprotonation of the substituted salicylaldehyde fragment. The formulations,- $[Cu(L)_2]$, $[Ni(L)_2]$, $[Co(L)_2]$ and $[Mn(L)_2]$ are in accordance with elemental analyses, physical and spectroscopic measurements. The complexes are soluble in organic solvents. Molar conductance values in DMF indicate the nonelectrolytic nature of the complexes. For the observed magnetic moment and electronic spectral data possible has been discussed. All the complexes show a single line EPR signals. From all the available data, the probable structures for the complexes have been proposed. The compounds synthesized in present study have shown promising cytotoxic activity when screened using the in vitro method and at the same time were shown to have good activity when tested using the Ehrlich Ascites carcinoma (EAC) model. The antimicrobial screening showed that the Manganese complexes possess enhanced antimicrobial activity towards fungi.

INTRODUCTION

Interest in the study of Schiff base hydrazones has been growing because of their antimicrobial, antituberculosis and antitumour activity. Schiff base play an important role in inorganic chemistry, as they easily form stable complexes with most transition metal ions. The development of the field of bioinorganic chemistry has increased interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [1-4].

Metal ions play a vital role in a vast number of biological processes. The antimicrobial properties of metal have been recognized for centuries and have represented some of the most fundamental breakthroughs in medicinal history [5]. Many studies stressed the role of metal ions in important

biological processes, whereas the inorganic pharmacology started to be an important field with more than 25 inorganic compounds, being used in therapy as antibacterial, antiviral and anticancer drugs [6, 7]. Schiff base complexes derived from aryl hydrazones have been reported to act as enzyme inhibitors and are useful due to their pharmacological applications [8-12].

Devappa Lamani and his co-workers have synthesized 2-chloroquinoline-3-carbaldehyde [(2-hydroxy-1-naphthyl) methylene] hydrazone (CQCMH) (2a-c) and 2-chloroquinoline-3-carbaldehyde [4-(dimethylamino) benzylidene] hydrazone (CQCDBH) (3a-c) from quinoline derivatives under suitable experimental conditions. . The synthesized compounds were characterized by elemental analysis, FT IR, ^{1}HNMR, and mass spectral data. The synthesized compounds have been screened for antibacterial and antifungal activities [13].

Erika M Becker and his co-workers have synthesized two series of novel ligands based on the very active 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) group. The synthesized compounds have been screened for antitumour activity [14].

A new hydrazone ligand, HL, was prepared by the reaction of 7-chloro-4-hydrazinoquinoline with o-hydroxy benzaldehyde by Mustafa M El-Behry and his co-workers. The ligand behaves as monoprotic bidentate. The ligand reacted with Cu (II), Ni (II), Co (II), Fe (III), and UO_2 (II) ions to yield mononuclear complexes. The HL and metal complexes were tested against one stain gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*), and fungi (*Candida albicans*). The tested compounds exhibited higher antibacterial activities [15].

Kirschner et al [16] have suggested that the transfer of the metal ion from the ligand to the cancer associated viruses was an important mechanism for designing new anticancer therapies. We have already drawn attention to the strong relationship between metal or its complexes and antibacterial, anticancer, antitumour and antifungal activities [17]. A number of *in vivo* studies have indicated [18] that biologically active compounds become more bacteriostatic and carcinistatic upon chelation.

The structures of the compounds were identified using spectroscopic techniques. Biological activity of the titled compounds was studied against gram (+) ve bacteria such as- *S.aureus* (ATCC-25923) and gram (-) ve bacteria such as-*E.coli* (ATCC-10536), *Pseudomonas* (ATCC-25619*)* as well as fungi such as- *Candida albicans*(ATCC-90028) and *Candida krusei*(ATCC-6250).

The aim of the present work is to synthesize new Schiff bases and their metal complexes and to study their coordination behaviour, spectral and biological activities against various micro-organisms.

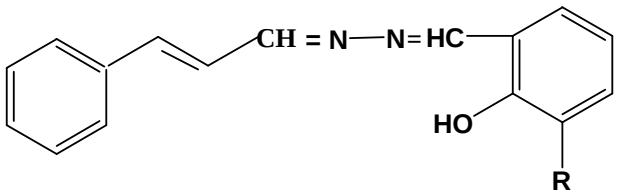


FIGURE-1 Representaive structure of ligand

MATERIALS AND METHODS

2.1 Material, analytical methods and physical measurements

All chemicals used were of reagent grade. Solvents were distilled prior to use. The metal content of the complexes were estimated gravimetrically, copper metal was estimated by ammonium thiocynate gravimetrically. Nickel, cobalt and manganese were estimated by gravimetrically using dimethylglyoxime, ammonium thiocynate and iron with triethanolamine respectively [19]. Magnetic susceptibility of complexes was measured at room temperature on a faraday balance using Hg [Co (SCN) $_4$] as a calibrant. Electronic spectra were recorded using digital spectrophotometer in DMSO. The IR spectra of ligands and their complexes were recorded as KBr pallets in the region 4000-400cm^{-1} on FT IR spectrophotometer Shimadzu 8201. ^{1}HNMR spectra of ligands and their Cu (II), Ni (II), Co (II) and Mn (II) complexes were recorded in DMSO-d_6 at room temperature using TMS as internal standard on a Bruker Advance 400 MHz FT NMR. Elemental analyses were carried out on a Vario EL III Elementar Carlo- Erba 1108. Conductivity measurements were made on 10^{-3} M solutions of the complexes in DMSO using Equiptronics model no Eq-660A conductivity meter, provides with a dip type cell having cell constant 1.0. EPR spectra of metal complexes of Schiff base hydrazones were recorded at room temperature on E-112 X-band spectrometer using TCNE as g-marker. Melting points of the ligands and their metal complexes were determined by open capillary method using Sunsim electric melting point apparatus and uncorrected. Molecular weight of ligands and their metal complexes were determined by Rast camphor method. The purity of ligands and their metal complexes was checked by thin layer chromatography using n-hexane-ethyl acetate mixture (3:1).

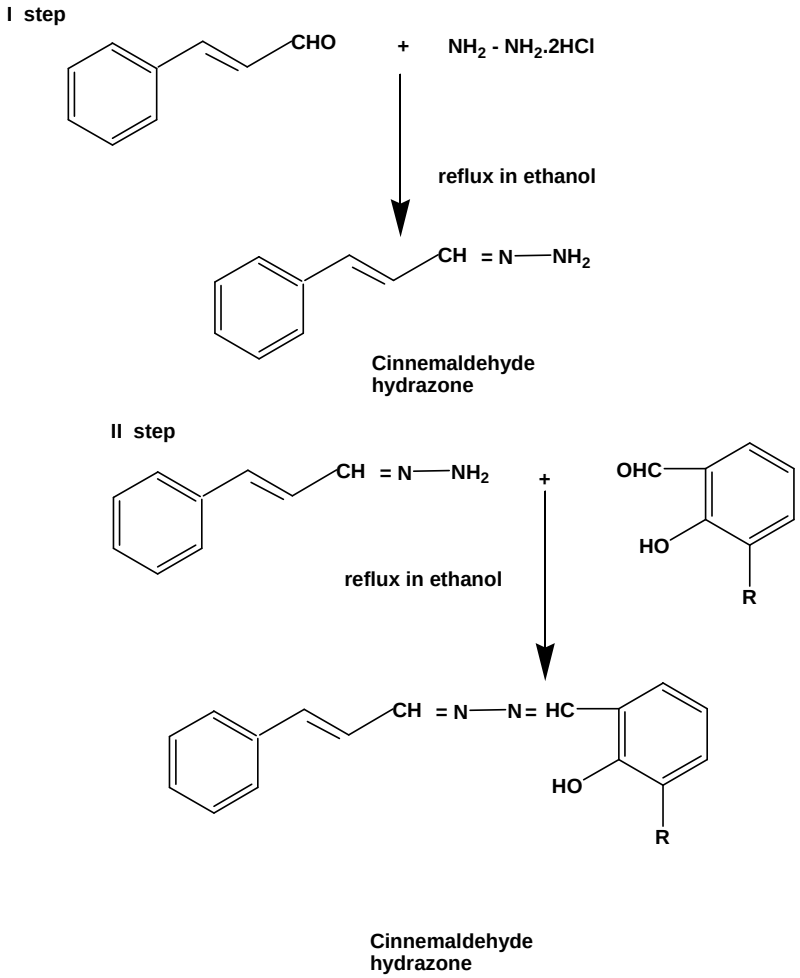
2.2 Synthesis of the ligands

The ligand was synthesized in two steps. The first step is the synthesis of cinnamaldehyde monohydrazone according to the reporting method [20], followed by the cinnamaldehyde hydrazones (II) in the second step from monohydrazone.-

Cinnamaldehyde monohydrazone (2gm, 1m mol) (80% yield, mp 120^{0}c, yellow crystal) was dissolved in absolute ethanol (10ml). To this solution salicylaldehyde, chloro salicylaldeyhde and nitro salicylaldehyde (1m mol 1.22ml, 1.32ml, 1.42ml) were added for preparing HL_1[cinnamaldehyde-(2-hydroxybenzylidene)hydrazine CHBH], HL_2[cinnamaldehyde-(2-hydroxy,3-chlorobenzylidene)hydrazine CHCBH] and HL_3 cinnamaldehyde-(2-hydroxy,3-nitrobenzylidene)hydrazine CHNBH] respectively. The reaction mixture was refluxed for 4hrs. After cooling, the formed yellow precipitate was collected, filtered and finally washed with absolute ethanol (10ml) and purified by recrystalization from ethanol (77%yield mp130^{0}c) (Scheme-1).

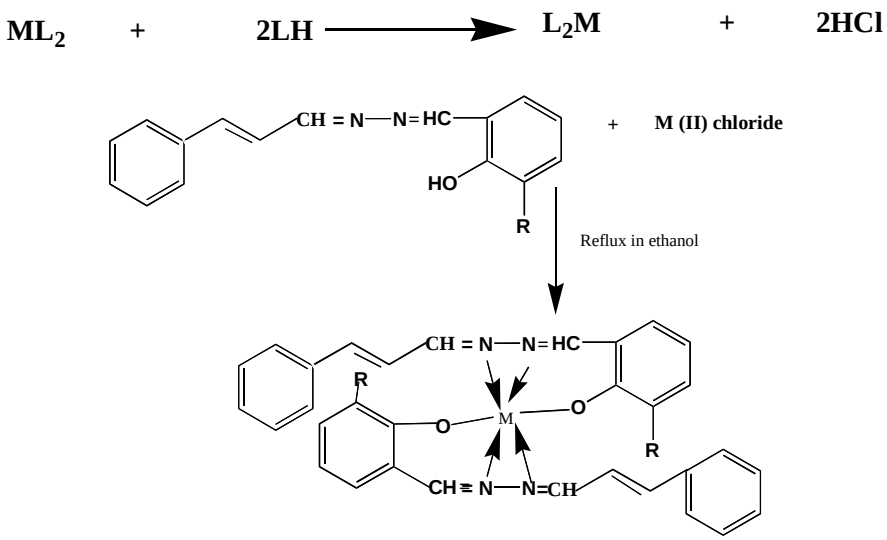
2.3 Synthesis of metal complexes

Warm ethanol solution (20ml) of the respective Schiff base (0.002M) were added to a magnetically stirred solution of the metal (II) salts (0.001M) in ethanol (25ml). The mixture was refluxed for 1hr and cooled at room temperature. On cooling, precipitates of metal complexes were formed, which were filtered from Buckner funnel, washed with ethanol and dried. Recrystallization in aqueous ethanol (30:70) gave the pure metal complexes. The analytical data of ligands and their metal complexes are given in table-I. (Scheme-2)



R= HL$_1$= H
R = HL$_2$ = Cl
R = HL$_3$ = NO$_2$

SCHEME-1 Synthesis of ligands



R = L$_1$ = H
R = L$_2$ = Cl
R = L$_3$ = NO$_2$

SCHEME-2 Representative synthesis of metal complexes

Table-I: Micro analytical data of the ligands and their metal complexes

compounds	Molecular weight Found (calc.)	Found (calc) %				M.P ^{0}C	Molar cond. Λm $Cm^2mol^{-1}ohm^{-1}$	Magnetic moment	Color	Yield	Molecular .formulae
		Carbon	Hydrogen	Nitrogen	Oxygen						
HL_1	248 (250)	76.0 (76.8)	5.58 (5.6)	11.1 (11.2)	6.38 (6.4)	130^{0}C	12	-	Yellow	77%	$C_{16}H_{14}N_2O$
HL_2	280 (284)	67.51 (67.6)	4.45 (4.57)	9.82 (9.85)	5.61 (5.63)	140^{0}C	15	-	Dark yellow	80%	$C_{16}H_{13}N_2OCl$
HL_3	292 (295)	65.0 (65.08)	4.35 (4.40)	14.21 (14.23)	16.22 (16.27)	135^{0}C	11	-	Yellow	75%	$C_{16}H_{13}N_4O_3$
L_1-Cu^{+2}	554 (556)	68.9 (69.06)	4.65 (4.67)	10.0 (10.07)	5.72 (5.75)	150^{0}C	20	1.98	Dark Brown	70%	$C_{16}H_{13}N_2OCu$
L_1-Ni^{+2}	552 (554)	69.28 (69.31)	4.67 (4.69)	10.0 (10.10)	5.70 (5.72)	160^{0}C	24	2.98	Brownish black	65%	$C_{16}H_{13}N_2ONi$
L_1-Co^{+2}	550 (552)	69.54 (69.56)	4.7 (4.71)	10.11 (10.14)	5.77 (5.79)	170^{0}C	26	4.98	Black	73%	$C_{16}H_{13}N_2OCo$
L_1-Mn^{+2}	546 (548)	69.98 (70.07)	4.72 (4.74)	10.18 (10.21)	5.81 (5.83)	175^{0}C	32	5.25	Dark black	80%	$C_{16}H_{13}N_2OMn$
L_2-Cu^{+2}	620 (624)	61.51 (61.53)	3.80 (3.84)	8.92 (8.97)	5.11 (5.12)	145^{0}C	22	1.88	Dark Brown	78%	$C_{16}H_{12}N_2OClCu$
L_2-Ni^{+2}	621 (622)	61.88 (61.93)	3.84 (3.87)	9.0 (9.03)	5.13 (5.16)	165^{0}C	25	3.01	Brownish black	84%	$C_{16}H_{12}N_2OClNi$
L_2-Co^{+2}	618 (620)	62.11 (62.13)	3.85 (3.88)	9.01 (9.06)	5.13 (5.17)	173^{0}C	28	5.01	Black	88%	$C_{16}H_{12}N_2OClCo$
L_2-Mn^{+2}	613 (616)	62.52 (62.54)	3.85 (3.90)	9.08 (9.12)	5.16 (5.21)	180^{0}C	31	5.52	Dark black	72%	$C_{16}H_{12}N_2OClMn$
L_3-Cu^{+2}	652 (656)	59.41 (59.44)	3.69 (3.71)	12.98 (13.0)	14.84 (14.86)	147^{0}C	21	2.01	Dark Brown	67%	$C_{16}H_{12}N_4O_3Cu$
L_3-Ni^{+2}	651 (654)	59.59 (59.62)	3.68 (3.72)	13.0 (13.04)	14.88 (14.90)	186^{0}C	27	3.15	Brownish black	72%	$C_{16}H_{12}N_4O_3Ni$
L_3-Ni^{+2}	650 (652)	59.79 (59.81)	3.71 (3.73)	13.0 (13.08)	14.92 (14.95)	190^{0}C	33	4.87	Black	82%	$C_{16}H_{12}N_4O_3Co$
L_3-Mn^{+2}	646 (648)	60.11 (60.18)	3.73 (3.76)	13.14 (13.16)	15.0 (15.04)	200^{0}C	38	5.3	Dark black	66%	$C_{16}H_{12}N_4O_3Mn$

Table –II: Spectral data of ligands and their metal complexes

Compounds	I.R. spectra cm^{-1}			^{1}H.N.M.R. spectra ppm		U.V. spectra nm	
	ν(C=N)	ν(M-N)	ν(C-O-C)	δ(Ar-H)	δ(HC=N)	(-C=C-)	(-C=N)
HL_1	1623, 1613	-	1202	7.2	7.68	250	300
HL_2	1612, 1605	-	1207	7.0	7.61	240	295
HL_3	1608, 1603	-	1210	7.5	7.54	245	310
L_1-Cu^{+2}	1588, 1593	450	1220	6.8	7.58	250	355
L_1-Ni^{+2}	1580, 1577	447	1225	6.85	7.45	250	350
L_1-Co^{+2}	1572, 1568	443	1224	6.75	7.32	250	345
L_1-Mn^{+2}	1565, 1562	462	1229	6.60	7.28	250	340
L_2-Cu^{+2}	1594, 1588	455	1230	6.65	7.22	240	365
L_2-Ni^{+2}	1586, 1583	463	1217	6.72	7.18	240	360
L_2-Co^{+2}	1576, 1572	470	1227	6.68	7.08	240	358
L_2-Mn^{+2}	1570, 1566	478	1232	6.57	7.99	240	344
L_3-Cu^{+2}	1604, 1600	465	1214	6.52	7.47	245	372
L_3-Ni^{+2}	1600, 1595	472	1234	6.97	7.39	245	366
L_3-Co^{+2}	1590, 1580	481	1225	6.86	7.26	245	350
L_3-Mn^{+2}	1584, 1577	486	1221	6.67	7.18	245	348

3.0 Evaluation of Antibacterial and Antifungal activities

3.1 Antibacterial activity

Antibacterial activity of test compounds was assessed against gm (+) ve bacteria such as-*S.aureus* (ATCC-25923) and gm (-) ve bacteria such as-*E.coli*. (ATCC-10536), *Pseudomonas* (ATCC-25619*)* by disc diffusion method [21, 22].

3.1.1 Materials

1. Muller-Hinton agar
2. Sterilised petridishes.
3. 20-24 hour old subcultures in Muller-Hinton agar medium
4. Sterilized test tubes containing solution of the test compounds in desired concentration

3.1.2 Preparation of inoculation medium

The definite volumes of peptone (0.5%), yeast extract (0.15%), beaf extract (0.15%), sodium chloride (0.35%), dipotassium phosphate (0.13%) and potassium dihydrogen phosphate (0.13%) were dissolved in distilled water and the pH was adjusted to7.2. This solution was sterilized by autoclaving at 15 p.s.i for 20 minutes.

3.1.3 Preparation of subcultures
One day prior to these tests, inoculation of above bacterial cultures was made in the inoculation medium as described above and incubated at 37^0C for 18-24 hours.

3.1.4 Preparation of base layer medium
Base layer medium was prepared by dissolving definite volumes of peptone (0.6%), yeast extract (0.3%), beaf extract (0.13%) and agar (2.5%) in distilled water. The pH of this medium was also adjusted to 7.2 and sterilized by autoclaving at 15 p.s.i for 20 minutes.

3.1.5 Preparation of test compound
Each test compounds (5mg) were dissolved in DMF (5ml) to give a solution of 1000µg/ml. Out of this 0.1ml of solution was used for antimicrobial testing.

3.1.6 Testing method
Base layer was obtained by pouring about 10-15ml of base layer medium into each sterilized Petri dishes and were allowed to attain room temperature. This solid layer after attaining room temperature is called base layer. Over night grown subcultures of bacteria were mixed with seed layer medium and immediately poured into petridishes containing the base layer and then allowed to attain room temperature.

Antimicrobial discs having diameter of 6mm (whatman no.1), soaked in test solutions, were dispensed on to the surface of this inoculated agar plate. Each disc must be pressed down to ensure its complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24mm from centre to centre. Ordinarily, no more than 12 discs should be placed on a 150mm plate, or more than 5 discs on a 100mm plate. Then these plates were subsequently incubated at 37^0C for 36 hours. The zone of inhibition, if any, was measured in mm for the particular compound. Linezolid was used as positive control and solvent control (12mm) was also used to know the activity of the solvent. The results of antibacterial testing are summarized in table-III

3.2 Antifungal activity-
Fungicidal activity of test compounds was assessed against *C.albicans* (ATCC-90028) *and C. krusei* (ATCC-6258) by disc diffusion method.
3.1.1 Materials
5. Muller-Hinton agar
6. Sterilized petridishes.
7. 16-18 hour old subcultures in Muller-Hinton agar medium supplemented with 1% glucose
8. Sterilized test tubes containing solution of the test compounds in desired concentration

3.2.2 Preparation of inoculation medium
Inoculation medium was prepared by dissolving definite volumes of peptone (1.0%), yeast extract (0.6%), sodium chloride (0. 5%), potassium dihydrogen phosphate (0. 3%) methylene blue (0.005%) and glucose (1.0%) were dissolved in distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 p.s.i for 20 minutes.

3.1.3 Preparation of subcultures
One day prior to these tests, inoculation of above fungal cultures was made in the inoculation medium as described above and incubated at 37^0C for 18-24 hours.

3.1.4 Preparation of base layer medium

Base layer medium was prepared by dissolving definite volumes of peptone (0.6%), yeast extract (0.3%), beaf extract (0.13%) and agar (2.5%) in distilled water. The pH of this medium was also adjusted to 7.2 and sterilized by autoclaving at 15 p.s.i for 20 minutes.

3.1.5 Preparation of test compound

Each test compounds (5mg) were dissolved in DMF (5ml) to give a solution of 1000µg/ml. Out of this 0.1ml of solution was used for antimicrobial testing.

3.1.6 Testing method

The method of testing for antifungal activity is the same as that adopted for assessing antibacterial activity. Clotrinazole was used as positive control and solvent control (12mm) was also used to know the activity of the solvent. The results of antifungal testing are summarized in table-IV.

RESULTS AND DISCUSSION

The ligands were synthesized by condensation of cinnamaldehyde mono hydrazone with substituted salicylaldehyde and then were characterized by means of IR, ^{1}HNMR spectroscopy, conductance measurement and elemental analysis.

The presence of the –C=N group is confirmed by a peaks around 1620cm^{-1} [23, 24]. In the spectra of ligands bands were observed at around 3422(HL_1), 3426(HL_2), 3430 (HL_3) cm^{-1} due to –OH group of salicylaldehyde [25].

The reaction between the metals (II) chlorides and the ligands (1:2) lead to formation of complexes, (scheme-1). The complexes were found to be soluble in DMF and DMSO but insoluble in common organic solvents such as- benzene, acetone, cyclohexane and so forth. The composition and coordination geometry of these complexes has been established on the following experimental observations. The molar conductance values in DMSO fall in the expected range (14-30cm^{2}ohm^{-1}mol^{-1}) of nonelectrolytes [26].

The complexes were analyzed for metal, nitrogen, carbon and hydrogen. The analytical data, conductivity and magnetic moment of the complexes are summarized in table-I.

4.1 Infrared spectral studies

The IR spectral data of the ligand derived from cinnamaldehyde monohydrazone with substituted salicylaldehyde show sharp absorption bands at 1623, 1613 cm^{-1}(HL_1),1612, 1605 cm^{-1} (HL_2),1608, 1603 cm^{-1}(HL_3)and 1210cm^{-1}(HL_1),1199 cm^{-1} (HL_2), 1200 cm^{-1} (HL_3) were assigned to HC=N and N-N[27] stretching vibration respectively. The bands at 1583cm^{-1}(HL_1), 1590cm^{-1}(HL_2) and 1577cm^{-1} (HL_3)are observed due –C=C stretching vibrations[28]. In the spectra of ligands, bands at about 1345, 1518cm^{-1}(HL_1), 1355,1519cm^{-1}(HL_2) and 1340,1517cm^{-1} (HL_3) cm^{-1} and 620cm^{-1}(HL_1), 630cm^{-1}(HL_2), 640cm^{-1} (HL_3) were observed due to(N=O) NO_2 and C-Cl stretching vibrations[29]. The IR spectral assignments of metal complexes were aided by comparison with the vibration frequencies of the free ligand. Like- bands at 1202cm^{-1} due to phenolic C-O shift to higher side (1240±10cm^{-1}) in the complexes [30]. In spectra of metal complexes, the vibration frequency of azomethine group decreases by 20-35 cm^{-1} due to coordination of nitrogen atom to the central metal atom [30]. The band of –OH group is disappeared in spectra of metal complexes due to deprotonation. The ν (M-N) Stretching vibrations are observed at 490-455cm^{-1} in the spectra of the metal complexes [31].

4.2 ^{1}HNMR spectral studies

The ^{1}HNMR spectra of the ligands show the signals for the aromatic protons around 7.2 ppm (HL_1), 7.0 ppm (HL_2), 7.5 ppm (HL_3), which is in the range of of their deshielding which is attributed to the donation of the lone pair of electrons by the azomethine nitrogen to the metal atoms[33].

In the ^{1}HNMR spectra of the metal complexes, the peaks due to –OH group of salicylaldehyde, were disappeared, which were earlier observed at 12.2ppm (HL_1), 12.16ppm (HL_2), 12.25ppm (HL_3) in the spectra of ligands [34]

4.3 Electronic spectral studies

Electronic spectral data of the ligands and their transition metal complexes were recorded in DMF solutions. In the electronic spectrum of the ligand, two prominent absorption bands at 250 and 350 nm were characterized. The band at 250nm corresponds to the π- π * transition of the –C=C group [35]. In the spectra of ligands, bands at 300nm corresponds to the n- π * transition of azomethine group, shift to higher wave length in spectra of metal complexes. In the spectra of ligands, bands observed at about 340nm due to the secondary band of benzene and which gets red shifted due to the presence of –C=N-N=C-, however, this appears at 380nm in the complexes due to the polarization in C=N bond caused by the metal ligand electrone interaction[33].

In case of complexes, the bands appeared in the almost same position as they were appeared in spectra of ligands and some new the bands appeared at 470, 355, 490, 463 nm in case of Cu^{+2}, Ni^{+2}, $Co^{+2}$and Mn^{+2} complexes respectively which were assigned due to ligand to metal charge transfer transition[36].

4.4 Magnetic measurement studies

The magnetic moments of the complexes were recorded at room temperature on a faraday balance using $Hg[Co(SCN)_4]$ as a calibrant and the observed magnetic moment values for the Co(II) complex are 4.98BM (HL_1),5.01BM (HL_2)and 4.87Bm (HL_3), which are in the range of 4.4-5.5BM observed for the octahedral complexes[37].

The nickel (II) complexes exhibit the magnetic moment values 2.98BM (HL_1), 3.01BM (HL_2) and 3.15BM (HL_3), which are in the range of 2.8-3.40BM observed for the octahedral complexes [30]. The values for the Cu (II) complex are 1.98BM (HL_1), 1.88BM (HL_2) and 2.01Bm (HL_3), which are in the range of 1.8-2.2BM observed for the octahedral complexes [30]. The values for the Mn (II) complex are 5.25BM (HL_1), 5.52BM (HL_2) and 5.3Bm (HL_3), which are in the range of 5.2-5.8BM observed for the octahedral complexes [38].

4.5 ESR spectral studies

X-band powder ESR spectra of the metal complexes of Schiff base hydrazones were recorded at room temperature on E-112-Xband spectrometer at TCNE as g-marker. The ESR spectra of the Cu^{+2}, Ni^{+2}, Co^{+2} and Mn^{+2} complexes show g values in the range of 2.89-5.18 [39].

4.6 Antimicrobial studies

In the light of interesting antimicrobial activities of the co-ordination complexes, the ligands and their corresponding complexes were screened antifungal and antibacterial activity against gm (+)ve bacteria such as- *S.aureus*(ATCC-25923) and gm(-)ve bacteria such as-*E.coli*.(ATCC-10536), *Pseudomonas*(ATCC-25619*)* as well as fungi such as- *Candida albicans*(ATCC-90028) and *Candida krusei*(ATCC-6250) by disc diffusion method. The radial growth of the colony was

recorded on completion of the incubation and the mean diameter for each complex at a single concentration was recorded. The average % of the inhibition of the bactericidal and fungicidal growth medium were compared using the Vincent equation [40]

$$I = C\text{-}Tx100/C, \text{ where } I = \% \text{ inhibition},$$

T = Average diameter of the bacterial and fungal growth on the control plates and
C= the average diameter of the growth on control plates.

The screening data of the inhibition of the fungi and bacteria are given in table-3and 4. From the data, it is clear that the metal complexes of Schiff base hydrazones have greater inhibiting power than the free ligand.

Although it is difficult to make out an exact structure reactivity relationship between the microbial activity and the structure of these complexes, it can possibly be concluded that the complexation as well as addition of a substrate enhances the activity of the complexes. The variation in the toxicity of different antimicrobial agents against different organisms as suggested by Garrod et al [41] depends either on the impermeability of the cell or differences in ribosome to the antimicrobial agents. Though the results suggested that the ligands have remarkable toxic property, their complexes of Schiff base hydrazones inhibit the growth of organisms to a greater extent.

Table-III: Antibacterial screening data of ligands and their metal complexes

Compounds	**Micro-organisms**		
	E.coli. (ATCC-10536)	**S.aureus (ATCC-25923)**	**Pseudomonas (ATCC-25619)**
	Representation zone of inhibition		
HL_1	+	+	+
HL_2	+	+	+
HL_3	+	+	++
L_1-Cu^{+2}	++	++	+++
L_1-Ni^{+2}	+++	+++	++++
L_1-Co^{+2}	+++	+++	+++
L_1-Mn^{+2}	++++	+++	++++
L_2-Cu^{+2}	++	+++	+++
L_2-Ni^{+2}	+++	+++	++++
L_2-Co^{+2}	++++	+++	++++
L_2-Mn^{+2}	++	+++	+++
L_3-Cu+2	++	+++	++++
L_3-Ni^{+2}	+++	++++	++++
L_3-Co^{+2}	+++	+++	++++
L_3-Mn^{+2}	++++	+++	++++

Inhibition zone diameter mm (% inhibition): + 10-15(33-49%), ++ 16-20 (53-66%); +++ 21-25 (69-83%), + +++ 26-30 (86-100%)
DMF = 12mm,
Linezolid = 30mm
Index

1. *Concentration of the compound = 1mg/ml in DMF.*
2. *Solvent used = Dimethyl formamide.*
3. *Control of the antibacterial activity = Linezolid*

Table-IV: Antifungal screening data of ligands and their metal complexes

Compounds	Micro-organisms	
	C.albicans (ATCC-90028)	C.krusei (ATCC-6250).
	Representation zone of inhibition	
HL_1	+	+
HL_2	+	+
HL_3	++	+
L_1-Cu^{+2}	++	+++
L_1-Ni^{+2}	+++	++++
L_1-Co^{+2}	++++	++++
L_1-Mn^{+2}	++	+++
L_2-Cu^{+2}	+++	++++
L_2-Ni^{+2}	+++	++++
L_2-Co^{+2}	++++	++++
L_2-Mn^{+2}	++++	++++
L_3-Cu^{+2}	+++	++++
L_3-Ni^{+2}	++++	+++
L_3-Co^{+2}	++++	++++
L_3-Mn^{+2}	++++	++++

Inhibition zone diameter mm (% inhibition): + 10-15 (32-48%), ++ 16-20 (51-64%); +++ 21-25 (67-80%), ++++ 26-32(83-100%)
DMF = 12mm,
Clotrinazole = 31mm
Index
1. Concentration of the compound = 1mg/ml in DMF.
2. Solvent used = Dimethyl formamide.
3 Control of the antifungal activity = Clotrinazole

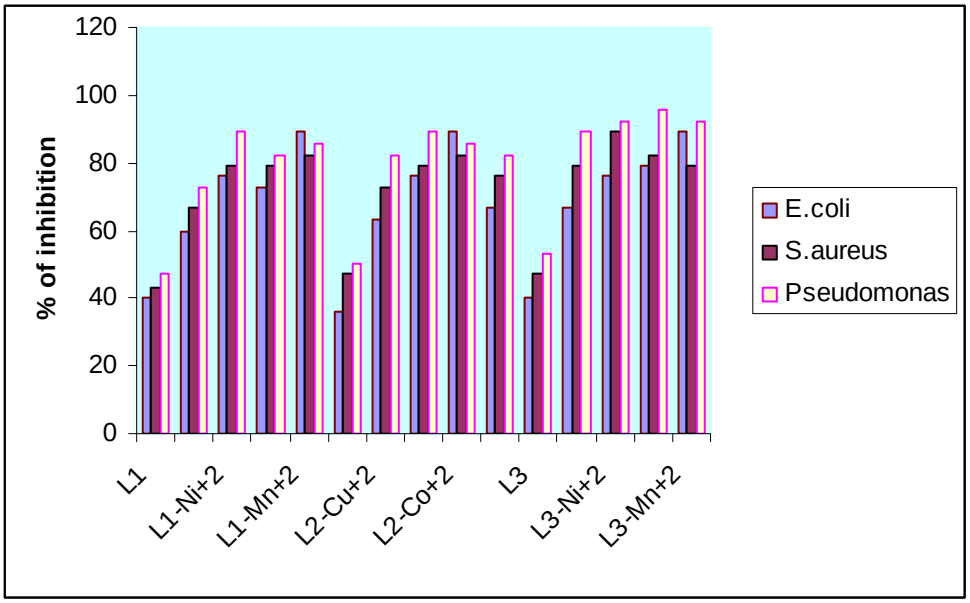


Fig.1 Antibacterial activity of ligands and their metal complexes

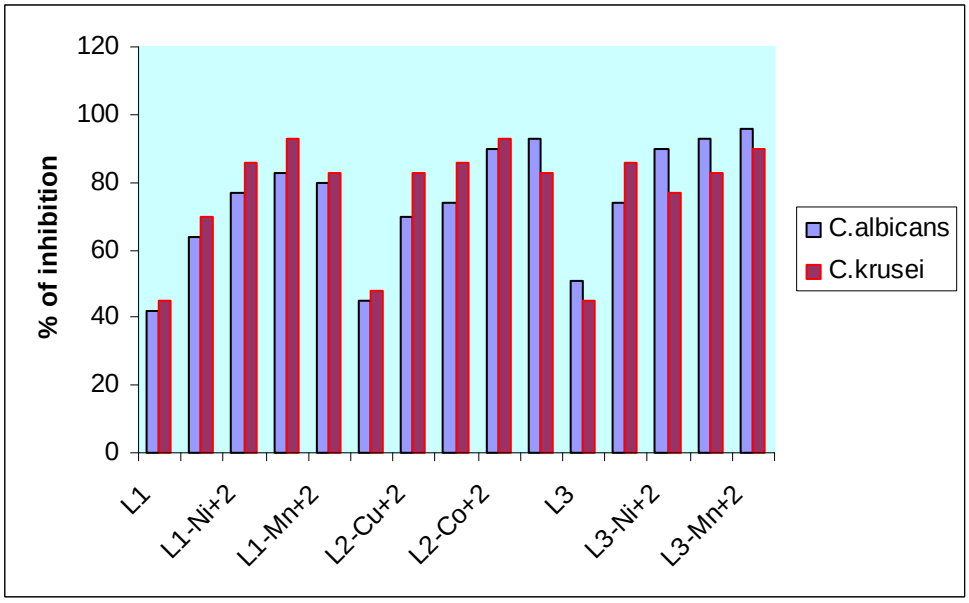


Fig.2 Antifungal activity of ligands and their metal complexes

CONCLUSION

From the elemental analysis, molar conductivity, UV-Visible, magnetic, IR and ^{1}HNMR spectral data it was possible to determine the type of coordination of the ligands in their metal complexes. In all the complexes, only one part of the ligand is coordinated to the metal ion resulting mononuclear complexes. The ligand coordinates through the nitrogen atoms (N-N) of the hydrazone moiety and oxygen atom after deprotonation of the substituted salicylaldehyde fragment. The ligand acts as a monobasic, tridentate (FIGURE-1)

Acknowledgements
We are grateful to Principal, Head Department of Chemistry, Government College, Ajmer for encouragement. We also thank the Regional Sophisticated Instrumentation Centre (RSIC), Central Drug Research Institute (CDRI) Lucknow, for providing Spectral Analysis, and micro analytical data**.** We are also grateful to Microbiology department of J.L.N. Medical College, Ajmer for providing all facilities and support in biological activity.

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