

Isatin Endowed Metal Chelates as Antibacterial and Antifungal Agents (Isatin Pembawa Logam Pengkelat sebagai Agen Anti-Bakteria dan Anti-Kulat)

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ABSTRACT

A new series of 5-chloroindoline-2,3-dione (isatin) derived ligands (L^1)-(L^4) were prepared by reacting isatin with various diamines such as ethane-1,2-diamine, propane-1,3-diamine, butane-1,4-diamine, and benzene-1,2-diamine in an equimolar ratio to give 3-[(2-aminoethyl)imino]-5-chloro-1,3-dihydro-2H-indol-2-one (L^1), 3-[(3-aminopropyl)imino]-5-chloro-1,3-dihydro-2H-indol-2-one (L^2), 3-[(4-aminobutyl)imino]-5-chloro-1,3-dihydro-2H-indol-2-one (L^3) and 3-[(2-aminophenyl)imino]-5-chloro-1,3-dihydro-2H-indol-2-one (L^4). All ligands acted as tridentate possessing three active sites, isatin-O, azomethine-N, and amino-N for binding with the metal atoms. The structures of the isatin based Schiff bases were elucidated through their spectral (infrared, ultraviolet, nuclear magnetic resonance, and mass spectra), physical (melting point and solubility) and analytical (C, N, H %) data. The prepared ligands were reacted with Co(II), Ni(II), Cu(II), and Zn(II) transition metals in 1:2 molar ratio (metal:ligand) to form their complexes. IR, UV, NMR, conductance, magnetic moment, and elemental analysis was used to characterize the metal complexes. Metals based isatins were evaluated for their in-vitro antimicrobial properties against selected fungal and bacterial species. The anti-bacterial and anti-fungal results showed the metal chelates to be more biologically active than their parent uncomplexed ligands.

Keywords: Antibacterial and antifungal activity; isatin transition metal(II) complexes

ABSTRAK

Siri baru 5-kloroindolin-2,3-dione (isatin) ligan terbitan (L^1)-(L^4) dihasilkan melalui tindak balas isatin dengan pelbagai diamina seperti etana-1,2-diamin, propana-1,3-diamin, butana-1,4-diamin dan benzena-1,2-diamin dalam nisbah ekuimolar untuk membentuk 3-[(2-aminoetil) imino]-5-kloro-1,3-dihidro-2H-indol-2-one (L^1), 3-[(3-aminopropil) imino]-5-kloro-1,3-dihidro-2H-indol-2-one (L^2), 3-[(4-aminobutil) imino]-5-kloro-1,3-dihidro-2H-indol-2-one (L^3) dan 3-[(2-aminofenil)imino]-5-kloro-1,3-dihidro-2H-indol-2-one (L^4). Semua ligan bertindak sebagai tridentat yang mempunyai tiga tapak aktif, isatin-O, azometine-N dan amino-N dengan mengikat atom logam. Struktur isatin berasaskan bes Schiff dijelaskan melalui spektrumnya (inframerah, ultraungu, resonansi magnetik nuklear dan spektra jisim), fizikal (takat lebur dan kelarutan) dan data analisis (C, N, H%). Ligan tersebut juga bertindak balas dengan logam peralihan Co(II), Ni(II), Cu(II) dan Zn(II) dalam nisbah molar 1: 2 (logam: ligan) untuk membentuk kompleks mereka. IR, UV, NMR, konduktans, momen magnetik dan analisis unsur digunakan untuk mencirikan kompleks logam. Isatin berasaskan logam dinilai untuk sifat antimikrobnya secara in vitro terhadap beberapa spesies kulat dan bakteria terpilih. Hasil kajian anti-bakteria dan anti-kulat ini menunjukkan bahawa pengkelat logam adalah lebih aktif secara biologinya berbanding ligan induk yang bukan kompleks.

Kata kunci: Aktiviti antibakteria dan antikulat; kompleks logam(II) peralihan isatin

INTRODUCTION

The chemistry of 1H-indole-2,3-dione (isatin) and its derivatives have significant application in medicine (Bekircan & Bektas 2008). Isatin can also be used as raw material for synthesizing new drugs. Naturally, it is found in certain plants such as *Couroupita guianensis* and *Calanthe discolor* (Ganim et al. 2019). Isatin nucleus containing both lactam ring and ketone parts has attracted much attention (Arjunan et al. 2009) in

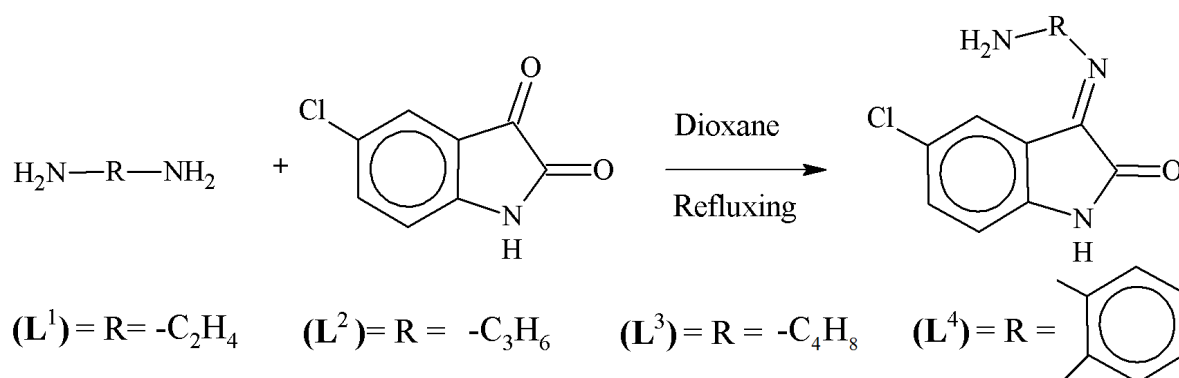
bioactive perspectives. The bio-active role of Schiff base derived isatins have been recognized long ago in medicinal activities against leprosy (Raghavendra et al. 2013), tuberculosis (Xu et al. 2017), bacterial (Chohan et al. 2009), viral (Da Silva et al. 2001) and fungal (Pandeya et al. 2005) infections. Isatin is integral component of many synthetic compounds used in medicine and displayed vast range of activities like cytotoxicity (Ghomathi et al. 2014), anti-microbial (Chohan et al. 2004), antiprotozoal (Kumar et al. 2015), antianxiety (Matesic et al. 2012) and

anthelmintic (Rao et al. 2014). Conventionally prepared isatin derived compounds are also considered good pharmacological agents such as anti-HIV (Pandeya et al. 2000), anticancer (Vine et al. 2009), enzyme inhibitors (Hyatt et al. 2007), antineoplastic (Beauchard et al. 2006), antiulcer (Aboul-Fadl & Bin-Jubair 2010), antileishmanial (Prakash & Raja 2011), and antifungal or antibacterial (Dileepan et al. 2018).

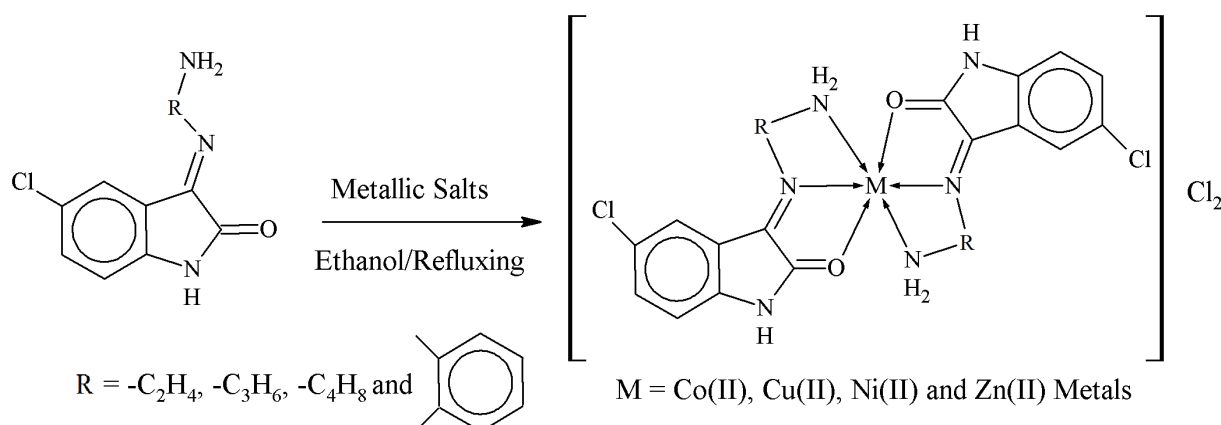
Metals play a key part in biological systems and their roles in subcellular and cellular functions are invariably recognized. The significance of metals is inevitable as they constitute essential part of enzymes which is involved in biochemical or metabolic processes. These are required in trace amounts for cellular uptakes, metabolic reactions and also act catalysts (Muralisankar et al. 2016). As 'privileged building blocks', practically all positions of isatin can be modified and C-5, C-3, and N-1 positions are the main domains for chemical variations. Wide spectrum of biological properties combined with a vast range of structural variations as well as effective applications in medical practice have inspired scientists to study isatin molecule and to create larger number of structurally diverse compounds (Guo 2019). There have

been many studies that involved various metal complexes of isatin derived Schiff base macrocycles. In view of their biological and pharmacological actions, isatin derived Schiff base macrocycles opened a new promising aspect in synthetic organic chemistry (Shakir et al. 2017).

Considering the potential bio-active nature (Moon et al. 2006) of isatins, their versatile chemistry (Zhen et al. 2015) and capability of smoothly crossing the blood-brain-barrier compelled us to further extend this chemistry and report four newly synthesized isatin ligands (L^1)-(L^4) by reacting isatin in an equimolar ratio with ethane-1,2-diamine, propane-1,3-diamine, butane-1,4-diamine and benzene-1,2-diamine, respectively (Scheme 1). The ligands were additionally reacted with Co(II), Ni(II), Cu(II) and Zn(II) transition metals in 1:2 molar ratio [metal:ligand] to synthesize their metal chelates (Scheme 2). The structural framework of these compounds was characterized by IR, UV, NMR, mass spectrometry, and elemental analysis. All prepared combinations were further evaluated for their *in vitro* anti-bacterial and anti-fungal bioassay against specific bacterial and fungal strains.



(Scheme 1)



(Scheme 2)

MATERIALS AND METHODS

The chemicals were purchased from Sigma Aldrich and used without purification. The reactions were performed in analytical grade solvents. Melting points were taken on Fischer Scientific apparatus. IR spectra were taken in Shimadzu Spectrophotometer using KBr disc. Elemental analysis of compounds was performed on Perkin Elmer made by USA. NMR spectra were taken in a Bruker Spectrospin Avance DPX-400 spectrometer in which DMSO-d₆ was utilized as solvent and tetramethylsilane as internal standard. For mass spectra, JEOL mass spectrometer was utilized. Ultraviolet-Visible Spectra were recorded on UVD-2950 LAMBOED double beam spectrophotometer in the frequency range of 250-800 nm. Orion 160 conductivity meter was used for molar conductivity of the metal complexes having 0.001 M solution in dimethylformamide (DMF). Stanton SM12/S Gouy balance was used to measure the magnetic moments of the synthesized metal complexes. *In vitro* antimicrobial bioassay was performed at HEJ, Research Institute, University of Karachi, Pakistan.

SYNTHETIC CHEMISTRY OF LIGANDS

The isatin based ligand, 3-[(2-aminoethyl)imino]-5-chloro-1,3-dihydro-2*H*-indol-2-one (L¹) was prepared by using a reported method (Chohan et al. 2009) in which

20 mL dioxane solution of 5-chloroindoline-2,3-dione (20 mmol) was transferred to a refluxed solution of the ethane-1,2-diamine (20 mmol), in the same solvent in an equimolar ratio followed by 2 drops of acetic acid. The completion of reaction was monitored via TLC. The obtained product was then filtered out, washed with dioxane and then dried. The product was further recrystallized in a mixture of hot dioxane:ethanol (1:1). The same method was used for the synthesis of other ligands (L²)-(L⁴). Spectral data of IR, ¹HNMR, ¹³CNMR and elemental analysis of ligands is depicted in Tables 1-3.

SYNTHETIC CHEMISTRY OF TRANSITION METAL(II) COMPLEXES

Transition metal complexes were synthesized by following standard procedure (Chohan et al. 2009), an ethanolic solution (10 mL) of the relevant metallic salt (5 mmol) was transferred into a refluxed ethanolic solution (20 mL) of the isatin ligand (10 mmol). During refluxing, a precipitated product was formed within 3 h. The obtained product was dried in an oven and further recrystallized by using a combination of hot ethanol:methanol (1:1) to get pure compound. All the metal complexes were synthesized on the same pattern and their IR, Zn-HNMR, Zn-CNMR and elemental data is reported in Tables 1-3.

TABLE 1. Physico-chemical determination and elemental data of the ligands and metal chelates

Compounds	% age Yield	Molecular weight/ formula	Dec. Points (°C)	Calculated (Present)			
				C	H	N	M
(L ¹)	79	[223.66] C ₁₀ H ₁₀ ClN ₃ O	140	53.70 (53.61)	4.51 (4.45)	18.79 (18.74)	-
(L ²)	76	[237.69] C ₁₁ H ₁₂ ClN ₃ O	210	55.59 (55.50)	5.05 (5.01)	17.68 (17.63)	-
(L ³)	71	[251.71] C ₁₂ H ₁₄ ClN ₃ O	150	57.26 (57.20)	5.61 (5.56)	16.69 (16.63)	-
(L ⁴)	65	[271.71] C ₁₄ H ₁₀ ClN ₃ O	260	61.87 (61.82)	3.68 (3.66)	15.46 (15.41)	-
[Co(L ¹) ₂]Cl ₂	76	[577.16] C ₂₀ H ₂₀ Cl ₄ N ₆ O ₂ Co	290-292	41.62 (41.55)	3.49 (3.44)	14.56 (14.51)	10.21 (10.16)
[Ni(L ¹) ₂]Cl ₂	71	[576.92] C ₂₀ H ₂₀ Cl ₄ N ₆ O ₂ Ni	295-297	41.64 (41.59)	3.49 (3.45)	14.57 (14.52)	10.17 (10.13)
[Cu(L ¹) ₂]Cl ₂	72	[581.77] C ₂₀ H ₂₀ Cl ₄ N ₆ O ₂ Cu	302-304	41.29 (41.22)	3.77 (3.74)	14.45 (14.40)	10.92 (10.87)

[Zn(L ¹) ₂]Cl ₂	73	[583.61] C ₂₀ H ₂₀ Cl ₄ N ₆ O ₂ Zn	305-307	41.16 (41.16)	3.45 (3.41)	14.40 (14.34)	11.20 (11.15)
[Co(L ²) ₂]Cl ₂	70	[605.21] C ₂₂ H ₂₄ Cl ₄ N ₆ O ₂ Co	293-295	43.46 (43.39)	4.00 (3.95)	13.89 (13.84)	9.74 (9.70)
[Ni(L ²) ₂]Cl ₂	68	[604.97] C ₂₂ H ₂₄ Cl ₄ N ₆ O ₂ Ni	316-318	43.68 (43.32)	4.00 (3.94)	13.90 (13.85)	9.70 (9.66)
[Cu(L ²) ₂]Cl ₂	71	[609.82] C ₂₂ H ₂₄ Cl ₄ N ₆ O ₂ Cu	310-312	43.33 (43.27)	3.97 (3.93)	13.78 (13.74)	10.42 (10.38)
[Zn(L ²) ₂]Cl ₂	72	[611.67] C ₂₂ H ₂₄ Cl ₄ N ₆ O ₂ Zn	300-302	43.20 (43.12)	3.95 (3.91)	13.74 (13.70)	10.69 (10.65)
[Co(L ³) ₂]Cl ₂	75	[633.26] C ₂₄ H ₂₈ Cl ₄ N ₆ O ₂ Co	299-301	45.52 (45.45)	4.46 (4.42)	13.27 (13.23)	9.31 (9.25)
[Ni(L ³) ₂]Cl ₂	78	[633.02] C ₂₄ H ₂₈ Cl ₄ N ₆ O ₂ Ni	292-294	45.54 (45.49)	4.46 (4.43)	13.28 (13.23)	9.27 (9.24)
[Cu(L ³) ₂]Cl ₂	67	[637.88] C ₂₄ H ₂₈ Cl ₄ N ₆ O ₂ Cu	309-312	45.19 (45.14)	4.42 (4.39)	13.18 (13.13)	9.96 (9.92)
[Zn(L ³) ₂]Cl ₂	70	[639.72] C ₂₄ H ₂₈ Cl ₄ N ₆ O ₂ Zn	305-307	45.06 (45.00)	4.41 (4.37)	13.14 (13.10)	10.22 (10.17)
[Co(L ⁴) ₂]Cl ₂	71	[673.24] C ₂₈ H ₂₀ Cl ₄ N ₆ O ₂ Co	315-317	49.95 (49.90)	2.99 (2.95)	12.48 (12.42)	8.75 (8.70)
[Ni(L ⁴) ₂]Cl ₂	72	[673.00] C ₂₈ H ₂₀ Cl ₄ N ₆ O ₂ Ni	296-298	49.97 (49.40)	3.00 (2.96)	12.49 (12.44)	8.72 (8.67)
[Cu(L ⁴) ₂]Cl ₂	69	[677.86] C ₂₈ H ₂₀ Cl ₄ N ₆ O ₂ Cu	310-312	49.61 (49.55)	2.97 (2.93)	12.40 (12.36)	9.37 (9.33)
[Zn(L ⁴) ₂]Cl ₂	76	[679.70] C ₂₈ H ₂₀ Cl ₄ N ₆ O ₂ Zn	304-306	49.48 (49.40)	2.97 (2.94)	12.36 (12.32)	9.62 (9.57)

TABLE 2. Conductance, magnetic moments and IR data of synthesized compounds

Compounds	Ω_M ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	BM μ_{eff}	λ_m (cm^{-1})	IR (cm^{-1})
(L ¹)	-	-	-	3300 (NH ₂), 3190 (N-H), 1732 (C=O), 1655 (C=N), 1616 (C=C), 1116 (C-N), 673 (C-Cl)
(L ²)	-	-	-	3305 (NH ₂), 3192 (NH), 1735 (C=O), 1656 (C=N), 1563 (C=C), 1120 (C-N), 673 (C-Cl)
(L ³)	-	-	-	3303 (NH ₂), 3195 (NH), 1738 (C=O), 1656 (C=N), 1616 (C=C), 1131 (C-N), 673 (C-Cl)

(L ⁴)	-	-	-	3361 (NH ₂), 3203 (NH), 1739 (C=O), 1646 (C=N), 1525 (C=C), 1194 (C-N), 670 (C-Cl)
[Co(L ¹) ₂]Cl ₂	89.4	4.05	7323, 17450, 20461, 29312	3286 (NH ₂), 1720 (C=O, isatin), 1641 (C=N, azomethine), 528 (M-N), 434 (M-O)
[Ni(L ¹) ₂]Cl ₂	89.2	3.16	10372, 15675, 26470, 29881	3285 (NH ₂), 1722 (C=O, isatin), 1640 (C=N, azomethine), 525 (M-N), 434 (M-O)
[Cu(L ¹) ₂]Cl ₂	87.9	1.76	14964, 19200, 30335	3288 (NH ₂), 1720 (C=O, isatin), 1643 (C=N, azomethine), 526 (M-N), 434 (M-O)
[Zn(L ¹) ₂]Cl ₂	88.2	Dia	29911	3290 (NH ₂), 1721 (C=O, isatin), 1642 (C=N, azomethine), 528 (M-N), 433 (M-O)
[Co(L ²) ₂]Cl ₂	86.5	4.13	7400, 17500, 20590, 29353	3292 (NH ₂), 1726 (C=O, isatin), 1647 (C=N, azomethine), 538 (M-N), 445 (M-O)
[Ni(L ²) ₂]Cl ₂	89.2	3.11	10431, 15633, 26431, 29864	3296 (NH ₂), 1725 (C=O, isatin), 1644 (C=N, azomethine), 539 (M-N), 443 (M-O)
[Cu(L ²) ₂]Cl ₂	91.3	1.75	15138, 19150, 30367	3294 (NH ₂), 1723 (C=O, isatin), 1645 (C=N, azomethine), 536 (M-N), 444 (M-O)
[Zn(L ²) ₂]Cl ₂	90.9	Dia	29864	3295 (NH ₂), 1726 (C=O, isatin), 1643 (C=N, azomethine), 535 (M-N), 443 (M-O)
[Co(L ³) ₂]Cl ₂	91.6	4.08	7280, 17470, 20527, 29300	3290 (NH ₂), 1725 (C=O, isatin), 1645 (C=N, azomethine), 536 (M-N), 442 (M-O)
[Ni(L ³) ₂]Cl ₂	92.8	3.18	10397, 15790, 26521, 29901	3294 (NH ₂), 1722 (C=O, isatin), 1642 (C=N, azomethine), 536 (M-N), 440 (M-O)
[Cu(L ³) ₂]Cl ₂	90.2	1.74	15081, 19172, 30355	3293 (NH ₂), 1721 (C=O, isatin), 1644 (C=N, azomethine), 535 (M-N), 442 (M-O)
[Zn(L ³) ₂]Cl ₂	90.1	Dia	29841	3294 (NH ₂), 1723 (C=O, isatin), 1640 (C=N, azomethine), 536 (M-N), 440 (M-O)
[Co(L ⁴) ₂]Cl ₂	89.1	4.05	7271, 17425, 20626, 29328	3347 (NH ₂), 1724 (C=O, isatin), 1631 (C=N, azomethine), 530 (M-N), 439 (M-O)
[Ni(L ⁴) ₂]Cl ₂	91.5	3.21	10414, 15769, 26501, 29935	3350 (NH ₂), 1725 (C=O, isatin), 1634 (C=N, azomethine), 532 (M-N), 437 (M-O)
[Cu(L ⁴) ₂]Cl ₂	89.3	1.78	15029, 19185, 30348	3352 (NH ₂), 1726 (C=O, isatin), 1634 (C=N, azomethine), 533 (M-N), 438 (M-O)
[Zn(L ⁴) ₂]Cl ₂	88.3	Dia	29832	3350 (NH ₂), 1725 (C=O, isatin), 1633 (C=N, azomethine), 535 (M-N), 439 (M-O)

TABLE 3. ¹HNMR and ¹³CNMR (δ, ppm) spectral data of ligands and Zn(II) complexes

Compounds	¹ HNMR (δ, ppm)	¹³ CNMR (δ, ppm)
(L ¹)	3.09 (t, 2-H), 3.75 (t, 2-H), 5.89 (s, NH ₂), 7.09 (d, 1-H, <i>J</i> = 8.6 Hz), 7.44 (dd, 1-H, <i>J</i> = 8.6, 2.5 Hz), 7.65 (d, 1-H, <i>J</i> = 2.5 Hz), 11.97 (s, N-H)	44.5, 57.2, 115.9, 123.2, 126.2, 129.5, 132.9, 135.7, 163.1, 166.2
(L ²)	2.59 (m, 2-H), 3.04 (t, 2-H), 3.72 (t, 2-H), 5.85 (s, NH ₂), 7.05 (d, 1-H, <i>J</i> = 8.7 Hz), 7.41 (dd, 1-H, <i>J</i> = 8.7, 2.4 Hz), 7.63 (d, 1-H, <i>J</i> = 2.4 Hz), 11.95 (s, NH)	44.6, 56.9, 115.5, 123.1, 125.8, 129.9, 133.3, 135.5, 163.4, 166.5
(L ³)	2.49 (m, 2-H), 2.64 (m, 2-H), 3.05 (t, 2-H), 3.73 (t, 2-H), 5.85 (s, NH ₂), 7.07 (d, 1-H, <i>J</i> = 8.6 Hz), 7.41 (dd, 1-H, <i>J</i> = 8.6, 2.6 Hz), 7.62 (d, 1-H, <i>J</i> = 2.6 Hz), 11.92 (s, NH)	32.9, 34.5, 44.4, 57.0, 115.6, 123.1, 126.4, 129.7, 132.7, 135.4, 163.6, 166.7
(L ⁴)	5.97 (s, NH ₂), 6.55-7.28 (m, 4-H, phenyl), 7.21 (d, 1-H, <i>J</i> = 8.5 Hz), 7.63 (dd, 1-H, <i>J</i> = 8.5, 2.4 Hz), 7.79 (d, 1-H, <i>J</i> = 2.4 Hz), 12.05 (s, N-H)	115.9, 119.3, 122.6, 123.2, 125.3, 126.2, 129.5, 130.8, 132.9, 135.7, 143.7, 146.3, 163.1, 166.2
[Zn(L ¹) ₂] Cl ₂	3.16 (t, 2-H), 3.83 (t, 2-H), 6.04 (s, NH ₂), 7.14 (d, 1-H, <i>J</i> = 8.6 Hz), 7.51 (dd, 1-H, <i>J</i> = 8.6, 2.5 Hz), 7.72 (d, 1-H, <i>J</i> = 2.5 Hz), 12.10 (s, N-H)	45.2, 58.0, 116.7, 123.9, 126.8, 130.0, 133.6, 136.4, 164.2, 167.2
[Zn(L ²) ₂] Cl ₂	2.67 (m, 2-H), 3.12 (t, 2-H), 3.80 (t, 2-H), 5.97 (s, NH ₂), 7.10 (d, 1-H, <i>J</i> = 8.7 Hz), 7.48 (dd, 1-H, <i>J</i> = 8.7, 2.4 Hz), 7.70 (d, 1-H, <i>J</i> = 2.4 Hz), 12.05 (s, N-H)	45.6, 57.8, 116.1, 123.8, 126.4, 130.4, 133.9, 135.1, 164.4, 167.6
[Zn(L ³) ₂] Cl ₂	2.71 (m, 2-H), 2.57 (m, 2-H), 3.13 (t, 2-H), 3.81 (t, 2-H), 5.98 (s, NH ₂), 7.13 (d, 1-H, <i>J</i> = 8.6 Hz), 7.47 (dd, 1-H, <i>J</i> = 8.6, 2.6 Hz), 7.67 (d, 1-H, <i>J</i> = 2.6 Hz), 12.01 (s, N-H)	33.9, 35.3, 45.0, 57.9, 116.4, 123.7, 126.9, 130.5, 133.3, 136.0, 164.5, 167.7
[Zn(L ⁴) ₂] Cl ₂	6.05 (s, NH ₂), 6.64-7.38 (m, 4-H, phenyl), 7.25 (d, 1-H, <i>J</i> = 8.5 Hz), 7.69 (dd, 1-H, <i>J</i> = 8.5, 2.4 Hz), 7.85 (d, 1-H, <i>J</i> = 2.4 Hz), 12.14 (s, NH)	116.5, 119.8, 123.3, 123.9, 125.8, 126.9, 130.0, 131.5, 133.6, 136.5, 144.4, 147.0, 164.2, 167.3

BIOLOGICAL ACTIVITY

In vitro antibacterial, antifungal, and minimum inhibitory bioactivity testing procedures have previously been reported (Yasmeen et al. 2017).

RESULTS AND DISCUSSION

Isatin based four Schiff bases (L¹)-(L⁴) (Scheme 1) were produced from the condensation reaction of 5-chloroindoline-2,3-dione with respective amines such as ethane-1,2-diamine, propane-1,3-diamine, butane-1,4-diamine, and benzene-1,2-diamine in an equimolar ratio, respectively. These isatin based ligands were air and moisture stable colored microcrystalline solids which melted in the range 140-260 °C. They were soluble in ethanol and methanol on heating. These tridentate ligands were reacted with Co(II), Ni(II), Cu(II), and Zn(II)

metals in ethanol to synthesize metal(II) complexes (Scheme 2) which were produced in good yields. All metal complexes decomposed in the range 290-318 °C (Table 1).

The metal complexes were of different colors excluding Zn complexes which showed off-white color. All complexes were microcrystalline in nature, decomposed without melting, soluble in DMSO and DMF but insoluble in some common organic solvents.

IR SPECTRA

Distinguishing IR frequencies of isatin based ligands along with metal complexes depicted in experimental unit (Table 2). Originally the reactant, 5-chloroindoline-2,3-dione (5-chloro-isatin) in its IR spectrum showed three bands (Amjad et al. 2016) at 1755, 1740, and

3190 cm^{-1} due to two carbonyls and NH group. Whereas, all diamines exhibited bands at 3305 and 3315 cm^{-1} corresponding to two NH_2 groups. All ligands (L^1)-(L^4) displayed disappearance of band at 1740 cm^{-1} assigned to one carbonyl and appearance of a new band at 1646-1656 cm^{-1} owing to azomethine ($-\text{C}=\text{N}$) linkage (Hanif & Chohan 2013). However, bands at 1755 cm^{-1} assigned to $-\text{C}=\text{O}$ and persisted unaffected providing a clue for condensation of carbonyl group of isatin with amino group of diamine. All ligands showed bands at 3190-3203, 1116-1194, and 670-673 cm^{-1} , respectively, as a result of $\nu(\text{NH})$, $\nu(\text{C}-\text{N})$, and $\nu(\text{C}-\text{Cl})$ vibrations. Comparison of IR spectral data of isatin ligands with metal(II) complexes (1)-(16) showed that ligands were tridentately coordinated to the metal atoms through azomethine-N and carbonyl-O. IR spectra of metal(II) complexes further showed $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{O})$ vibrations at 1646-1656 and 1755 cm^{-1} to move to lower frequency (10-17 cm^{-1}) at 1631-1640 and 1740 cm^{-1} , respectively, representing coordination (Bagihalli et al. 2009) of these groups with the metal(II) ions. Two new bands also appeared at 434-445 and 525-538 cm^{-1} in the spectra of metal complexes assigned to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$ vibrations. Appearance of two new bands and shifting of other bands in the metal complexes confirmed the coordination/chelation (Nakamoto 2009) of metal atoms with isatin ligands through isatin-O and azomethine-N (Nyquist 2001).

^1H NMR SPECTRA

^1H NMR spectral data of isatin ligands along with Zn complexes in $\text{DMSO}-d_6$ is listed in Table 3. Amino (NH_2) and (NH) protons of all the ligands demonstrated (Freeman 1997) distinctive singlet at 5.85-5.97 ppm and 11.92-12.05 ppm. The phenyl protons of isatin ring were found at 6.55-7.63 ppm as multiplet. The ethylene, propylene and butylene protons were found at 2.49-3.65 ppm as triplet and multiplet, respectively. Azomethine-N proton showed a sharp singlet at 6.75 ppm. Upon coordination with the Zn metal atom, all protons moved to downfield by 0.5-1.0 ppm, due to drifting of electron cloud and conjugation.

^{13}C NMR SPECTRA

^{13}C NMR spectral data of the isatin ligands and its Zn(II) complexes were depicted in Table 3. Spectra of ligands displayed well-distinguished azomethine and carbonyl carbons at 163.1-163.6 and 166.2-166.7 ppm, respectively. The ethylene, propylene and butylene carbons were found at 32.9-57.2 ppm in their expected region. The remaining other carbons of aromatic rings were observed at 115.5-163.6 ppm. Downfield shifting of the azomethine and carbonyl carbons were found in the spectra of Zn complexes because of drifting of electron density to Zn atom. In the same way, other carbons of

methylene and aromatic phenyl rings being adjacent to the coordination positions also indicated downfield drift by 0.5-1.1 ppm.

MASS SPECTRA

The molecular masses of isatin ligands (L^1)-(L^4), were found at m/z 223 (calcd. 223.66) of fragment $[\text{C}_{10}\text{H}_{10}\text{ClN}_3\text{O}]^+$, m/z 237 (237.69) of fragment $[\text{C}_{11}\text{H}_{12}\text{ClN}_3\text{O}]^+$, m/z 251 (251.71) of fragment $[\text{C}_{12}\text{H}_{14}\text{ClN}_3\text{O}]^+$, and m/z 271 (271.71) for fragment $[\text{C}_{14}\text{H}_{10}\text{ClN}_3\text{O}]^+$, correspondingly. The most stable fragment $[\text{C}_{10}\text{H}_{10}\text{N}_3\text{O}]^+$ of (L^1) appeared at m/z 188 which was considered as base peak. On the other hand, ligand (L^2) showed this stable peak at m/z 202 of fragment $[\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}]^+$ and (L^3) at m/z 216 of fragment $[\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}]^+$. Likewise, ligand (L^4) displayed base peak of fragment $[\text{C}_{14}\text{H}_{10}\text{N}_3\text{O}]^+$ at m/z 236. Mass fragmentation arrangement of isatin based ligands (L^1)-(L^4) experienced the breaking of $\text{C}=\text{C}$, $\text{C}-\text{N}$, $\text{C}=\text{O}$, $\text{C}-\text{Cl}$ and $\text{C}=\text{N}$ (exocyclic) bonds. Appearances of distinct molecular ion peaks of ligands strongly confirmed the formation of the isatin Schiff bases.

MEASUREMENT OF MOLAR CONDUCTANCES AND MAGNETIC MOMENTS

Due to solubility of metal complexes in DMF, measurement of molar conductance was done in it. The molar conductance results (86.5-92.8 $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$) representing the conductors (Geary 1971) nature of the complexes. Magnetic moments of Co compounds found in 4.05 to 4.13 BM ranges (Table 2), therefore, showed high spin octahedral complex (Balhausen 1962). Similarly, an octahedral geometry (Balhausen 1962) was experienced by Ni(II) complexes due to two unpaired electrons which were justified by their magnetic moment 3.11-3.21 BM results. Also, Cu complexes showing octahedral (Lever 1984) geometry as measured magnetic moments 1.74-1.78 BM are indicative of one unpaired electron. The Zn complexes did not respond to the instrument because of their diamagnetic (Nair & Joseyphus 2008) nature.

ELECTRONIC SPECTRA

The electronic spectra of the Co(II) complexes generally displayed three transitions in the regions 7271-7400, 17425-17500, and 20461-20626 cm^{-1} due to intra-ligand transitions $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$, $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{A}_{2g}(\text{F})$ and $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{P})$ in an octahedral environment. A high intensity band was also observed at 29300-29353 cm^{-1} due to metal \rightarrow ligand charge transfer (Chohan et al. 2009). The electronic spectral data of the Ni(II) complexes showed (Chohan et al. 2006) bands in the region at 10372-10431, 15675-15769, and 26431-26521 cm^{-1} which were assigned to transitions, $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{F})$, $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F})$ and $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{P})$, respectively,

suggesting their octahedral geometry (Chohan et al. 2009). Also, a strong band due to metal to ligand charge transfer appeared at 29864-29935 cm^{-1} . The spectra of Cu(II) complexes demonstrated two weak bands at 14964-15138, 19150-19200 cm^{-1} which may be assigned to the transitions ${}^2B_{1g} \rightarrow {}^2A_{1g}$ and ${}^2B_{1g} \rightarrow {}^2E_g$, respectively, suggesting their octahedral geometry (Abdul-Ghani & Khaleel 2009; Sumrra et al. 2018). A high intensity band was observed at 30335-30367 cm^{-1} which was assigned to the metal \rightarrow ligand charge transfer. The Zn(II) complexes as diamagnetic only observed d-d transitions and a strong band of high intensity at 29832-29911 cm^{-1} due to metal \rightarrow ligand charge transfer (Sumrra et al. 2018).

ANTIBACTERIAL SCREENING (IN-VITRO)

Isatin based ligands (L^1)-(L^4) and their metallic chelates (1)-(16) were assessed for anti-bacterial activity against following bacterial strains; *Pseudomonas aeruginosa* = A, *Shigella flexneri* = B, *Bacillus subtilis* = C, *Escherichia coli* = D, *Staphylococcus aureus* = E, and *Salmonella typhi* = F by using standard procedure. The anti-bacterial results were matched with imipenem (standard drug) and stated in Table 4. Ligand (L^1) presented overall moderate (10-13 mm) activity against selected bacterial strains. Similarly, ligand (L^2) presented moderate (10-13 mm) activity against all bacterial species excluding *Escherichia coli* which showed weaker (09 mm) activity. On the other hand, the ligand (L^3) displayed significant (15 mm) activity against *Bacillus subtilis*, moderate (10-13 mm) activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella flexneri*, and weaker (09 mm) activity against *Salmonella typhi*, and *Staphylococcus aureus* strains. Moreover, ligand (L^4) experienced moderate (10-14 mm) activity against *Escherichia coli*, *Shigella flexneri*, and *Salmonella typhi*, and weaker (08-09 mm) activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* selected bacterial types. The metal complex (1), (3), (4), (7), (8), (11) and (13) demonstrated overall significant (14-22 mm) activity against all bacterial strains.

However, complex (2) displayed significant (15-20 mm) activity against all strains except *Staphylococcus aureus*. The metal complexes (5) and (6) exhibited moderate (10-14 mm) activity in opposition to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* and significant (15-23 mm) activity against *Shigella flexneri*, *Salmonella typhi*, and *Bacillus subtilis*. The compounds (9) and (10) showed significant (15-20 mm) activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Shigella flexneri* and moderate (10-12 mm) activity against *Escherichia coli* and *Salmonella typhi*. Also, the complex (12) displayed moderate (11-14 mm) bio-activity against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* and significant (19-21 mm) activity against *Shigella flexneri*, *Salmonella typhi*, and *Bacillus subtilis*. However, the complexes (14) and (15) displayed significant (15-20 mm) activity against *Escherichia coli*, *Salmonella typhi*, and *Bacillus subtilis* and moderate (10-13 mm) activity against *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Moreover, the complex (16) displayed significant (19-20 mm) activity against *Escherichia coli* and *Salmonella typhi*, and moderate (10-14 mm) activity against all other strains. It was overall concluded that metal complexes showed better active behavior against one and/or more bacterial strains which in turn, support our previous results (Yasmeen et al. 2017) that upon metal chelation no or minor bioactive compounds converted to more bioactive. Due to chelation, overlapping of ligands occurs that decreases the polarity of metallic ions and increases the delocalization of π -electrons which consequently increases the lipophilic behavior of the metal complexes thus increasing their penetration ability into the cells resulting their destruction. These results (Table 4, Figure 1) clearly demonstrated that inhibitory action of the metal complexes was more prominent with respect to the simple ligands. Moreover, the Zn complex (4) of (L^1) was considered to be the most bioactive compound.

TABLE 4. Antibacterial studies of ligands (L^1)-(L^4) and metal(II) chelates

Compounds	Inhibition zones (mm)							Average
	A	B	C	D	E	F	SA	
(L^1)	10	10	11	13	10	11	1.17	10.83
(L^2)	09	13	10	11	10	12	1.47	10.83
(L^3)	10	12	13	09	09	15	2.42	11.33
(L^4)	11	10	09	14	08	09	2.14	10.16
$[\text{Co}(\text{L}^1)_2]\text{Cl}_2$	17	15	18	17	15	19	1.60	16.83

[Ni(L ¹) ₂]Cl ₂	20	15	19	20	13	18	2.88	17.50
[Cu(L ¹) ₂]Cl ₂	20	15	17	18	16	20	2.07	17.67
[Zn(L ¹) ₂]Cl ₂	18	16	18	20	15	21	2.28	18.00
[Co(L ²) ₂]Cl ₂	10	17	11	19	12	23	5.16	15.33
[Ni(L ²) ₂]Cl ₂	10	19	14	20	10	19	4.63	15.33
[Cu(L ²) ₂]Cl ₂	15	21	16	18	16	18	2.16	17.33
[Zn(L ²) ₂]Cl ₂	16	20	15	17	15	22	2.88	17.50
[Co(L ³) ₂]Cl ₂	10	20	18	10	16	19	4.46	15.50
[Ni(L ³) ₂]Cl ₂	12	17	18	10	15	20	3.77	15.50
[Cu(L ³) ₂]Cl ₂	16	19	17	16	15	22	2.59	17.50
[Zn(L ³) ₂]Cl ₂	14	20	19	11	12	21	4.36	16.16
[Co(L ⁴) ₂]Cl ₂	17	16	15	19	15	15	1.60	16.16
[Ni(L ⁴) ₂]Cl ₂	20	10	10	20	11	16	4.81	16.16
[Cu(L ⁴) ₂]Cl ₂	19	13	14	17	14	15	2.25	15.33
[Zn(L ⁴) ₂]Cl ₂	20	14	12	19	10	11	4.23	14.33
SD	24	23	22	23	20	25	1.72	22.83

Pseudomonas aeruginosa = A, *Shigella flexneri* = B, *Bacillus subtilis* = C, *Escherichia coli* = D, *Staphylococcus aureus* = E, and *Salmonella typhi* = F, SA = Statistical analysis, SD = Imipenem; standard drug

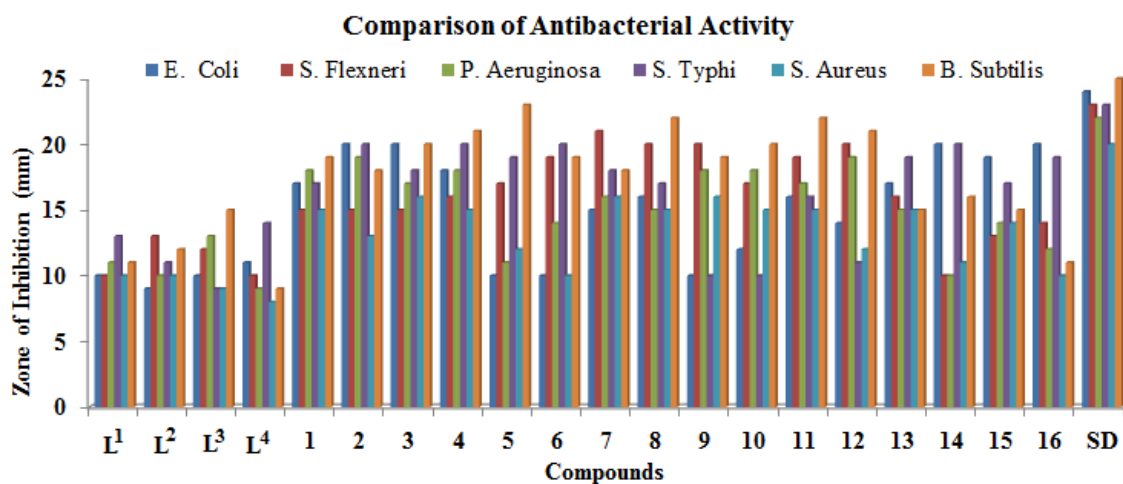


FIGURE 1. Comparative results of anti-bacterial activity of isatin ligands vs metal(II) Chelates

ANTIFUNGAL SCREENING (IN-VITRO)

The antifungal bioassay of all the synthesized isatin based compounds was performed against *Trichophyton longifusus* = G, *Aspergillus flavus* = H, *Candida albicans* = I, *Candida glabrata* = J, *Microsporium canis* = K, and *Fusarium solani* = L, fungal species (Table 5). The results of fungal inhibition were matched (Figure 2) with standard drugs (miconazole and amphotericin B). Ligand (L¹) displayed moderate (35-45%) activity against *Trichophyton longifusus*, *Aspergillus flavus*, and *Candida glabrata*, weaker (25%) activity against *Microsporium canis* and was inactive against remaining two strains *Candida albicans* and *Fusarium solani*. Likewise, the ligand (L²) showed significant (57%) activity against *Trichophyton longifusus*, moderate (36-46%) activity against *Candida glabrata*, *Candida albicans*, and *Fusarium solani* weaker (26%) activity against *Microsporium canis* but inactive against *Aspergillus flavus*. Instead, (L³) showed significant (55-60%) bioactivity against *Candida albicans* and *Candida glabrata*, moderate (36-41%) activity against *Aspergillus flavus* and *Microsporium canis* but inactive against *Trichophyton longifusus* and *Fusarium solani*. Also, (L⁴) exhibited significant (55%) activity against *Trichophyton longifusus*, moderate (39-51%) activity against *Aspergillus flavus*, *Fusarium solani*, and *Candida glabrata* but no activity was shown against *Candida albicans* and *Microsporium canis*. Conversely, the metal complexes (1) and (2) possessed significant (61-63%) bioactivity against *Trichophyton longifusus*, *Microsporium canis*, *Aspergillus flavus*, and *Candida glabrata*, moderate (35-43%) activity against *Candida albicans* and weaker (25-32%) activity against *Fusarium solani*. Similarly, the metal complexes (3) and (4) exhibited significant (56-74%) activity against *Trichophyton longifusus*, *Aspergillus flavus*, and *Candida*

glabrata fungal strains and moderate (35-48%) activity against *Candida albicans*, and *Microsporium canis*. The metal complexes (5)-(8) possessed overall significant (55-75%) activity against all fungal types. Following complexes (9)-(12) presented significant (55-76%) bioactivity against *Candida albicans*, *Candida glabrata*, *Aspergillus flavus*, and *Microsporium canis* fungal species and moderate (35-46%) activity against *Trichophyton longifusus* and *Fusarium solani* except metal complex (9) which observed weaker (22%) activity against *Fusarium solani*. However, the metal complexes (13)-(16) experienced significant (55-77%) activity against *Aspergillus flavus*, *Trichophyton longifusus*, *Candida glabrata*, and *Fusarium solani* strains and moderate (35-49%) activity against *Candida albicans*, and *Microsporium canis* except metal complex (15) which observed weaker (25-28%) activity against *Microsporium canis*, and *Candida albicans*. The Co complex (14) of (L⁴) displayed highest bioactivity among others. The data reported in Table 5 and Figure 2 clearly indicated the enhanced (Amjad et al. 2016) bioactivity of complexes rather than their non-complexed ligands.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The synthesized isatin ligands along with their metal chelates exhibiting potential antibacterial (>80%) activity was picked for MIC readings and achieved results listed in Table 6. The MIC findings of all the metal complexes except complex Cu-(L⁴)₂ showed to display bioactivity against one or more than bacterial types. The MIC results were found in the range 11.66 to 48.91 µg mL⁻¹. The complex Ni-(L¹)₂ was supposed to be the highest bioactive showing the maximum inhibition of 11.66 µg mL⁻¹ against *E. Coli*.

TABLE 5. Antifungal studies of isatin ligands (L¹)-(L⁴) and metal(II) chelates

Compounds	Inhibition in percentage (%)							Average
	G	H	I	J	K	L	SA	
(L ¹)	35	00	43	25	00	45	20.36	24.66
(L ²)	57	46	00	26	48	36	20.39	35.50
(L ³)	00	55	41	36	00	60	26.29	32.00
(L ⁴)	55	00	45	00	39	51	25.12	31.67
[Co(L ¹) ₂]Cl ₂	68	32	70	58	35	61	16.50	54.00
[Ni(L ¹) ₂]Cl ₂	55	25	75	64	43	73	19.20	55.88
[Cu(L ¹) ₂]Cl ₂	63	45	60	48	35	74	14.11	54.16
[Zn(L ¹) ₂]Cl ₂	70	39	56	42	38	59	12.99	50.66
[Co(L ²) ₂]Cl ₂	73	58	62	56	75	56	08.57	63.33

[Ni(L ²) ₂]Cl ₂	69	69	59	62	69	61	04.67	64.83
[Cu(L ²) ₂]Cl ₂	59	73	60	60	66	55	06.37	62.50
[Zn(L ²) ₂]Cl ₂	61	64	67	55	58	59	04.32	60.66
[Co(L ³) ₂]Cl ₂	46	73	60	67	22	76	20.35	57.33
[Ni(L ³) ₂]Cl ₂	35	61	55	51	30	70	15.31	50.33
[Cu(L ³) ₂]Cl ₂	39	64	60	56	35	75	15.25	54.83
[Zn(L ³) ₂]Cl ₂	40	75	58	64	44	67	13.61	58.00
[Co(L ⁴) ₂]Cl ₂	74	35	60	41	62	74	16.43	67.66
[Ni(L ⁴) ₂]Cl ₂	68	45	67	36	60	77	15.46	58.33
[Cu(L ⁴) ₂]Cl ₂	71	28	58	25	57	75	21.24	52.33
[Zn(L ⁴) ₂]Cl ₂	67	48	62	37	55	65	11.52	55.66

Trichophyton longifusus = G, *Aspergillus flavus* = H, *Candida albicans* = I, *Candida glabrata* = J, *Microsporum canis* = K, and *Fusarium solani* = L, SA = Statistical analysis, standard drugs = miconazole and amphotericin B

TABLE 6. Minimum inhibition concentration of metal complexes

Chelates	A	B	C	D	E	F
[Co(L ¹) ₂]Cl ₂	-	-	36.17	-	-	-
[Ni(L ¹) ₂]Cl ₂	11.66	-	19.82	43.19	-	-
[Cu(L ¹) ₂]Cl ₂	46.75	-	-	-	33.32	26.51
[Zn(L ¹) ₂]Cl ₂	-	-	23.98	26.12	-	48.91
[Co(L ²) ₂]Cl ₂	-	-	-	29.05	-	16.15
[Ni(L ²) ₂]Cl ₂	-	28.47	-	12.51	-	-
[Cu(L ²) ₂]Cl ₂	-	19.16	-	-	36.31	-
[Zn(L ²) ₂]Cl ₂	-	41.60	-	-	-	38.37
[Co(L ³) ₂]Cl ₂	-	27.19	35.71	-	43.77	-
[Ni(L ³) ₂]Cl ₂	-	-	25.57	-	-	35.66
[Cu(L ³) ₂]Cl ₂	-	37.61	-	-	-	27.17
[Zn(L ³) ₂]Cl ₂	-	42.44	34.22	-	-	-
[Co(L ⁴) ₂]Cl ₂	-	-	-	40.61	-	-
[Ni(L ⁴) ₂]Cl ₂	45.07	-	-	27.57	-	-
[Zn(L ⁴) ₂]Cl ₂	26.11	-	-	39.52	-	-

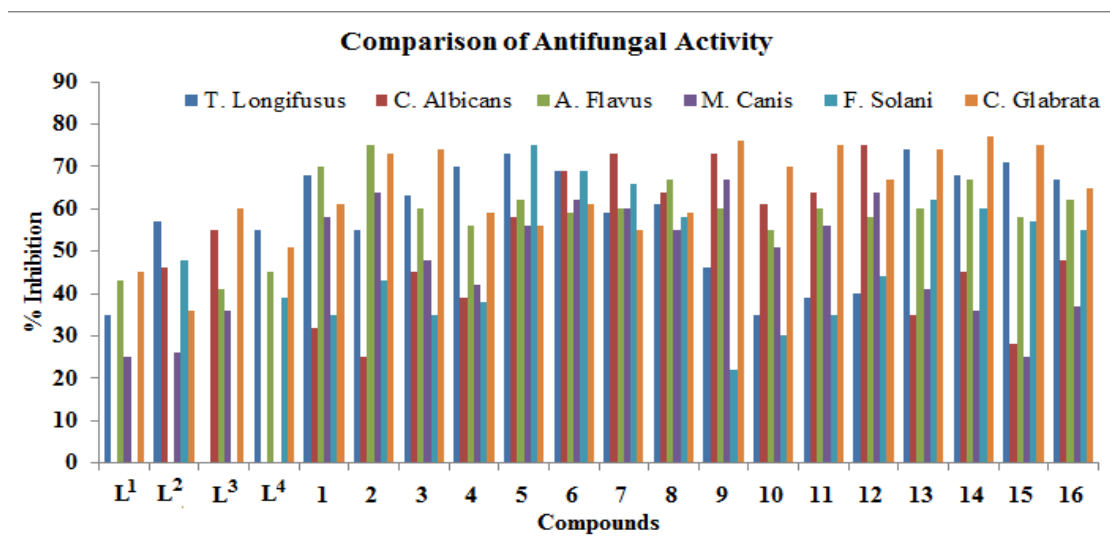


FIGURE 2. Comparatives results of antifungal activity of isatin ligands vs metal(ii) chelates

CONCLUSION

The synthesized isatins derived compounds act as tridentate ligands for coordination with the transition metals (Co, Ni, Cu, and Zn) via azomethine-N, ammine-N and carbonyl-O. Spectral characterization and elemental analysis established that the ligands are coordinated with the metallic atoms in an octahedral geometry. The anti-microbial results indicated that the metal complexes presented more bioactivity against selected bacterial/fungal strains than their uncomplexed ligands due to chelation. We believe that some of these compounds have the potential to become drug candidates and their difference in activity towards closely related species may help to highlight differences in the activation or inhibition of different signaling pathways.

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