

Preparation, Characterization and Biological Activity of New Tridentate Imine-Oxime Ligand (H₂L) and Its Metal Complexes

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The new tridentate Schiff-oxime ligand 1-[2-(2,4-dihydroxybenzylidene)aminophenyl]ethanone oxime (H₂L), prepared *via* condensation of equal amounts of 1-(2-aminophenyl)ethanone oxime and 4-hydroxysalicylaldehyde. H₂L ligand characterized by using FTIR, UV-visible spectroscopies, ¹H NMR, ¹³C NMR spectra and Mass spectrum. Metal ions complexes with Cr³⁺, Mn²⁺, Co²⁺, Ni²⁺ and Cu²⁺ ions, prepared in a M:L (1:2) ratio and characterized by IR, UV-visible spectroscopies, magnetic susceptibility, molar conductivity and flame atomic absorption measurements. Results of spectral studies proved the chelation behaviour of this ligand, which coordinated to metal ions as a monobasic NNO tridentate ligand, *via* imine and oxime nitrogen atoms in addition to phenolic oxygen atom, after losing its proton. The proposed geometry of all the prepared complexes was octahedral. The minimal inhibition zone against two Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative bacteria (*E. coli* and *Klebsella* spp.) also determined.

Keywords: NNO tridentate, Schiff-oxime, Metal complexes, *o*-Aminoacetophenone oxime, 4-Hydroxy-salicylaldehyde, Antibacterial activity.

INTRODUCTION

Schiff-oxime ligands characterized by the existence and participation of iminic- and oximic azomethine groups in coordination with metal ions [1-3]. Metal complexes of imine-oxime have various applications in different scientific fields. It has been used as antimicrobial [4], antibacterial [5], antifungal [5,6] as well as anticancer [7]. Some of Schiff oxime derivatives have been used in extraction [8] and spectrophotometric determination [9] of metal ions. Imine-oxime complexes have been also found used as catalysts for selective oxidation of alcohols to ketones and aldehydes [10].

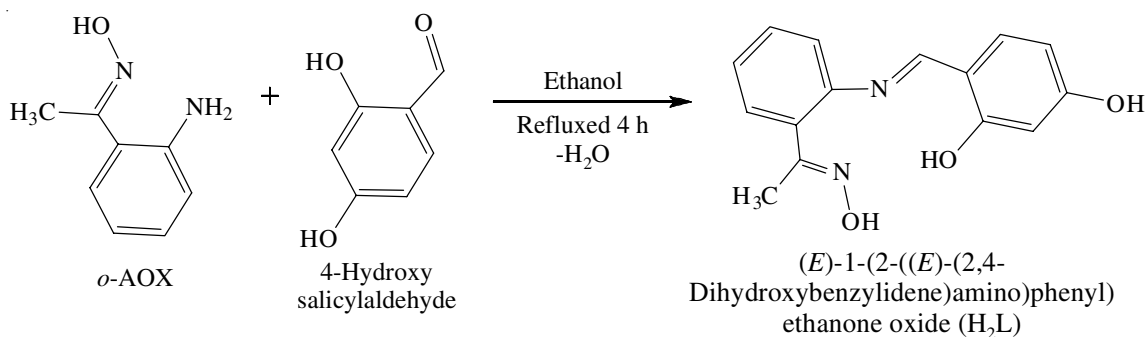
EXPERIMENTAL

Chemicals have been procured from Fluka and Sigma-Aldrich. Shimadzu (FT-IR), 4800S spectrophotometer has been used to get FTIR spectra of prepared compounds (4000-400 cm⁻¹, KBr). Cary 100con. spectrophotometer has been used for electronic spectra of prepared compounds at wavelength 200-800 nm. Stuart digital SMP30 apparatus used for measuring melting points of compounds. Mass spectrum of H₂L was performed on GC-MS QP-2010 (Shimadzu). NMR spectra of H₂L in DMSO-*d*₆ were recorded by Bruker DMX-500 spectrophotometer (300 MHz). Phoenix-986 AA spectrophotometer has been used to estimate the metal ions percentages.

Auto magnetic susceptibility balance (Sherwood Scientific) at 300 K has been used to measure magnetic susceptibility of prepared complexes. Inolab Multi 740, WTW 82362 Weilhiem-Germany at 25 °C has been used to record conductivity measurements of complexes solutions in DMSO solvent (10⁻³ M).

Synthesis of [1-(2-aminophenyl)ethanone oxime] (*o*-AOX) [11]: 10 mL Absolute ethanolic solution of *o*-aminoacetophenone (*o*-ACP, 1.352 g, 0.01 mol) gradually added to a mixed solvent solution EtOH/H₂O, (35/15) mL contains (1.042 g, 0.015 mol) of hydroxylamine hydrochloride and (1.23 g, 0.015 mol) of sodium acetate. Final reaction mixture reflux for 7 h. The white needle precipitate washed with distilled water, then dried and recrystallized from hot absolute ethanol.

Synthesis of 1-[2-(2,4-dihydroxybenzylidene)aminophenyl]ethanone oxime (H₂L): The solution of precursor *o*-AOX, (1.5 g, 0.01 mol) in 15 mL of hot absolute ethanol gradually added to solution of 4-hydroxysalicylaldehyde (2.01 g, 0.01 mol) in 15 mL of hot ethanolic solution. Although yellow precipitate formed after 3 h, 1 h has been added to period of reflux. The reaction mixture kept in refrigerator overnight. Next day the product filtered off, washed with diethylether and cold absolute ethanol, respectively. The purity of product tested with TLC technique by using 7:3 mL ratio of (hexane:ethyl acetate). **Scheme-I** represents the preparation reaction of H₂L.



Synthesis of metal complexes: One similar method has been followed to synthesis of all metal ion complexes. Metal chloride salt (0.0005 mol) dissolved in 5 mL of absolute ethanol and slowly added to H_2L (0.270 g, 0.001 mol) dissolved in 15 mL hot absolute ethanol. At mixing time colour change has been noticed at reaction mixture. All obtained solutions then heated under reflux for 4 h. All the complexes precipitate collected after slow evaporation of their solutions at room temperature. The precipitates washed repeatedly with diethyl ether then distilled water. Finally all coloured precipitates recrystallized by hot absolute ethanol solution.

RESULTS AND DISCUSSION

1H NMR and ^{13}C NMR spectra of H_2L : The chemical shifts data and their assignments of both NMR spectra were listed, respectively (Tables 1 and 2).

1H NMR spectrum of H_2L exhibited signals at (δ = 11.62 ppm, 6.19 s, 2H), (δ = 9.39 ppm, s, 1H), (δ = 7.61 ppm, d, 1H) and (δ = 2.33 ppm, 3H), which were assigned respectively to phenolic [12] hydroxyl groups (2-OH and 4-OH), oxime [13] hydroxyl group, azomethine (CH=N) [13] and CH_3 group [6,13] protons, respectively. The signals were observed at (δ = 7.34 ppm, d, 1H), (δ = 7.25 ppm, t, 1H), (δ = 6.98 ppm, d, 1H), (δ = 6.85 ppm, d, 1H), (δ = 6.78 ppm, t, 1H), (δ = 6.25 ppm, s, 1H) and (δ = 6.11 ppm, d, 1H), assigned to H_1 , H_2 , H_7 , H_6 , H_3 , H_5 and H_4 , respectively.

TABLE-1
 1H NMR DATA CHEMICAL SHIFTS ASSIGNMENT OF H_2L

δ (ppm)	Assignment
11.62 (1H, s)	2-OH
6.19 (1H, s)	4-OH
9.39 (1H, s)	(oxime) (OH)
7.61 (1H, s) 2.33 (3H, s)	(CH=N, CH_3) (H)
7.34, 6.11 (2H, d)	H_1 , H_4
6.98, 6.85 (2H, d)	H_7 , H_6
7.25, 6.78 (2H, t)	H_2 , H_3
6.25 (1H, s)	H_5

TABLE-2
 ^{13}C NMR DATA AND CHEMICAL SHIFTS ASSIGNMENT OF H_2L

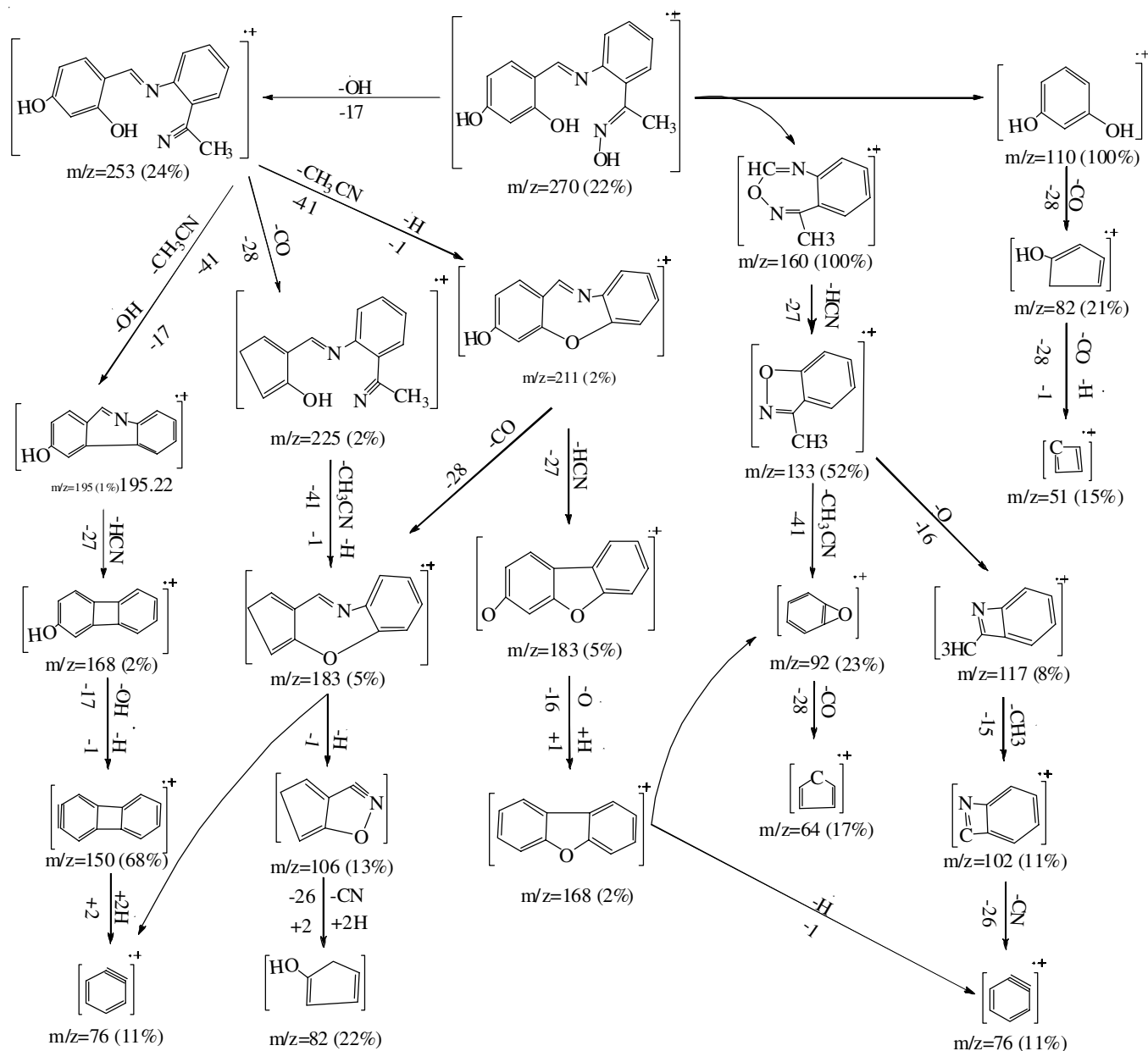
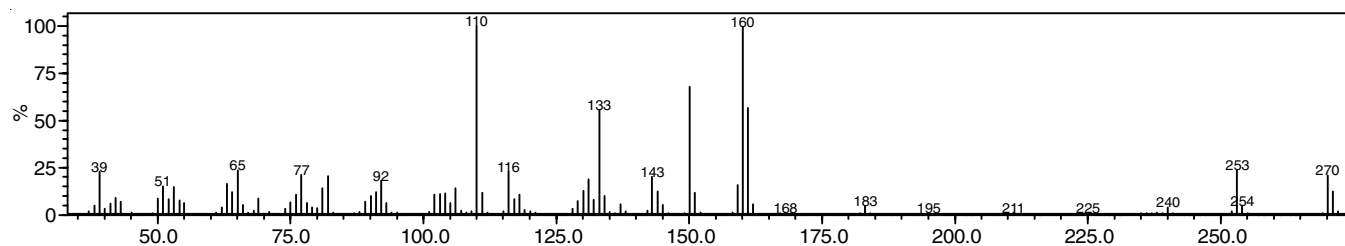
δ (ppm)	Assignment
159.20, 156.86	(C2-OH), (C4-OH)
140.93	CH=N (C7)
74.23	-CH- (C14)
11.88	CH_3 (C15)
140.64, 131.30	C8, C9
127.47, 125.38	C6, C10
118.25, 116.20	C12, C11
114.77, 113.83	C13, C1
105.85, 103.75	C5, C3

The ^{13}C NMR spectrum of H_2L exhibited chemical shifts at (δ = 159.20, 156.86 ppm), (δ = 140.93 ppm) and (δ = 11.88 ppm), which are assigned to C2-OH, C4-OH [11,12], (C=N) of azomethine carbon (C7) and sp^3 hybridized carbon atom related to CH_3 group [11,13], respectively. The spectrum also revealed new chemical shift at δ = 74.23 ppm due to oxime-nitroso tautomer [14].

Mass spectrum of H_2L : Fig. 1 clearly reveals the mother ion peak of H_2L at m/z = 270, related to M^+ . The proposal pathways of H_2L fragmentation are described in **Scheme-II**.

Characterization of metal ion complexes: The yield percentages, physical properties and metal content of H_2L and its metal complexes are tabulated in Table-3.

FTIR study: The FTIR spectrum of H_2L (Fig. 2) exhibited two strong and sharp bands at 3369 cm^{-1} and 3308 cm^{-1} , related to $\nu(\text{O-H})$, of phenolic hydroxyl group (4-OH) [15] and oxime hydroxyl group [11], respectively. These two bands disappeared in complexes spectra. Except C_2 complex, because it overlapped with each other inside broad band observed at range $3400\text{--}3100\text{ cm}^{-1}$, as shown in FTIR spectrum of C_3 complex (Fig. 3). The $\nu(\text{O-H})$ of phenolic hydroxyl group (2-OH) in ligand spectrum observed as broad band at 3174 cm^{-1} , because its involvement in hydrogen bonding (intra-ligand hydrogen bond) with nitrogen atom of azomethine linkage ($\text{O-H}\cdots\text{N}$) [16]. The latter band disappeared [15,16] in complexes spectra, this proved the involvement of it in coordination with metal ions. The phenolic $\nu(\text{C-O})$ stretching vibration which observed



Scheme-II: Proposal Pathways of H₂L fragmentation

TABLE-3
YIELD PERCENTAGES, PHYSICAL PROPERTIES AND METAL CONTENT OF COMPOUNDS

Comp.	m.f.	m.w. (g mol ⁻¹)	Colour	m.p. (°C)	Yield (%)	M (%): Calc. (found)
H ₂ L	C ₁₅ H ₁₄ N ₂ O ₃	270.28	Yellow	188-190	93	—
C ₁	C ₃₀ H ₂₆ N ₄ O ₆ CrCl	626.00	Light brown	214-216	43	8.31 (7.80)
C ₂	C ₃₀ H ₂₆ N ₄ O ₆ Mn	593.49	Dark brown	182-184	41	9.26 (8.76)
C ₃	C ₃₀ H ₂₆ N ₄ O ₆ Co	597.48	Yellowish brown	298-300	47	9.86 (10.16)
C ₄	C ₃₀ H ₂₆ N ₄ O ₆ Ni	597.24	Greenish brown	225-228	54	9.83 (9.32)
C ₅	C ₃₀ H ₂₆ N ₄ O ₆ Cu	602.10	Olive	181-183	57	10.55 (11.87)

in the spectrum of H_2L at 1257 cm^{-1} , has been shifted by $2\text{-}13\text{ cm}^{-1}$ to lower frequencies [16] in complexes spectra. This also indicated the participation of phenolic oxygen atom in coordination with metal ions. The azomethine groups of Schiff and oxime noticed at 1624 cm^{-1} and 1600 cm^{-1} in H_2L , turned into lower frequencies in the complexes spectra [15,16]. This proved the participation of both groups in coordination with metal ions. The $\nu(\text{C-N})$ and $\nu(\text{C-O})$ vibrations suffered from significant changes as a result of coordination. New vibration bands have been noticed in complexes spectra at wavenumber

ranges $563\text{-}543\text{ cm}^{-1}$ and $466\text{-}462\text{ cm}^{-1}$ attributed to (M-O) and (M-N) vibrations, respectively [6]. Characteristic bands of prepared compounds with their frequencies and assignments are listed in Table-4.

Absorption spectra and magnetic properties of complexes: Spectral data and the magnetic moment of H_2L and its metal complexes are tabulated in Table-5. Fig. 4 represents the absorption spectra of H_2L and its complexes.

The UV-visible spectrum of H_2L , reveals five absorption peaks at (225 nm , 44444 cm^{-1}), (240 nm , 41666 cm^{-1}), (262

TABLE-4
FTIR SPECTRAL DATA (cm^{-1}) OF H_2L AND IT'S COMPLEXES

Comp.	$\nu(\text{O-H})$ phenol	$\nu(\text{O-H})$ oxime	$\nu(\text{C-H})$ Arom. Alph.	$\nu(\text{C=N})$ sch. $\nu(\text{C=N})$ ox.	$\nu(\text{C=C})$	$\nu(\text{C-O})$ $\nu(\text{C-N})$ $\nu(\text{N-O})$	$\nu(\text{M-O})$ $\nu(\text{M-N})$
H_2L	3369 (s) 3174 (br)	3308 (s)	3070 (w) 2976-2901 (w)	1624 (s) 1600 (s)	1489-1438	1257 (s) 1180 (s) 1039 (m)	— —
C_1	—	—	3080 (w) 2804-2777 (w)	1608 (s) 1583 (s)	1533-1431	1247 (m) 1197 (m) 1035 (w)	553 (w) 462 (w)
C_2	3369 (s) —	—	3086 (w) 2918 (w)	1600 (s) 1539 (w)	1539-1437	1255 (m) 1182 (m) 1026 (w)	545 (w) 466 (w)
C_3	—	—	3064 (w) 2914 (w)	1606 (s) 1587 (s)	1535-1440	1253 (s) 1199 (s) 1035 (w)	563 (w) 462 (w)
C_4	—	—	—	1612 (s) 1570 (m)	1535-1437	1244 (w) 1193 (s) 1012 (w)	553 (w) 466 (w)
C_5	—	—	3084 (w) —	1610 (s) 1579 (m)	1539-1429	1249 (m) 1192 (s) 1037 (w)	543 (w) 462 (w)

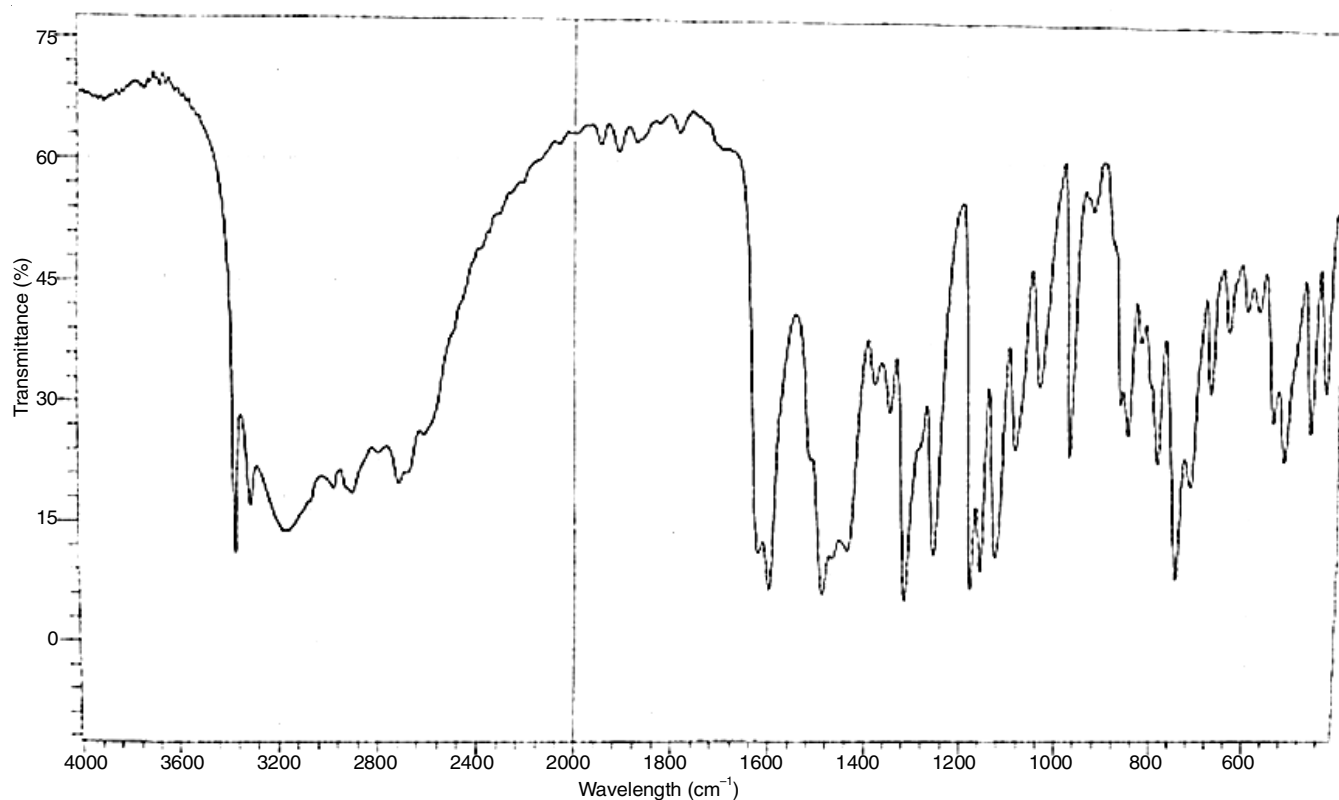
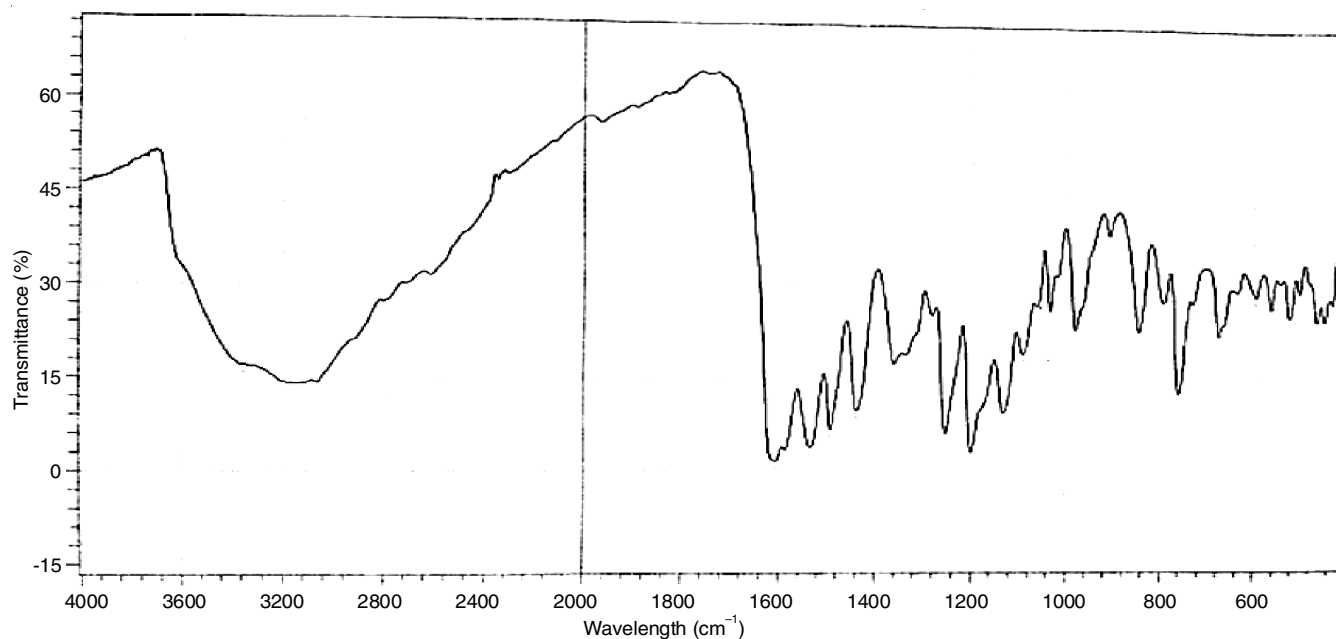
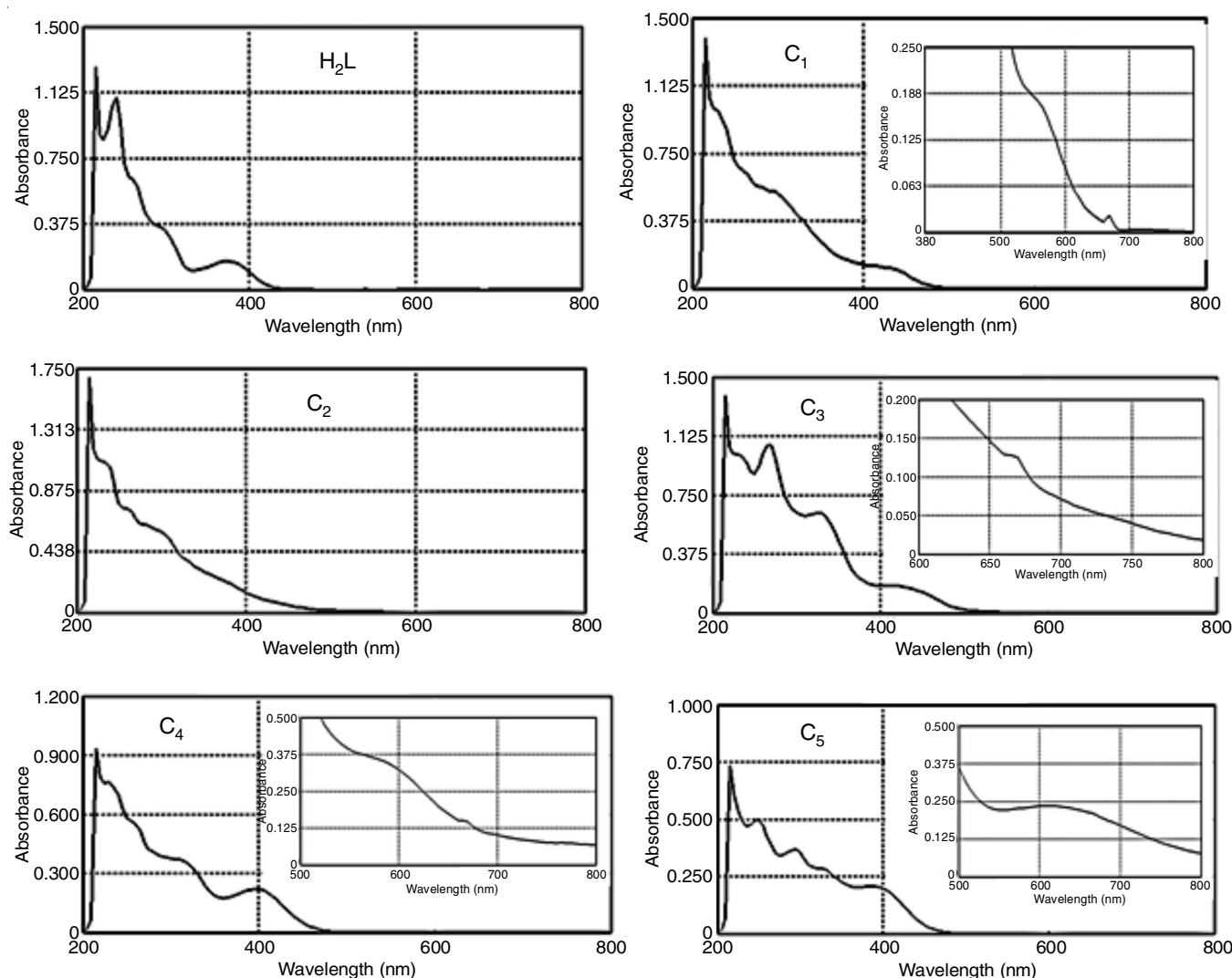


Fig. 2. FTIR spectrum of H_2L

Fig. 3. FTIR spectrum of C₃ complexTABLE-5
ABSORPTION DATA OF H₂L AND PREPARED COMPLEXES

Compound symbol (colour in solid state) (colour in EtOH)	$\lambda_{\max}$ (nm)	ν (cm ⁻¹)	Assignment	μ_{eff} (BM)	Molar conductance (S cm ² mol ⁻¹)	Geometry	
H ₂ L (Yellow) (Yellow)	225	44444	$\pi \rightarrow \pi^*$	—	—	—	
	240	41666					
	262	38167					
	296	33783					
	375	26666	ILCT				
C ₁ (Light Brown) (Light Brown)	215	46511	Ligand field	3.91	39.4	Oh	
	263	38022					
	294	34013					
	435	22988					$(^4\text{A}_2\text{g}^{(\text{F})} \rightarrow ^4\text{T}_1\text{g}^{(\text{F})})$ (ν_3) & CT
	559	17889					$(^4\text{A}_2\text{g}^{(\text{F})} \rightarrow ^4\text{T}_1\text{g}^{(\text{F})})$ (ν_2)
	669	14947	$(^4\text{A}_2\text{g}^{(\text{F})} \rightarrow ^4\text{T}_2\text{g}^{(\text{F})})$ (ν_1) (10Dq)				
C ₂ (Dark Brown) (Brown)	215	46511	Ligand field	5.40	12.12	Oh	
	236	38022					
	262	38167					
	307	32573					
C ₃ (Yellowish Brown) (Brown)	214	46728	Ligand field	3.90	8.76	Oh	
	250	40000					
	270	37037					
	325	30769					
	426	23474					$(^4\text{T}_1\text{g}^{(\text{F})} \rightarrow ^4\text{T}_1\text{g}^{(\text{P})})$ (ν_3) & CT
	670	14925					$^4\text{T}_1\text{g}^{(\text{F})} \rightarrow ^4\text{A}_2\text{g}^{(\text{F})}$ (ν_2)
	946	10373	$(^4\text{T}_1\text{g}^{(\text{F})} \rightarrow ^4\text{T}_2\text{g}^{(\text{F})})$ (ν_1)**				
C ₄ (Greenish Brown) (Brown)	214	46728	Ligand field	2.85	8.55	Oh	
	229	43668					
	261	38314					
	312	32051					
	395	25316					$^3\text{A}_2\text{g}^{(\text{F})} \rightarrow ^3\text{T}_1\text{g}^{(\text{P})})$ (ν_3) & CT
	588	17006					$^3\text{A}_2\text{g}^{(\text{F})} \rightarrow ^3\text{T}_1\text{g}^{(\text{F})})$ (ν_2)
	667	14992	$^3\text{A}_2\text{g}^{(\text{F})} \rightarrow ^3\text{T}_2\text{g}^{(\text{F})})$ (ν_1)				
C ₅ (Olive) (Light green)	214	46728	Ligand field	1.79	12.80	Oh	
	248	40322					
	295	33898					
	330	30303					
	392	25510					CT
	620	16129	$^2\text{Eg} \rightarrow ^2\text{T}_{\text{g}}$				

**Calculated theoretically.

Fig. 4. Absorption spectra of H₂L and its metal complexes

nm, 38167 cm⁻¹), (296 nm, 33783 cm⁻¹) and (375 nm, 26666 cm⁻¹). The first three absorption peaks assigned to $\pi \rightarrow \pi^*$ transitions of benzene rings and oxime azomethine group [17,18], the fourth absorption peak assigned to $\pi \rightarrow \pi^*$ transition of Schiff azomethine group [17,19]. The fifth low intensity absorption peak assigned to intra-ligand charge transfer transitions (ILCT) [20].

Spectrum of Cr³⁺ (C₁) complex, exhibits three absorption peaks [21] at (669 nm, 14947 cm⁻¹), (559 nm, 17889 cm⁻¹) and (435 nm, 22988 cm⁻¹). First two them assigned to (⁴A_{2g}(^F) → ⁴T_{2g}(^F)) (ν₁) (10Dq) [20] and (⁴A_{2g}(^F) → ⁴T_{1g}(^F)) (ν₂) [21], transitions, respectively. The third peak assigned to (⁴A_{2g}(^F) → ⁴T_{1g}(^P)) (ν₃) and charge transfer (CT) transitions. The spectrum also exhibits other three peaks at (215 nm, 46511 cm⁻¹), (263 nm, 38022 cm⁻¹) and (294 nm, 34013 cm⁻¹), respectively, were ascribed to ligand field spectra [21]. The value of 10 Dq is equal to energy of first transition [22]. The Racah parameter (β), (B') and (% Covalency) have been calculated depending upon Tanabe-Sugano diagram and the values were 0.39, 354 cm⁻¹ and 61 %, respectively. The value of Racah (β), indicated the existence of covalence character in bonding between H₂L and Cr(III). The value of effective magnetic moment

was 3.91 BM within the normal range of octahedral geometry [21,23].

The ILCT transition of free ligand absent in spectrum of Mn²⁺ (C₂) complex, as a result of involvement the non-bonding lone pairs of azomethine groups nitrogen atoms in coordination with Mn(II) ion [23]. The spectrum also exhibited four absorption peaks at (215 nm, 46511 cm⁻¹), (236 nm, 38022 cm⁻¹), (262, 38167 cm⁻¹), (307 nm, 32573 cm⁻¹), related to ligand field spectra [21]. Any d → d peak might be assigned to the manganese(II) center was not observed [24]. The room temperature magnetic value was 5.4 BM, which confirm the presence of octahedral high spin Mn(II) complex [21].

Ultraviolet region of Co²⁺ (C₃) complex, exhibits four peaks (214 nm, 46728 cm⁻¹), (250 nm, 40000 cm⁻¹), (270 nm, 37037 cm⁻¹) and (325 nm, 30769 cm⁻¹) which which assigned to ligand field spectra [21]. The spectrum also shows new low intensity peak at (426 nm, 23474 cm⁻¹), attributed to (⁴T_{1g}(^F) → ⁴T_{1g}(^P)) (ν₃) and MLCT transition [25,26]. At high concentration [26] another new peak has been noticed at (670 nm, 14925 cm⁻¹), assigned to ⁴T_{1g}(^F) → ⁴A_{2g}(^F) (ν₂), transition. The Racah parameter (β), (B') and (% Covalency) obtained from Tanabe-Sugano diagram and their values were 0.46, 451 cm⁻¹ and 54 %, respec-

tively. Moderate covalence character in bonding between Co(II) and H₂L has been proved from β value. This confirm in addition to value of magnetic moment (5.4 BM), the existence of Co(II) ion in an octahedral geometry [25].

Absorption spectrum of Ni²⁺ (C₄) complex revealed four absorption peak at ultraviolet region (214 nm, 46728 cm⁻¹), (229 nm, 43668 cm⁻¹), (261 nm, 38314 cm⁻¹), (312 nm, 32051 cm⁻¹), were assigned to ligand field spectra [21]. This spectrum exhibited three peaks at visible region (395 nm, 25316 cm⁻¹), (588 nm, 17006 cm⁻¹) and (667 nm, 14992 cm⁻¹), related to [³A₂g^(F)→³T₁g^(P) (ν_3) & CT], [³A₂g^(F)→³T₁g^(F) (ν_2)] and [³A₂g^(F)→³T₂g^(F) (ν_1)], respectively [21]. The Racah parameter (β), (B') and (% Covalency) obtained from Tanabe-Sugano diagram and their values were 0.38, 389 cm⁻¹ and 62 %, respectively. Strong covalence character between Ni(II) and H₂L has been proved from small β value. The obtained value of magnetic moment was 2.85 BM, consistent with two unpaired electron without orbital contribution of Ni(II) octahedral system [25].

Absorption spectrum of Cu²⁺ (C₅) complex, displays four absorption peaks at ultraviolet region (214 nm, 46728 cm⁻¹), (248 nm, 40322 cm⁻¹), (295 nm, 33898 cm⁻¹) and (330 nm, 30303 cm⁻¹), were attributed to ligand field spectra [21]. Two absorption peaks have been noticed in visible region exactly at (392 nm, 25510 cm⁻¹) and (620 nm, 16129 cm⁻¹) attributed to charge transfer (CT) and ²E_g→²T₂g and transitions [27], respectively. Magnetic moment was 1.79 BM, consistent with one electron of distorted octahedral geometry [21].

Molar conductivity measurements: The molar conductance value of C₁ complex in EtOH (39.4 S cm² mol⁻¹) reveals electrolyte nature [28] (1:1) ratio. Molar conductivity of (C₂-C₅) in EtOH solution were within the range (8-13 S cm² mol⁻¹), these proved the non-electrolyte nature [29] of these complexes. Depending upon all previous results we can proposed suggested structures for complexes, as shown in Fig. 5.

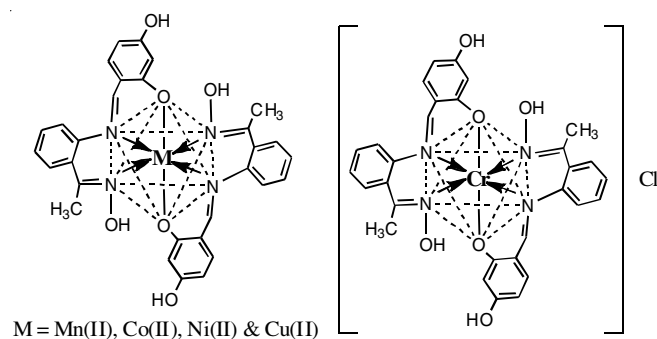


Fig. 5. Proposed structures of prepared complexes

Biological study: The test solutions (1 × 10⁻³ M and 1 × 10⁻⁴ M) were prepared by dissolving desired quantity from each compound with DMSO. The plates were incubated for 24 h. The sample was leveled in the sterile disc and incubated at 37 °C. The antibacterial activity obtained after incubation by measuring the inhibition zone diameter (IZ) around the hole (mm). The results of the tested samples was compared with the control (DMSO) (Table-6). The *in vitro* antibacterial activity has been done at 37 °C against four pathogenic bacteria, two Gram-positive and two Gram-negative. DMSO has been used as blank control solvent, because it is inactive in culture medium.

TABLE-6
INHIBITION ZONE (mm) DATA OF PREPARED
COMPOUNDS AGAINST SELECTED BACTERIA

Compd.	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>Klebsella</i> spp
(DMSO) (C)	0	0	0	0
H ₂ L (1)	0	0	15	0
C ₁ (2)	11	12	14	12
C ₂ (3)	12	13	8	14
C ₃ (4)	0	0	0	0
C ₄ (5)	0	0	14	0
C ₅ (6)	0	0	12	10

The concentration (1 × 10⁻⁴ M), didn't show activity toward any one of selected bacteria. At 1 × 10⁻³ M, the solution of H₂L exhibited higher antibacterial activity than its complexes against growth of *E. coli* (-) [30]. The prepared ligand H₂L, showed no effect against growth of *S. aureus* (+), *S. epidermidis* (+) and *Klebsella* spp (-) [31], while the prepared complexes (C₁ and C₂) showed positive effect against four organisms which exhibits moderately to high activity and the zone of inhibition 8-14 mm (Table-6). The prepared complex C₃, showed no effect against growth of selected bacteria. C₄ complex showed high activity with a zone of inhibition 14 mm against growth of *E. coli* (-) but not has effectiveness against other bacteria. C₅ complex showed high activity with inhibited zone (10-12 mm) against growth of *Klebsella* spp. (-) and *E. coli* (-), respectively but not has effectiveness against *S. aureus* (+) and *S. epidermidis* (+).

Conclusion

New chelation complexes of Cr³⁺, Mn²⁺, Co²⁺, Ni²⁺ and Cu²⁺ with a new Schiff-oxime ligand have been prepared in moderate yields percentages. Analytical and spectroscopic data showed that the complexes are mononuclear and the Schiff-oxime ligand acting as a monobasic (NNO) tridentate ligand. The imine-oxime ligand and its metal complexes exhibited different inhibition behaviour against selected bacteria.

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