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Green Synthesis of Salicylaldehyde Derivative Schiff Base Metal Complexes Using Microwave Irradiation: Characterisation & Antimicrobial Studies

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Three series of salphen derivatives were synthesised and underwent complexation with Cd(II) ion using microwave irradiation. The synthesised compounds were characterised using FT-IR, NMR, elemental analysis, and X-ray crystallography. The FT-IR and ^{1}H-NMR spectroscopies revealed the shifting of azomethine peaks, as well as the disappearance of hydroxyl functional groups in ligands upon complexation, suggesting the metal bonded with the ligands in a N_2O_2 coordination manner. The synthesised compounds were tested for antimicrobial activities against selected bacterial strains and the results showed moderate to good activity in Cd(II) complexes containing electron donating groups compared to their respective free ligands.

Key words: Antibacterial study; Schiff base; metal complex; microwave assisted synthesis

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Microwave assisted synthesis has now become the new non-conventional method in organic and inorganic synthesis, replacing the conventional reflux and proving to be eco-friendly, clean, and convenient [1]. The use of microwave irradiation as a heat source not only shortens the reaction time but also enhances product yield as well as enhancing the purity by reducing unnecessary side reactions [2].

The condensation reaction of primary amines with active carbonyl compounds leads to the formation of Schiff base compounds. Schiff base compounds are considered as "privileged ligands" by many due to their stability and structural design [3]. The azomethine (C=N) group found in Schiff base compounds is known to be biologically active. Thus, it has received significant attention in chemistry and biology for its various applications such as being antitumor [4], antifungal [5], and antimicrobial [6, 7].

Schiff bases derived from diamine compounds are valuable precursors for multinuclear complexes. The condensation between phenylenediamine with salicylaldehyde results in the formation of a N_2O_2 donor-type ligand. This type of ligand is interesting because of its capability to coordinate with one or more metal ions, depending on the positional relation between two amino groups in the precursor [8].

The present research paper describes the synthesis of Cd(II) Schiff base complexes derived from salicylaldehyde derivatives using microwave irradiation. The ligands and their respective complexes were characterised using physiochemical and spectroscopic techniques. Later, their antimicrobial properties were screened against *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, and *Klebsiella pneumoniae*.

EXPERIMENTAL

1. General

All the chemicals used to synthesise the Schiff base ligands and their Cd(II) complexes were of analytical grade purchased from Sigma Aldrich, while the reactions were carried out using Anton Paar Monowave 450 microwave reactor. Stuart Melting Point SMP10 apparatus was used to determine the products' melting points. The elemental analyses were performed on Thermo Flash EA 110 Elemental Analyzer. Infrared and ^{1}H-NMR spectroscopy analyses of ligands and metal complexes were recorded on Perkin-Elmer FT-IR 1600 and Bruker Avance 300 MHz NMR spectrometers, respectively. Single-crystallography X-ray analyses of ligands were carried out using D8 Quest diffractometer by Bruker.

2. Synthesis of Schiff Base Ligands

The general synthesis route of ligands is shown in Figure 1. An appropriate salicylaldehyde derivative (10 mmol) and m-phenylenediamine (5 mmol) were mixed in 10 mL methanol. The reaction mixture was heated in the microwave reactor at 150°C for 5

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minutes. A yellowish precipitate was filtered off and washed several times using cold methanol. The precipitate was then dried over anhydrous silica gel.

2.1. Synthesis of *N, N'*-bis(salicylidene)-1,3-phenylenediamine, L1H

Yield: 98%. m.p.: 110–114°C. Anal. Calcd for $C_{20}H_{16}N_2O_2$ (%): C: 75.93; H: 5.10; N: 8.86. Found: C, 75.39; H, 5.14; N, 9.26. IR (λ max cm^{-1}) (KBr): 1617 (C=N), 1568(C=C), 1357 (C-O phenolic), 1286 (-OH_{bend}). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 13.14 (s, -OH), 8.71 (s, HC=N), 7.52–6.99 (m, aromatic).

2.2. Synthesis of *N, N'*-bis(5-methoxysalicylidene)-1,3-phenylenediamine, L1OMe

Yield: 98%. m.p.: 150–152°C. Anal. Calcd for $C_{22}H_{20}N_2O_4$ (%): C: 70.20; H: 5.36; N: 7.44. Found: C:70.02; H, 5.55; N, 7.85. IR (λ max cm^{-1}) (KBr): 1619(C=N), 1571 (C=C), 1334 (C-O phenolic), 1278 (-OH_{bend}), 823 (C-Cl). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 12.33 (s, -OH), 9.00 (s, HC=N), 7.52–6.91 (m, aromatic), 3.74 (s, -OCH_3).

2.3. Synthesis of *N, N'*-bis(5-bromosalicylidene)-1,3-phenylenediamine, L1Br

Yield: 95%. m.p.: 232–235°C. Anal. Calcd for $C_{20}H_{14}Br_2N_2O_2$ (%): C, 50.66; H, 2.98; N, 5.91. Found: C: 51.02; H: 3.07; N, 5.35. IR (λ max cm^{-1}) (KBr): 1617 (C=N), 1594 (C=C), 1313 (C-O phenolic), 1189 (-OH_{bend}), 785 (C-Cl). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 11.09 (s, -OH), 8.97 (s, HC=N), 7.85–6.92 (m, aromatic).

3. Synthesis of Cd(II) Complexes

The Cd(II) complexes were prepared according to the scheme shown in Figure 2. 1.0 mmol solution of an appropriate ligand in ethanol (10 mL) was mixed with an ethanolic solution of $Cd(CH_3COO)_2{\cdot}4H_2O$ (1.0 mmol). The mixture was heated in the microwave reactor at 150°C for 15 minutes. The yellow precipitate produced was filtered and washed with cold methanol and later dried over anhydrous silica gel. A similar method was used to synthesise Cd(L1OMe) and Cd(L1Br) by replacing the free ligand with equimolar LiOMe and L1Br, respectively.

3.1. Synthesis of Cd(L1H)

Yield: 80%. m.p.: > 300°C. Anal. Calcd. for $C_{40}H_{28}Cd_2N_4O_4$ (%): C: 56.29; H: 3.31; N: 6.56. Found: C:55.64; H, 3.60; N, 7.92. IR (λ max cm^{-1}) (KBr): 1607 (C=N), 1580 (C=C), 1320 (C-O phenolic), 533 (M-N), 442 (M-O). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 9.02 (s, HC=N), 7.64–6.90 (m, aromatic). Molar Cond. ($S^{-1}cm^2\ mol^{-1}$): 3.44.

3.2. Synthesis of Cd(L1OMe)

Yield: 85%. m.p.: 260°C (decomposed). Anal. Calcd for $C_{44}H_{36}Cd_2N_4O_8$ (%): C: 54.28; H: 3.73; N: 5.74. Found: C, 64.81; H, 4.89; N: 8.10. IR (λ max cm^{-1}) (KBr): 1609 (C=N), 1571 (C=C), 1333 (C-O phenolic), 532 (M-N), 436 (M-O). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 8.98 (s, HC=N), 7.51–6.89 (m, aromatic), 3.72 (t, -OCH_3). Molar Cond. ($S^{-1}cm^2\ mol^{-1}$): 2.41.

3.3. Synthesis of Cd(L1Br)

Yield: 79%. m.p.: > 300°C. Anal. Calcd for $C_{40}H_{24}Br_4Cd_2N_4O_4$ (%): C: 41.10; H: 2.07; N: 4.79. Found: C: 41.66; H: 2.14; N: 5.82. IR (λ max cm^{-1}) (KBr): 1606 (C=N), 1584 (C=C), 1315 (C-O phenolic), 780 (C-Br), 546 (M-N), 443 (M-O). ^1H 300Mhz NMR, DMSO-d^6, δ(ppm): 8.98 (s, HC=N), 7.85–6.88 (m, aromatic). Molar Cond. ($S^{-1}cm^2\ mol^{-1}$): 2.25.

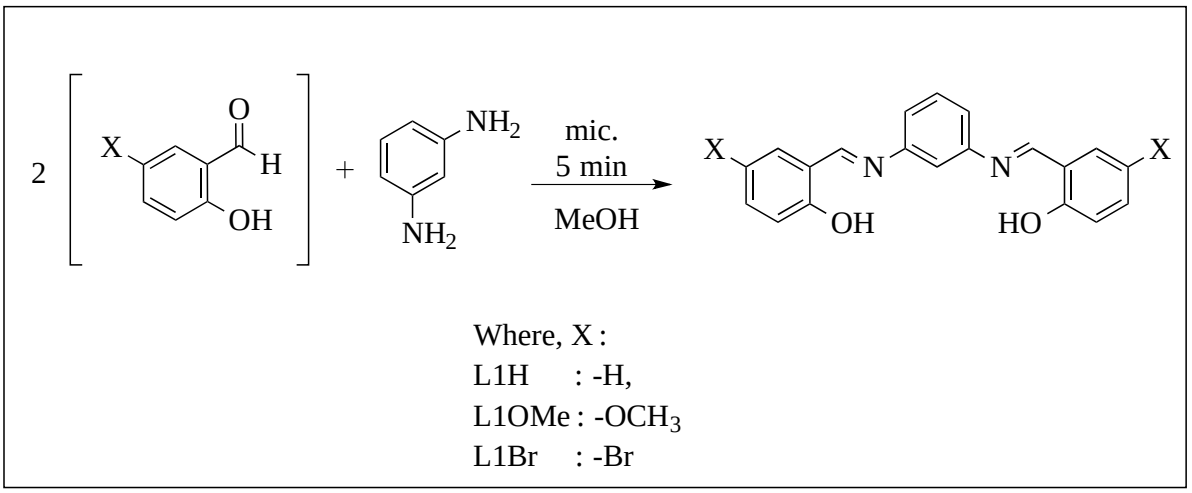

Figure 1. Synthesis route for L1H, L1OMe, and L1Br ligands

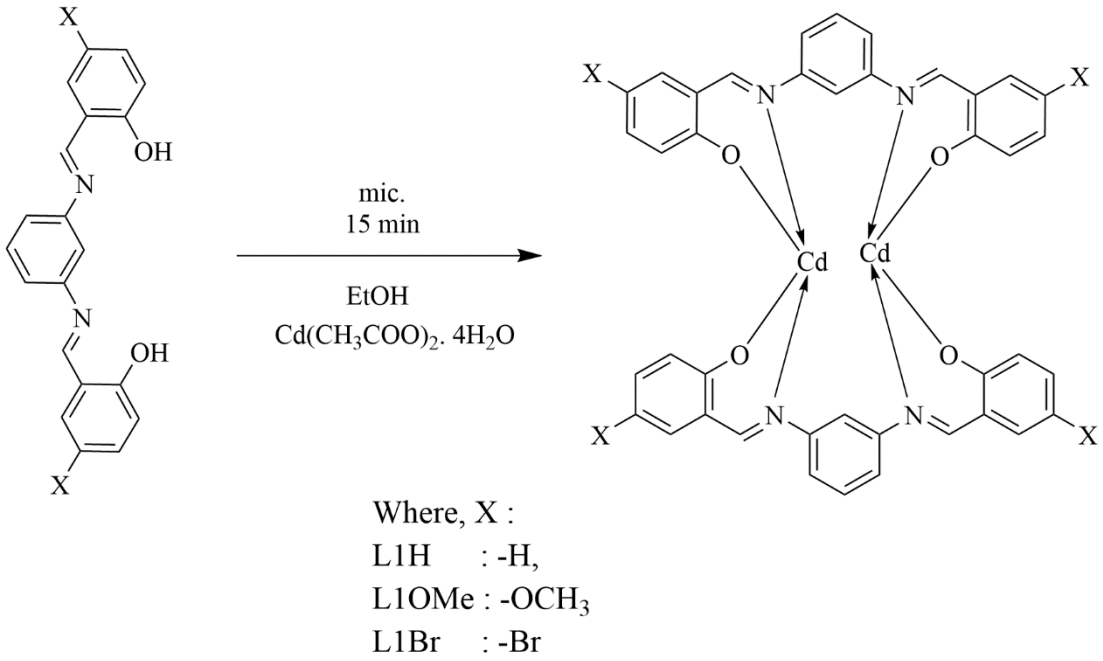

Figure 2. Synthesis route for Cd(II) complexes

4. Disc Diffusion Screening

An antibacterial test against pathogens was carried out by using the disc diffusion method [9]. Bacteria from stock cultures were lightly inoculated into Mueller Hinton Broth (MHB) and allowed to grow overnight at 37°C in an ambient air incubator. The cultures were diluted with fresh MHB in order to achieve an optical density of 0.1 at a wavelength of 625 nm in the spectrophotometer. Later, sterile cotton swabs were dipped into the broth cultures and the bacteria were inoculated onto individual Mueller Hinton Agar (MHA) plates. Sterile 6 mm in diameter paper discs were placed on the agar at equal distances. Subsequently, 10 µL aliquot of the parent ligands and their respective Cd(II) complexes dissolved at a concentration of 2000 µg/mL in DMSO were dispensed individually to each of the discs. The agar plates were incubated at 37°C overnight. For each plate, gentamicin acted as the positive control, while DMSO acted as the negative control. The diameter of the inhibition zone of the discs on the plates was measured in millimetre (mm) and recorded.

RESULTS AND DISCUSSION

1. General

Based on the physiochemical analyses of the ligands, it is suggested that the reaction between salicylaldehyde and diamine took place in a 2:1 ratio, whereas the Cd(II) complexes were formed in a 1:1 ratio between the free ligands and metal ions. This is according to the percent composition of C, H and N obtained through elemental analysis which were in concordance with the calculated percentage. The solubility test showed that the ligands mostly dissolved in MeCN, THF, MeOH, and DMSO, while the Cd(II) complexes only dissolved in DMSO and DMF. The molar conductivity of the complexes was found to be low, suggesting the complexes were non-electrolyte at room temperature. The reduction in reaction time and volume of solvent used in the microwave irradiation method while maintaining the purity not only improved the way of synthesising the ligands, but also minimised cost and wastage in line with the Principles of Green Chemistry [10].

2. Infrared Spectroscopy

The IR spectra of the Cd(II) complexes showed a sharp vibrational peak of ν(C=N) at 1606–1609 cm^{-1} which shifted 9–10 cm^{-1} to a lower frequency than their respective parent ligands indicating the involvement of azomethine nitrogen in the complexation [11]–[13]. ν(C-O) phenolic vibration in the range of 1357–1313 cm^{-1} in the ligands also showed a band shifting in the spectra of the complexes. This shifting frequency was attributed to the deprotonation of phenolic hydrogen due to coordination with the metal ions as a result of complexation. The involvement of azomethine nitrogen and phenolic oxygen was further confirmed by the appearance of new absorption peaks of ν(M-N) and ν(M-O) stretching modes in the complexes' spectra in the range of 546–533 cm^{-1} and 443–436 cm^{-1}, respectively.

3. ^{1}H-NMR Spectroscopy

The ^{1}H-NMR data for all the ligands were characterised by four important signals assigned to phenolic, azomethine, aromatic rings, and methoxy protons. A singlet peak was recorded at the downfield

region within the range of 13.14–11.09 ppm, associated to the protons of phenolic hydrogen (-OH). This peak was found to be absent in the metal complexes' spectra, suggesting deprotonation of phenolic hydrogen and indicated the formation of M-O bond [14].

In L1H ligand, a single peak was observed at 8.71 ppm. This singlet peak was assigned to be the azomethine protons (-HC=N), whereas in L1OMe and L1Br, this peak appeared in the low field region at 9.00 ppm and 8.97 ppm, respectively. Coordination of both nitrogen atoms of the ligand to the metal centre in the Cd(II) complexes was indicated by the shifting of the azomethine proton signal to 9.02 ppm in Cd(L1H) along with 8.98 ppm in both Cd(L1OMe) and Cd(L1Br).

4. X-Ray Crystallography

Suitable ligand crystals were obtained through slow evaporation and underwent crystallographic analyses. The crystal data and structures of the ligands are given in Table 1. In L1H, both outer benzene rings C(1)-C(6) and c(15)-C(20) of L1Sal were found to be twisted from the central benzene ring C(8)-C(13), with dihedral angles of 43.7(2)° and 40.6(2)°, respectively, forming a non-planar molecule structure. Moreover, the non-planarity of the compound was further supported by the torsion angles of C(9)-C(8)-N(1)-C(7) = 37.7(6)° and C(12)-C(13)-N(2)-C(14) = 35.7(6)°. The bond length of C(7)-N(1) and C(14)-N(2) as well as their angles implied that the nitrogen atoms were sp^2-hybridised, [d(C(7)-N(1)) = 1.287(5) Å, ∠ N(1)-C(7)-C(6) = 121.6(4)°; d(C(14)-N(2)) = 1.286(5) Å, ∠ C(14)-N(2)-C(13) = 120.1(4)°].

Meanwhile, the whole structure of L1OMe was a mirror symmetry generated in which the reflection plane was positioned at C(10) and C(11) of the central ring. The crystal of L1OMe was found to be nearly planar, with the dihedral angle between the central ring (C(8)-C(11)) and the terminal aromatic ring (C(1)-C(6)) of 2.17(19)°. The torsion angle of azomethine (C(6)-C(7)-N(1)-C(8)) moiety that connected the terminal rings with the central ring was 179.3(3)°, confirming the moiety was in *E*-configuration. On top of that, the methoxy group attached to C(2) of the aromatic ring was nearly coplanar with a torsion angle of C12-O2-C4-C5 = 1.3(6)°.

The L1Br structure appeared to be non-planar. The two outer aromatic rings C(1)-C(6) and C(1a)-C(6a) were tilted toward the central ring with the dihedral angle of 39.60(2)° on both rings, in concordance with crystal data reported previously [15]. Both of the rings were twisted from the central ring along C(7)-N(1)-C(8)-C(9) with a torsion angle of -38.4(6)°. The bond length of the azomethine, C(7)-N(1) was 1.279(5) Å and an angle of 121.7(3)° between N(1)-C(7)-C(6), while having a torsion of 179.(4)° at C(8)-N(1)-C(7)-C(6). The bond length of C(4)-Br(1) was found to be 1.894(4)Å and coplanar with the benzene ring (C(1)-C(6)), to which it was attached.

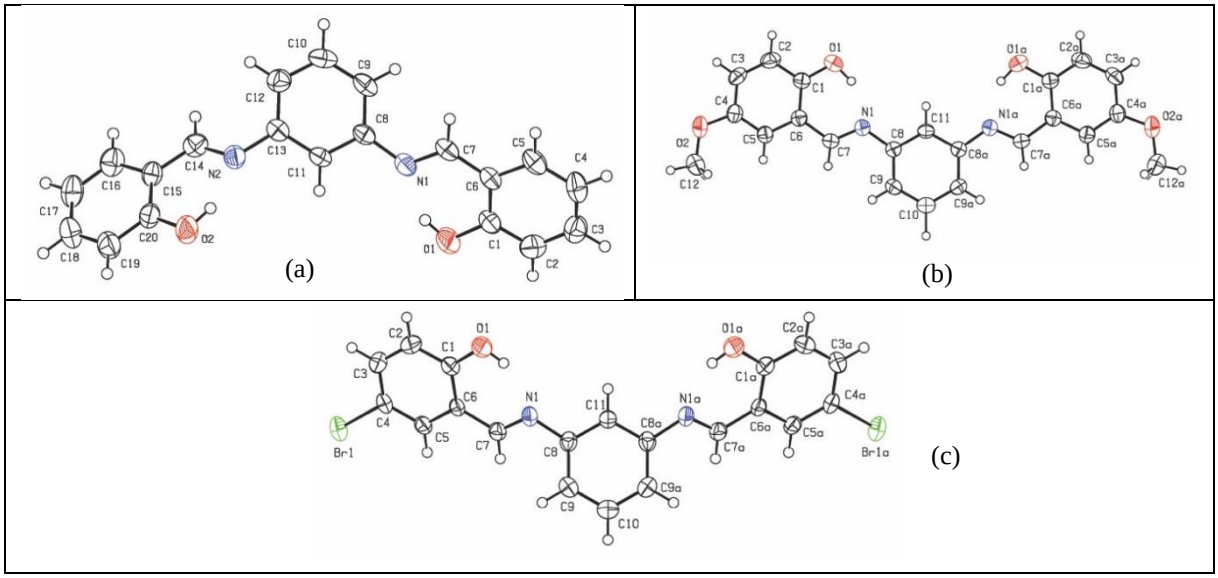

Figure 3. ORTEP diagrams of (a) L1H, (b) L1OMe, and (c) L1Br with the atom labelling and displacement ellipsoids drawn at 50% probability level

Table 1. Crystal data of L1H, L1OMe, and L1Br ligands

L1H		L1OMe		L1Br	
Empirical formula	$C_{20}H_{16}N_2O_2$	**Empirical formula**	$C_{22}H_{20}N_2O_4$	**Empirical formula**	$C_{20}H_{14}Br_2N_2O_2$
Formula weight	316.35	**Formula weight**	376.40	**Formula weight**	474.15
Crystal system	Monoclinic	**Crystal system**	Orthorhombic	**Crystal system**	Orthorhombic
Space group	$P2_1/n$	**Space group**	P m n 21	**Space group**	Pnma
Unit cell dimensions	a = 15.62(15)Å b= 6.06(6) Å c = 17.10(16)Å α= 90° β= 97.17(3)° γ= 90°	**Unit cell dimensions**	a = 38.92(4) Å b = 3.97(4) Å c = 5.85(6) Å α= 90° β= 90° γ= 90°	**Unit cell dimensions**	a = 12.37(3) Å b = 37.02(9)Å c = 3.90(8) Å α= 90° β= 90° γ= 90°
Volume	1608.1(3) $Å^3$	**Volume**	905.39(16) Å3	**Volume**	1791.0(7) $Å^3$
Z	4	**Z**	2	**Z**	4
Reflections collected	36969	**Reflections collected**	7914	**Reflections collected**	22033
Independent reflections	3855 [R(int) = 0.1249]	**Independent reflections**	2039 [R(int) = 0.0359]	**Independent reflections**	2152 [R(int) = 0.0488]
Final R indices [I>2sigma(I)]	R1 = 0.1910, wR2 = 0.2132	**Final R indices [I>2sigma(I)]**	R1 = 0.0511, wR2 = 0.1262	**Final R indices [I>2sigma(I)]**	R1 = 0.0525, wR2 = 0.0787
R indices (all data)	R1 = 0.2577, wR2 = 0.2284	**R indices (all data)**	R1 = 0.0642, wR2 = 0.1329	**R indices (all data)**	R1 = 0.0719, wR2 = 0.0848

Table 2. The antimicrobial activities of the synthesised L1 ligands and their metal complex derivatives expressed as zones of growth inhibition in mm against the tested bacterial strains

Bacteria strains	L1H	Cd(L1H)	L1OMe	Cd(L1OMe)	L1Br	Cd(L1Br)	Gentamicin
E. coli	0	8	0	0	0	8	23
K. pneumoniae	7	10	7	8	0	0	22
B. subtilis	0	13	0	13	0	0	25
B. cereus	0	13	7	13	0	0	23

5. Antimicrobial Activities

All the synthesised ligands and metal complexes were screened for antibacterial activities using the disc diffusion method and the zone of inhibition was measured in millimetres as tabulated in Table 2. A total of four bacterial strains were used to evaluate the biological properties of the ligands.

Based on the screening results, it was found that most of the free ligands showed almost no antibacterial activities. Meanwhile, some improvements in the size of the inhibition zone could be observed in the complexes. Apparently, Cd(II) complexes incorporated with electron donating group (EDG) ligands showed good inhibition compared to complexes with electron withdrawing substituents. These observations can be explained through Overton's concept of permeability and Tweedy's theory of chelation [16], [17]. On chelation, metal ion polarity is reduced to a greater extent due to the overlapping of the ligand orbital and partial sharing of positive charge of metal ions with donor groups. Hence, the delocalisation of the π-electrons is increased over the whole chelate sphere which enhances the lipophilicity of the complex. The lipophilic nature of the central metal atom is also increased upon chelation, which subsequently favours the permeation through the lipid layer of the cell membrane [18].

CONCLUSION

L1H, L1OMe, and L1Br Schiff base ligands and their Cd(II) complexes were successfully synthesised. Both ligands and Cd(II) complexes were synthesised using minimum solvent using microwave irradiation. The analytical data showed that the metal was coordinated in a N_2O_2 coordination. The prepared compounds were tested for their antimicrobial activities and the results showed that complexation improved the biological properties of the compounds and it was also found that compounds associated with electron donating groups were more favourable in resisting the growth of the tested bacteria.

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