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Original Article

HYDRAZIDE SCHIFF BASES OF ACETYLACETONATE METAL COMPLEXES: SYNTHESIS, SPECTROSCOPIC AND BIOLOGICAL STUDIES

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ABSTRACT

Objective: The study was focused on the synthesis and spectroscopic studies of metal acetylacetonates and their complexes using bidentate Schiff-base ligands (NO), evaluation of their *in vitro* antibacterial potentials against pathogenic microorganism.

Methods: Acetylacetonate salts of Cobalt (II), Manganese (II) and Magnesium (II) were prepared by reacting their metal hydroxides with acetylacetone. The metal complexes of *N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide (HL^1), *N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide (HL^2) obtained from the condensation reaction of 4-(diethylamino)-2-hydroxybenzaldehyde and 4-nitrobenzohydrazide/or 4-methoxybenzohydrazide. The synthesized compounds were characterized by fourier transform infrared spectroscopy (FT-IR), proton and carbon-13 nuclear magnetic resonance (NMR), thermo gravimetric analysis (TGA). The compounds were screened for antimicrobial properties against a list of Gram-positive bacterial strains.

Results: The FT-IR spectra revealed that the Schiff bases acts as bidentate chelating ligand via a nitrogen of the azomethine and phenolic oxygen atoms. NMR reveals the presence of azomethine (HC=N) and aromatic hydrogens at expected chemical shifts confirming the formation of the Schiff base ligands. Thermal decomposition behaviour was studied by thermogravimetry revealing stability up to 260 °C. The compounds were evaluated for antibacterial potentials against *Staphylococcus aureus* and *Enterococcus faecalis.* The manganese acetylacetonato (*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide: Mn (acac) (L^2) exhibited antimicrobial activities against both *Enterococcus faecalis* and *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 398.0 µg/ml.

Conclusion: The prepared compounds showed no inhibition against the selected pathogenic microorganisms except for Mn(acac)(L^2). Standard antibacterial compounds: ampicillin and ciprofloxacin was used as positive control. The antibacterial activity of the compound depends on the kind of substituent on the benzo hydrazide rings at the para position, thereby suggesting the compound as promising chemotherapeutic agents for further structural optimization.

Keywords: Metal acetylacetonates, Schiff base complexes, Spectroscopic studies, Thermal studies, Antibacterial

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INTRODUCTION

Infectious diseases which are directly related to bacteria exhibiting multiple resistance to antibiotics have increased the world's mortality rate, resulting in about 17 million deaths globally per year in children and the elderly [1]. Therefore, the need to develop novel antibacterial drugs with excellent mechanisms of action and the structural-activity relationship has become an urgent biomedical necessity [2]. Schiff base ligands bearing azomethine (>C=N–) functional group, possessing N, O, and S-donor atoms have been established as versatile pharmacophores for design and development of various biologically active compounds [3]. They are used in wide variety of applications such as anti-cancer, anti-corrosion, anti-HIV, anti-radical, anti-bacterial and anti-fungal material and DNA cleavage [2, 4]. Some biologically important Schiff base derivatives include the imidazoles, pyrimidines, hydrazides and hydrazine's [5-6]. Hydrazide derivatives have been reported to possess a broad spectrum of antibacterial activities. They also act as a good potential for oral drugs used for the treatment of genetic disorders like thalassemia [7-8].

Schiff bases and their complexes are very attractive materials for several applications due to their desirable features such as structural similarities with natural biological substances, relatively simple preparation procedures, the synthetic flexibility that enables the design of suitable structural properties, selectivity and sensitivity towards the central metal atom rich architectural variety and amazing coordination capacity [9-11]. The chemical stability of Schiff base complexes can be influenced by the presence of azomethine linkage which has a great impact in increasing the basicity of each nitrogen atom [12]. These metal complex derivatives showing considerable biological activity may represent an interesting approach for designing new antibacterial and antifungal drugs [13].

In this study, we describe the synthesis of two hydrazides Schiff base ligands: 4-(diethylamino)-2-hydroxybenzaldehyde with 4-nitro-benzohydrazide or 4-methoxybenzohydrazide and their Mn (acetyl-acetonate)$_2$, Co(acetylacetonate)$_2$, Mg(acetylacetonate)$_2$ complexes by reflux methods as chemotherapeutic agents with interesting biological properties such as antimicrobial activity. The synthesized compounds were characterized by FT-IR, TGA, ^{1}H NMR and ^{13}C NMR.

MATERIALS AND METHODS

Experimental

Materials and spectral measurements

All the chemicals used in this project were of analytical reagent (AR) grade and used as received from the suppliers without further purifications. The chemicals were obtained from different suppliers as indicated. Methanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), Hydrogen peroxide (H_2O_2), distilled acetylacetone, 4-methoxbenzhydrazide, 4-(diethylamino)-2-hydroxybenzaldehyde, 4-nitrobenzohydrazide, Cobalt(II) acetate tetrahydrate were purchased from Sigma Aldrich, South Africa; Magnesium Chloride Hexahydrate, Potassium permanganate, Potassium Hydroxide and Sodium Hydroxide were purchased from LABCHEM, Gauteng, South Africa. IR spectra were recorded by using a PerkinElmer 580 Spectrometer within the range of 4000–400 cm^{-1} in nujol mull, METTLER TOLEDO TGA with the top-of-the-line ultra-micro balance with unique built-in calibration weights for thermo grams and ^{1}H and ^{13}C NMR spectra in d_6-DMSO solution using

TMS as an internal standard were obtained with Agilent VnmrJ 3 Spectrometer operating at 500 MHz.

General procedure for the synthesis of Schiff base ligands (HL^1–HL^2)

***N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-nitro-benzohydrazide (HL^1)**

The Schiff base ligand was prepared in line with the method described in the literature [14]. Stoichiometric amounts of 4-(diethylamino)-2-hydroxybenzaldehyde (10 mmol) in methanol (50 ml) was carefully added to a stirred pre-warmed methanolic solution of 4-nitrobenzohydrazide (10 mmol) and refluxed at 70 °C for 2 h in a closed system. Consequently, the resultant mixture was cooled to room temperature and then concentrated to a volume of 20 ml. The solid product formed was recovered by filtration, washed with methanol and dried in a desiccator over anhydrous $CaCl_2$. The synthetic pathway of the ligand is explained in scheme 1.

***N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide (HL^2)**

The Schiff base ligand was prepared in line with the method described in the literature [14]. Stoichiometric amounts of 4-(diethylamino)-2-hydroxybenzaldehyde (10 mmol) in methanol (50 ml) was carefully added to a stirred pre-warmed methanolic solution of 4-methoxybenzohydrazide (10 mmol) and refluxed at 70 °C for 2 h in a closed system. Consequently, the resultant mixture was cooled to room temperature and then concentrated to a volume of 20 ml. The solid product formed was recovered by filtration, washed with methanol and dried in a desiccator over anhydrous $CaCl_2$. The synthetic pathway of the ligand is explained in scheme 1.

Synthesis of metal acetylacetonates: $M(acac)_2$

Acetylacetonate salts of Cobalt (Co), Manganese (Mn), and Magnesium (Mg) were prepared by reacting their metal hydroxides with acetylacetone [15].

Cobalt acetylacetonate, $Co(acac)_2$: Cobalt acetate tetrahydrate (10 g, 40.1 mmol) was dissolved in 200 ml water in a 500 ml beaker. KOH (20 %) was added slowly with constant stirring to precipitate the metal as its hydroxide. Initially, a blue precipitate was formed. The precipitate was stirred for 10 min and allowed to stand for 30 min. The colour of the precipitate changed from blue to green and finally to pink. The addition of the alkali was continued until the pH of the solution was raised to 8. The metal hydroxide formed was repeatedly washed with water to remove all traces of the alkali followed by decantation and finally filtered using Whatman No. 2 filter paper.

The pink cobalt (II) hydroxide was quantitatively transferred into a 250 ml beaker. Distilled acetylacetone (9.1 ml, 88.2 mmol) was added dropwise with continuous stirring. An exothermic reaction took place leading to the formation of the pink shiny crystalline compound. The crystals were allowed to stand at room temperature for 30 min and then placed in an ice-water bath for 15 min. The pink shiny crystals of cobalt acetylacetonate formed were filtered. The resulting crystals were separated by filtration, washed and dried in a vacuum desiccator over anhydrous $CaCl_2$ for 48 h.

Manganese acetylacetonate $Mn(acac)_2$: Following the above procedure, the compound was obtained as dark brown shinny crystals from powdered KMnO4 (5.0 g, 31.7 mmol) dissolved in 100 ml water and acetylacetone (14.67 ml, 147 mmol).

Magnesium acetylacetonate $Mg(acac)_2$: Following the same procedure, the compound was obtained as white crystals from magnesium chloride hexahydrate (10 g, 49.19 mmol) dissolved in 200 ml water and acetylacetone (11.15 ml, 108.21 mmol).

Synthesis of metal schiff base complexes

In a 250 ml round bottom flask fitted with a water condenser, a warm stirred methanolic solution (30 ml) of the ligands: HL^1 or HL^2 (0.01 mol) was mixed with (0.01 mol) of [$Co(acac)_2$]/[$Mn(acac)_2$]/[$Mg(acac)_2$] respectively dissolved in 30 ml methanol in a dropwise manner. The colour of the solutions changed immediately. The mixture was stirred vigorously for 20 min. and refluxed over a steam bath at 70 °C for 2 h in a closed. Consequently, the resultant solution was left overnight in a fume cupboard at ambient temperature until all traces of the solvent had evaporated. The solid formed, washed with methanol and dried over anhydrous calcium chloride in a vacuum desiccator. Scheme 2, describes the detailed synthetic pathway for the Schiff base complexes.

Antibacterial assay

Bacterial strains

The synthesized Schiff base ligands and their metal complexes were evaluated for their in vitro antibacterial activities. Antibacterial activity was tested against a list of Gram-positive bacterial strains: Staphylococcus aureus and Enterococcus faecalis using a modification of the Kirby-Bauer disc diffusion technique [5]. The micro-organisms were obtained from the Department of Microbiology, North-West University, South African.

A total of ten Staphylococcus aureus: (1RO4) S. aureus, (1RO6) S. aureus, (1RO9) S. aureus, (2RO4) S. aureus, (2RO5) S. aureus, (3RO6) S. aureus, (3RO9) S. aureus, (4R4) S. aureus, (4R5) S. aureus and (15Z1) S. aureus and five Enterococcus faecalis: (DEEL4-2) E. faecalis, (STL1-3) E. faecalis, (MP3-1) E. faecalis, (MP3-2) E. faecalis and (V2-2) E. faecalis were used in the study. However, the identities of the isolates used in the study were confirmed using preliminary gram staining, (catalase test, hemolysis on blood agar) and confirmatory serotyping, (specific PCR analysis) and the Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) Mass Spectrometry.

***In vitro* antimicrobial assay**

The synthesized compounds were tested to evaluate their growth inhibitory activities against the selected bacteria strains using a modification of the previous methods [5]. Ten Staphylococcus aureus: (1RO4) S. aureus, (1RO6) S. aureus, (1RO9) S. aureus, (2RO4) S. aureus, (2RO5) S. aureus, (3RO6) S. aureus, (3RO9) S. aureus, (4R4) S. aureus, (4R5) S. aureus and (15Z1) S. aureus and five Enterococcus faecalis: (DEEL4-2) E. faecalis, (STL1-3) E. faecalis, (MP3-1) E. faecalis, (MP3-2) E. faecalis and (V2-2) E. faecalis. However, reference strains E. faecalis (ATCC 6569) and S. aureus (ATCC® 25923) were used for quality controls in this study. Holes (three) were bored in the plates (6 mm diameter) using sterile cork borer, about 20 µl of the Schiff base complexes stock solution was inoculated across the wells and incubated at 37 °C for 24 h. Consequently, after incubation, the strains were classified as susceptible, or resistant and for the purposes of analysis, intermediate susceptibility was regarded as susceptible. The average diameter of three readings of the clear zone surrounding the hole was taken as the degree of inhibition against the bacteria and recorded as mean.

Minimum inhibitory concentrations

To evaluate the minimum inhibitory concentrations of the synthesized compounds. The following concentrations of 10.0 µg/ml, 30.0 µg/ml, 60.0 µg/ml, 100.0 µg/ml, 247.2 µg/ml, 305.3 µg/ml, 378.9 µg/ml and 398.0 µg/ml were used. About 100 µl aliquots of the bacterial suspensions were spread-plated on solidified Muller Hinton agar. Using sterile needles and paper discs that were previously autoclaved and soaked in different concentrations of the test samples were placed on the surface of the agar. The inoculated plates that contained the antibiotic (ampicillin and ciprofloxacin) and the test compounds were incubated aerobically at 37 °C for 24 h. After 24 h of incubation at 37 °C, the minimum inhibitory concentrations were documented.

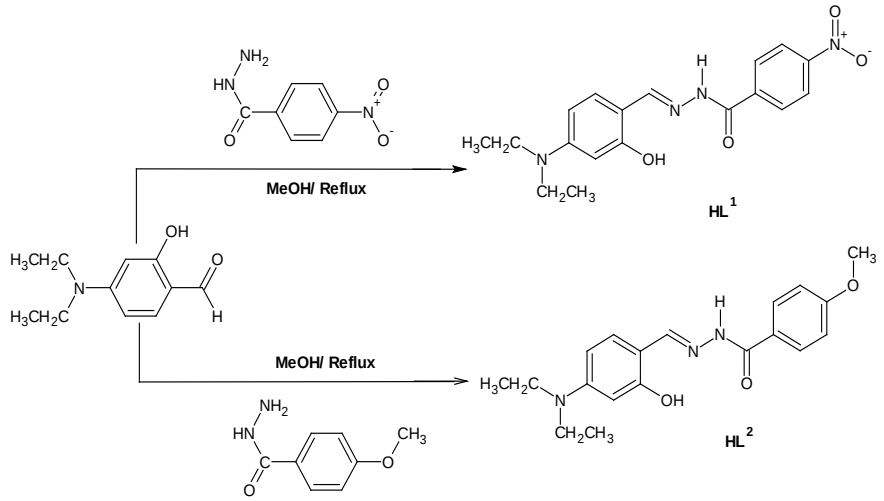
RESULTS AND DISCUSSION

Synthesis of the compounds

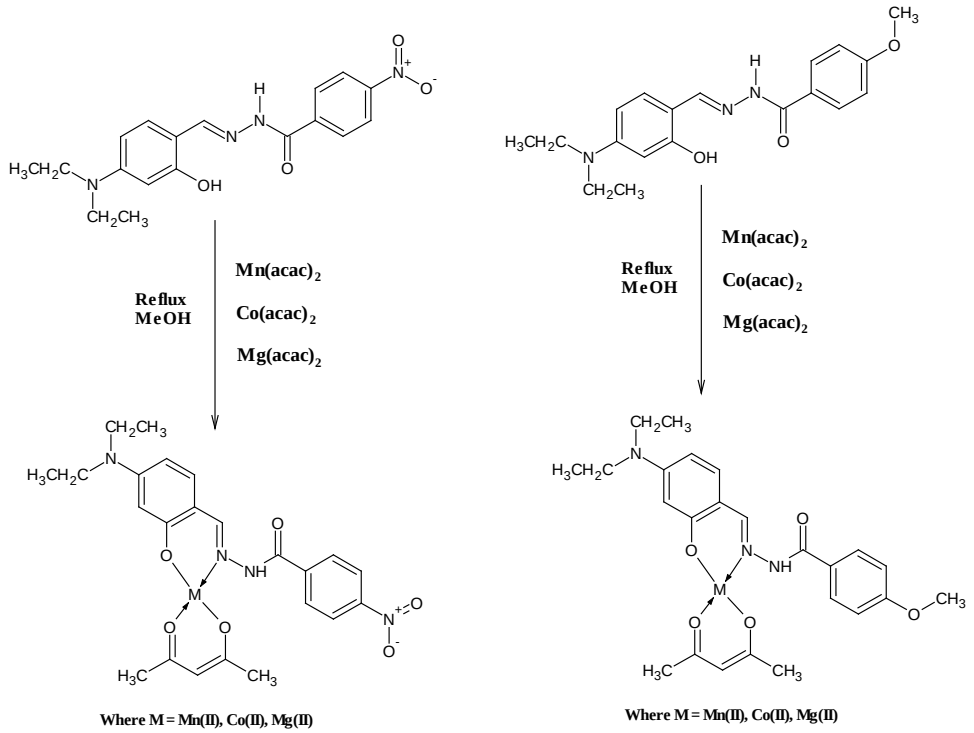
The bidentate N-O Schiff bases *viz*: *N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide (HL^1) and *N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxy-benzohydrazide (HL^2) were synthesised by the condensation reaction of 4-(diethylamino)-2-hydroxybenzaldehyde and 4-nitrobenzohydrazide/4-methoxybenzohydrazide (scheme 1). The Schiff base metal complexes were obtained by the reaction of metal acetylacetonates: $M(acac)_2$ in methanol with the Schiff base ligand in a 1:1-mole ratio as shown in scheme 2.

Fourier transform infrared spectroscopy (FT-IR)

IR spectra gives some valuable information in coordination chemistry. In order to study the binding pattern of the Schiff base (HL^1–HL^2) to the cobalt (Co), manganese (Mn), and magnesium (Mg) in the complexes, the IR spectrum of the Schiff base ligands was compared with the spectra of the complexes. The FT-IR results are presented in tables 1 and 2 alongside their individual vibrational peaks.



Scheme 1: Synthesis and structures of schiff base ligands (HL^1–HL^2)



Scheme 2: Detailed synthetic pathway for the schiff base complexes

IR spectra of the pure acetylacetone (acac) showed vibrational peaks at 2964 to 2877 cm^{-1} which are however similar to the observed stretching vibrational frequencies of 2868–2961 cm^{-1} (tables 1) in the spectrum of metal acetylacetonates [$Co(acac)_2$, $Mn(acac)_2$, $Mg(acac)_2$] assignable to the methyl and methylene groups [15]. The vibrational frequency at 1704 cm^{-1}, is due to carbonyl stretching vibration in the acetylacetone molecule.

This carbonyl frequency is absent or rather shifted downwards in the spectra of metal acetylacetonates indicating the formation of metal acetylacetonates bonds (M-O). This suggested that the coordination has taken place through oxygen atom of C=O group. The metal oxygen vibrational frequency, ν(M-O) was observed at different wavenumbers: 927 cm^{-1}, 921 cm^{-1}, 919 cm^{-1} for $Co(acac)_2$, $Mn(acac)_2$, $Mg(acac)_2$ respectively [15-16].

The infrared spectra of the ligands (HL^1–HL^2) showed that the vibrational frequency due to the phenolic OH group that appeared in the spectrum of the ligand at 3558 cm^{-1} and 3508 cm^{-1} for HL^1 and HL^2had disappeared in the spectra of the complexes (table 2). This may be due to the displacement of its proton by the metal acetylacetonates [9, 13]. ν(NH) of the uncoordinated NH groups appeared as a shoulder at 3274–3344 cm^{-1} in spectra of the ligands and at 3232–3358 cm^{-1} in spectra of the metal complexes [17]. The IR

spectra of the free ligands show the characteristic>C=N (azomethine group) bands in the 1623–1687 cm^{-1} region which are shifted to lower frequencies in the spectra of the metal complexes (1608–1643 cm^{-1}) [3, 18]. This ν(C=N) shift to in all the complexes by about 15–44 cm^{-1} indicates the involvement of azomethine nitrogen in the coordination sphere with the metal ions for all the complexes as well as lack of carbonyl group from original substituted benzohydrazide compounds [17-18]. The stretching vibration of the phenolic ν(C-O) observed at regions 1232-1237 cm^{-1} in the free ligands undergo a hypochromic shift to 1311-1340 cm^{-1} regions in the complexes upon complexation as recorded in table 2. This shifts further confirms the coordination of the phenolic oxygen leading to the formation of C-O-M bond [4, 14, 19] (where M = Co, Mn and Mg).

Also, the appearance of new bands in the range of 839-850 $cm^{-1}$and 815-823 cm^{-1} suggests ν(M-O) and ν(M-N) frequencies, respectively [4, 17]. The bands observed at 1576-1593 cm^{-1} is due to the ν(C-C) stretching of the aromatic ring systems. In all the metal acetylacetonate-Schiff base complexes, most of the band shifts observed in the wave number region 1379-1436 cm^{-1} and 1240-1259 cm^{-1} are in agreement with the structural changes observed in the molecular carbon skeleton after complexation, which cause some changes in (C–C) bond lengths. Valuable evidence concerning the environment of the functional group's coordination to the metal atoms through azomethine nitrogen and phenolic oxygen atoms has been obtained from the FT-IR spectra.

Thermogravimetric analysis (TGA)

Thermogravimetric and derivative thermogravimetric analysis (TGA/DTA) of the synthesized Schiff base compounds were measured under a nitrogen atmosphere at a heating rate of 5 °C min^{-1} from 0 °C to 900 °C. TG/DTG results were plotted as percentage weight loss against temperature; provides insight into nature, properties of different molecules and the residues obtained after thermal decomposition.

Changes in the sample in the DTA curve show either exothermic or endothermic reaction taking place in the sample [10].

Table 1: Characteristic peaks of pure acetylacetone, M (acac)$_2$ and Schiff base ligands

Acetylacetone and M(acac)$_2$ peaks (cm^{-1})					Schiff base ligands peaks (cm^{-1})		
Assignment	**$C_5H_8O_2$**	**Co(acac)$_2$**	**Mn(acac)$_2$**	**Mg(acac)$_2$**	**Assignment**	**HL^1**	**HL^2**
$\nu(CH_3)$	2964	2950	2961	2954	$\nu(O\text{-}H)_{str}$	3558	3508
$\nu(CH_3)$	2917	2904			$\nu(N\text{-}H)_{str}$	3344	3288
$\nu(CH_3)$	2877	2871	2868	2869		3274	
ν(C=O	1704	1627	1604	1601	$\nu(C\text{-}H)_{str}$	2973	2960
ν(CO)(acac)	1600					2927	
$\delta(CH_3)_{asy}$		1515	1502	1520			2890
$\delta(CH_3)_{asy}$	1411				$\nu(C=N)_{str}$	1623	1687
$\delta(CH_3)_{as}$	1357	1349	1339	1379	$\nu(C=C)_{Ar}$	1589	1589
$\nu(C\text{-}C\text{-}C)_s$	1243	1272	1255	1259			1585
ν(M-O)		927	921	919	ν(C-H)	1515	1515
ν(C-H)	912	769	778	762		1413	
δ(ring)	777	665			ν(C-C)	1340	1341
						1240	1297
					$\nu(C\text{-}O)_{str}$	1237	1232
					$\nu(N\text{-}N)_{str}$	1141	1140
					ν(C-C)skeletal	1130	1130
						1027	1054
					ν(C-H) bending	1012	1025
					Ring in plane	848	835
					Ring out of plane	701	703

M(acac)$_2$-Metal acetylacetonate; Co-Cobalt; Mn-Manganese; Mg-Magnesium; HL^1-*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-nitrobenzohydrazide; HL^2-*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide; cm-centimetre.

Table 2: Characteristic IR bands (cm^{-1}) of the Schiff base metal complexes

Assignment	**Co(acac)(L^1)**	**Mn(acac)(L^1)**	**Mg(acac)(L^1)**	**Co(acac)(L^2)**	**Mn(acac)(L^2)**	**Mg(acac)(L^2)**
$\nu(N\text{-}H)_{str}$	3358	3290	3349	3232	3236	3357
$\nu(C\text{-}H)_{str}$	2927	2915	2971	2964	2973	2910
	2892	2888	2865	2876	2832	2829
$\nu(C=O)_{str}$	1714	1705	1687	1699	1706	1681
$\nu(C=N)_{str}$	1617	1608	1613	1639	1643	1612
$\nu(C=C)_{Ar}$	1587	1577	1589	1576	1591	1593
$\nu(C\text{-}O\text{-}C)_{str}$	1487	1498	1434	1463	1496	1481
ν(C-C)skeletal	1409	1403	1365	1436	1421	1379
$\nu(C\text{-}O)_{str}$	1340	1327	1336	1321	1317	1311
ν(C-C)skeletal	1240	1243	1240	1259	1255	1245
$\nu(N\text{-}N)_{str}$	1137	1135	1128	1138	1133	1125
ν(C-H)bending	1014	1041	1010	1025	1018	1012
ν(M-O)	848	850	848	840	842	839
ν(M-N)	817	823	815	820	817	821
Ring in plane	788	773	784	761	763	761
Ring out of plane	701	703	700	665	667	619

Co-Cobalt; Mn-Manganese; Mg-Magnesium; (acac)-acetylacetonate; L^1-*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide; L^2-*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide; cm-centimetre.

The TG-DTG curve of Schiff base (HL^1) is shown in fig. 1. There are two main weight loss stages for the decomposition process. The peak at about 42.83 °C could be assigned as the vaporization of moisture while the peak at 253.83 °C indicates the melting of the ligand. The decomposition progressed to 315 °C, with the temperature of the highest decomposition observed at about 287 °C. The thermogram of the Schiff base (HL^2), reveals a thermal stability of 280 °C with single-stage decomposition temperature with a reaction interval of 60 °C. The compound was stable up to 260 °C and its decomposition started at 272 °C and finally completed at 340 °C, with the temperature of the highest decomposition observed at about 316 °C (fig. 2) [4, 12].

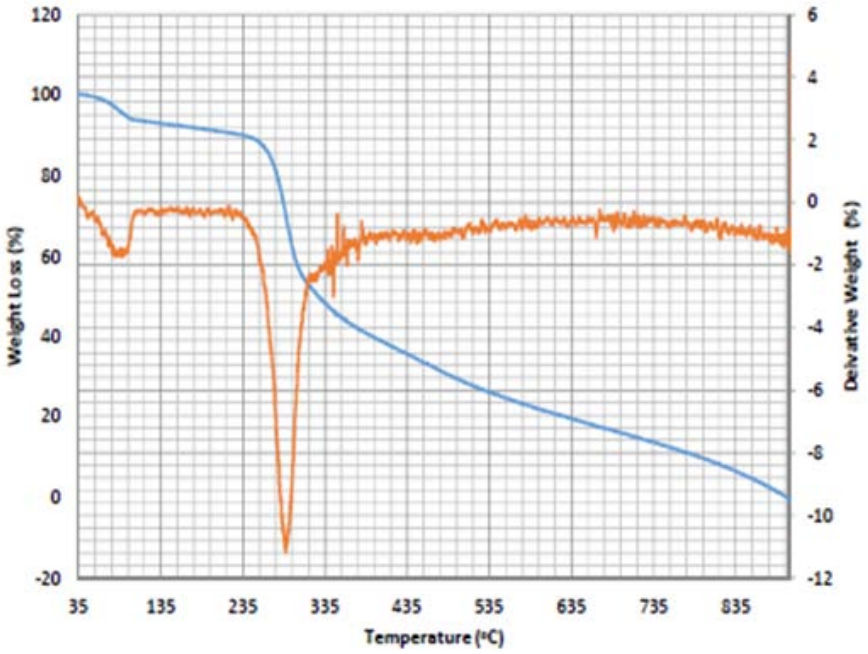


Fig. 1: TG/DTG curves for Schiff base, HL^1: *N*′-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide

Mn(acac)(L^1) was stable up to 60 °C and its decomposition started at 62 °C and finally completed at 425 °C. In the DTA thermogram of the Mn(acac)(L^1) reveals four endothermal peaks caused by de-nitration (NO_2), diethylamino, acetylacetonate and Schiff ligand molecule, the remaining mass seems likely to correspond to Mn_2O_3 (fig. 3). Mg(acac)(L^2) weight loss started at about 85 °C revealing four endothermal peaks corresponding to the removal of methoxylation, diethylamino, acetylacetonate and Schiff ligand molecule, the remaining mass seems likely to correspond to MgO as a residue (fig. 4) [4, 10]. However, the number of decomposition processes (stages) gives an indication of the degree of purity synthesised Schiff base ligands and their corresponding metal complexes [12, 20].

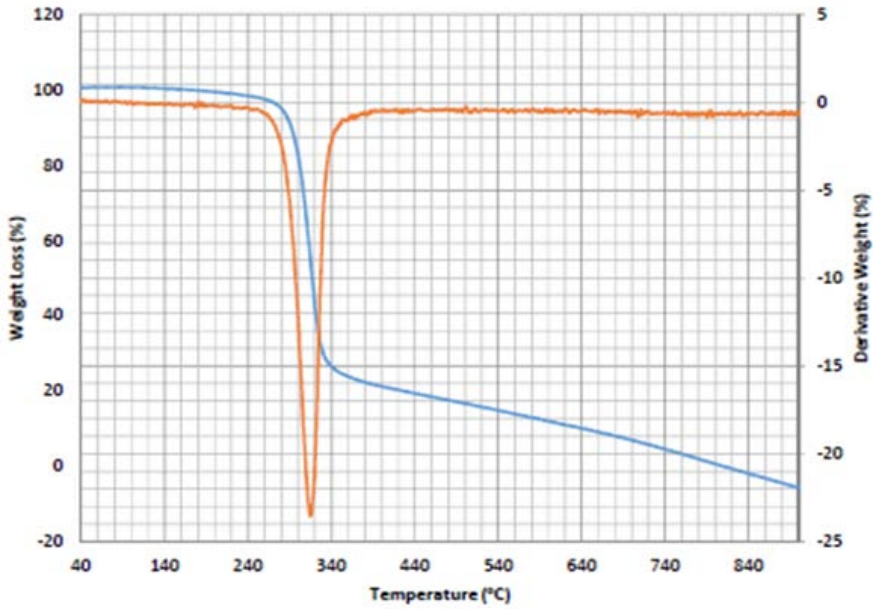


Fig. 2: TG/DTG curves for Schiff base, HL^2: *N*′-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide

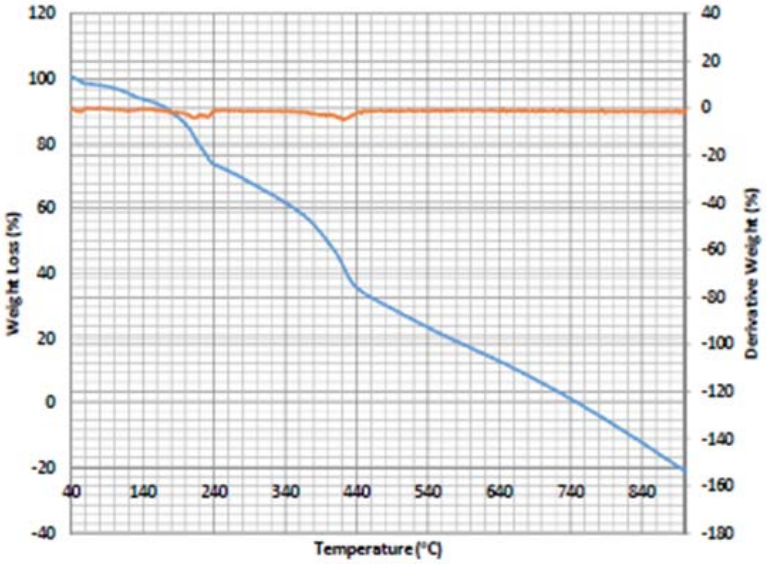


Fig. 3: TG/DTG curves for Mn(acac)(L¹): Manganese acetylacetonato(*N*′-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide)

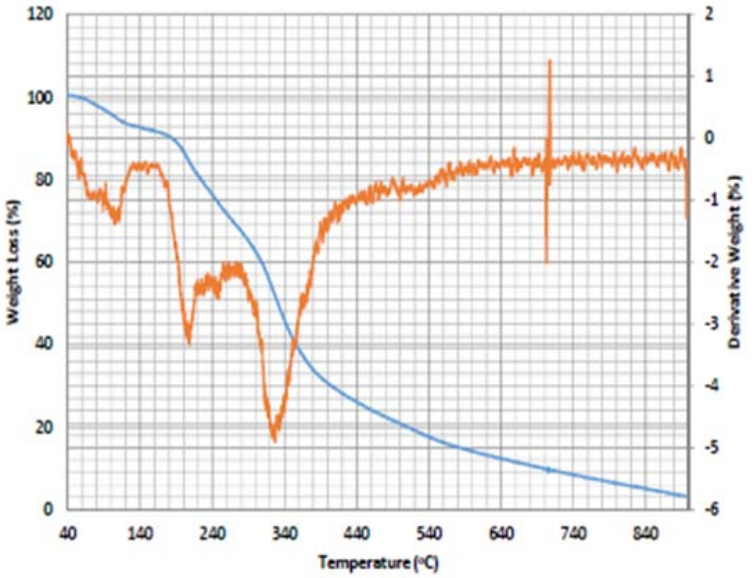


Fig. 4: TG/DTG curves for Mg(acac)(L²): Magnesium acetylacetonato(*N*′-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide)

¹H NMR and ¹³C NMR spectral studies

Proton and carbon-13 nuclear magnetic resonance spectroscopy of Schiff base ligands and their metal complexes were measured in d_6-DMSO solution at room temperature with tetramethylsilane (TMS) as an internal standard.

¹H NMR spectrum of Schiff base (HL^1) reveals the presence of a phenolic proton at δ 12.07 ppm (1H, s). While the signal due to the NH group appeared at δ 11.32 ppm (1H, s), azomethine proton (–HC=N) at δ 8.46 ppm (1H, s), aromatic protons at δ 8.38 (1H, d), δ 8.36 (1H, d), δ 8.16 (1H, d) ppm. Many signals appearing as multiplets in the δ 6.29-7.23 ppm region are due to the aromatic protons, methylene protons at the range δ 3.34-3.39 ppm (multiplets), methyl protons at 1.11(3H, t) ppm **(**fig. 5). In the ¹³C NMR spectrum, the signal of 161.0 ppm is due to the phenolic carbon, amide carbon at δ 160.3 ppm, δ 151.3 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 150.8, 149.7, 139.4, 132.7, 130.0, 124.1, 106.8, 104.3 ppm, methylene carbon at δ 44.3 ppm, methyl carbon at δ 13.01 ppm **(**fig. 6) [4, 14, 17].

Similarly, ¹H NMR spectrum of Schiff base (HL^2) show some signals at δ 11.76 ppm (1H, s) that is attributed to the phenolic proton. While the signal due to the NH group appeared at δ 11.27 ppm (1H, s), azomethine proton (–HC=N) at δ 8.43 ppm (1H, s), aromatic protons at δ 7.50 (1H, d), δ 7.42 (1H, d), δ 7.20 (1H, d) ppm. Many signals appearing as multiplets in the δ 6.26-7.18 ppm region are due to the aromatic protons, methoxy proton 3.84 (3H, s) ppm, methylene protons at the range δ 3.33-3.38 ppm (multiplets), methyl protons at 1.11(3H, t) ppm **(**fig. 7). In the ¹³C NMR spectrum, the signal of 162.41 ppm is due to the phenolic carbon, amide carbon at δ 160.2 ppm, δ 159.7 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 150.6, 150.4, 136.0, 132.0, 130.5, 120.1, 119.0, 113.2, 108.6, 104.1 ppm, methoxy carbon at 97.4 ppm, methylene carbon appeared at 55.3 and 44.3 ppm, methyl carbon at δ 13.02 ppm **(**fig. 8) [4, 14, 17].

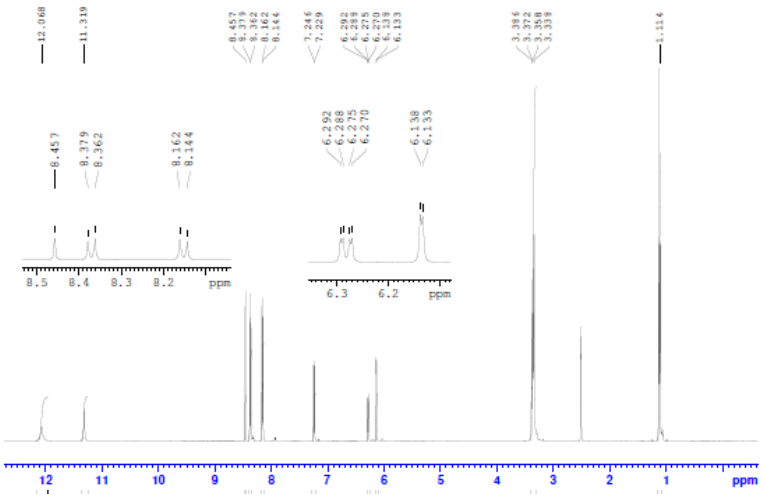


Fig. 5: Proton NMR for Schiff base, HL¹: *N′*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide

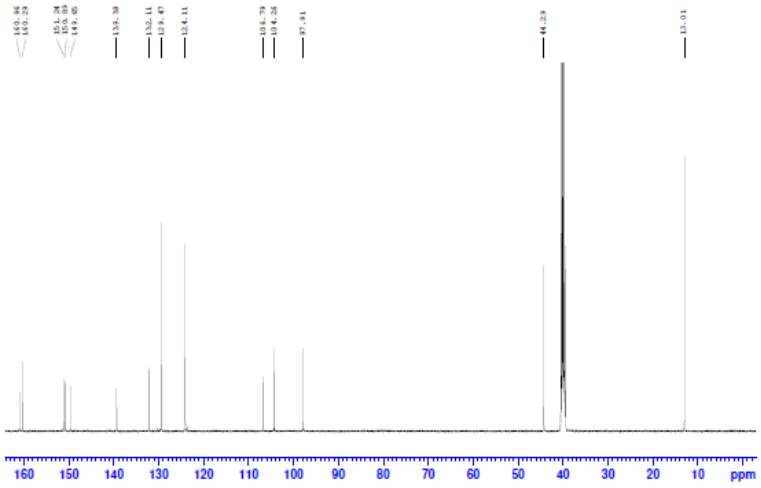


Fig. 6: Carbon-13 NMR for Schiff base, HL¹: *N′*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-nitrobenzohydrazide

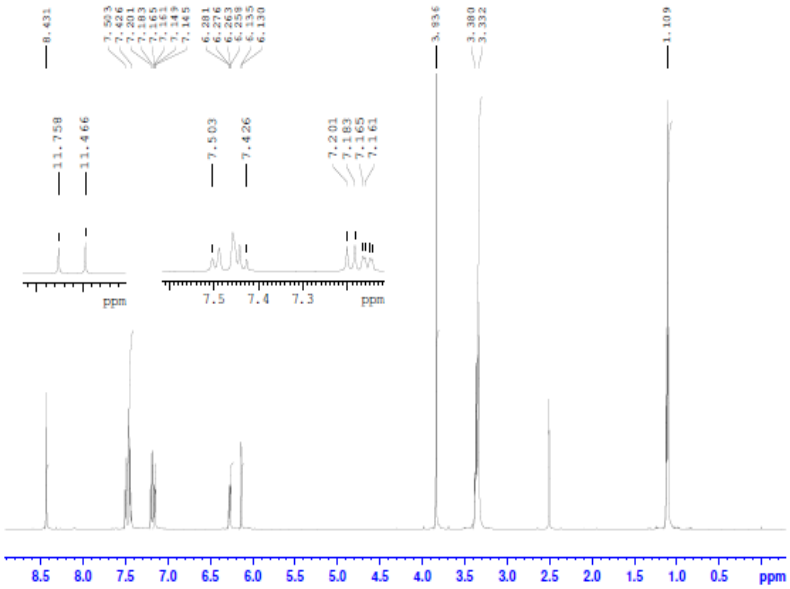


Fig. 7: Proton NMR for Schiff base, HL²: *N′*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide

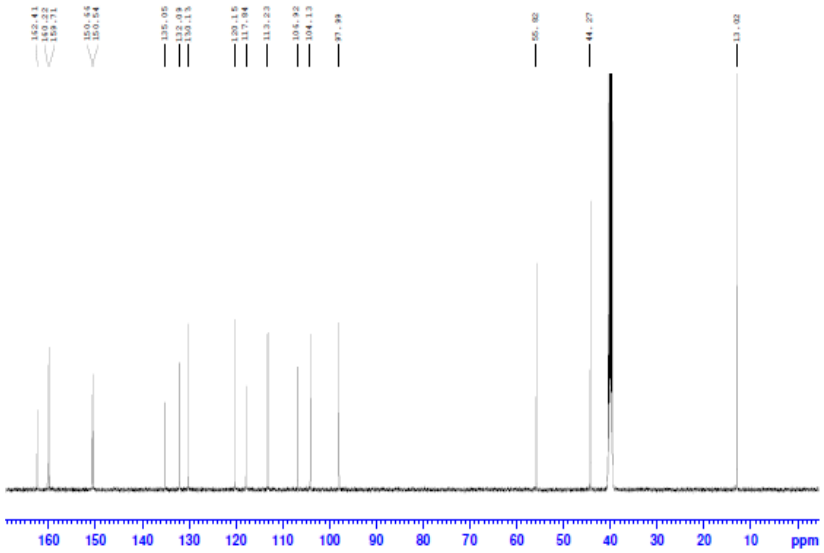


Fig. 8: Carbon-13 NMR for Schiff base, HL^2: *N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl] methylidene}-4-methoxybenzohydrazide

The spectrum of the complexes differs from that of the free ligands as explained. The disappearance of the signal due to the phenolic OH group is attributed to its involvement in coordination sphere with the corresponding metal ions [12, 17]. The ^{1}H NMR spectrum of the complex: Co(acac)(L^1) exhibited singlet signals at δ 12.06 ppm which could be attributed to the proton of an amide nitrogen. The azomethine proton signal at δ 8.71 ppm (1H, s), in the spectrum of the cobalt complex is shifted downfield compared to the free ligand, suggesting deshielding of the azomethine group due to the coordination with metal ions [21]. Aromatic protons at δ 8.45 (1H, s), δ 8.99 (1H, s), δ 8.37 (1H, s), δ 8.16 (1H, s), signals appearing as multiplets in the δ 6.14-7.26 ppm region are due to the aromatic protons, methylene protons at the range δ 2.51-3.36 (multiplets), methyl protons at δ 2.04 (3H, d), δ 1.11 (3H, t) ppm. The ^{13}C spectrum reveals the presence of ketonic carbon at δ 189.00 ppm, the signal of δ 167.3 ppm is due to the phenolic carbon, amide carbon at δ 160.9 ppm, δ 160.2 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 150.9, 149.6, 139.4, 132.1, 129.5, 124.1, 104.3 ppm, methylene carbon at δ 44.3, 26.0 ppm, methyl carbon at δ 13.01 ppm [4, 14, 17].

The ^{1}H NMR spectrum of the complex: Mn(acac)(L^1) exhibited singlet signals at δ 11.83 ppm which could be attributed to the proton of an amide nitrogen, azomethine proton signal at δ 8.38 ppm (1H, s), aromatic protons at 8.36 (1H, s), δ 8.33 (1H, s), δ 8.15 (1H, s), δ 8.13 (1H, s), signals appearing as multiplets in the δ 6.77-7.58 ppm region are due to the aromatic protons, methylene protons at the range δ 2.51-3.33 (multiplets), methyl protons at δ 2.06 (3H, d), δ 1.22 (3H, t) ppm. The ^{13}C spectrum reveals the presence of ketonic carbon at δ 189.01 ppm, the signal of δ 161.4 ppm is due to the phenolic carbon, amide carbon at δ 161.2 ppm, δ 152.2 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 150.3, 149.6, 140.0, 131.1, 129.5, 129.1, 123.3, 121.7, 112.3 ppm, methylene carbon at δ 26.0 ppm, methyl carbon at δ 13.2 ppm [4, 14, 17, 21].

The ^{1}H NMR spectrum of the complex: Mg(acac)(L^1) exhibited singlet signals at δ 11.36 ppm which could be attributed to the proton of an amide nitrogen, azomethine proton signal at δ 8.44 ppm (1H, s), aromatic protons at 8.36 (1H, s), δ 8.35 (1H, s), δ 8.17 (1H, s), δ 8.15 (1H, s), signals appearing as multiplets in the δ 6.13-7.23 ppm region are due to the aromatic protons, methylene protons at the range δ 2.51-3.35 (multiplets), methyl protons at δ 2.14 (3H, d), δ 1.72 (3H, t), δ 1.11 (3H, t) ppm. The ^{13}C spectrum reveals the presence of ketonic carbon at δ 188.7 ppm, the signal of δ 161.0 ppm is due to the phenolic carbon, amide carbon at δ 160.3 ppm, δ 151.3 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 150.9, 149.6, 139.4, 132.1, 129.5, 124.1, 106.8, 104.2 ppm, methylene carbon at δ 44.3, 28.15 ppm, methyl carbon at δ 13.01 ppm [4, 14, 17, 21].

The ^{1}H NMR spectrum of the complex: Co(acac)(L^2) exhibited singlet signals at δ 11.41 ppm which could be attributed to the proton of an amide nitrogen, azomethine proton signal at δ 8.30 ppm (1H, s), aromatic protons at 7.89 (1H, s), δ 7.88 (1H, s), δ 7.54 (1H, s), δ 7.52 (1H, s), signals appearing as multiplets in the δ 6.75-7.05 ppm region are due to the aromatic protons, methoxy proton 3.83 (3H, s) ppm, methylene protons at the range δ 2.51-3.32 (multiplets), methyl protons at δ 2.05 (3H, d), δ 1.02 (3H, t) ppm (fig. 9). The ^{13}C spectrum reveals the presence of ketonic carbon at δ 189.01 ppm, the signal of δ 162.6 ppm is due to the phenolic carbon, amide carbon at δ 162.2 ppm, δ 151.9 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 148.5, 129.8, 128.8, 126.3, 122.3, 112.3 ppm, methoxy carbon at 97.3 ppm, methylene carbon at δ 55.9 and 26.0 ppm, methyl carbon at δ 13.3 ppm (fig. 10) [4, 14, 17, 21].

The ^{1}H NMR spectrum of the complex: Mn(acac)(L^2) exhibited singlet signals at δ 11.43 ppm which could be attributed to the proton of an amide nitrogen, azomethine proton signal at δ 7.86 ppm (1H, s), aromatic protons at 7.73 (1H, s), δ 7.48 (1H, s), δ 7.26 (1H, s), δ 7.17 (1H, s), signals appearing as multiplets in the δ 6.05-7.00 ppm region are due to the aromatic protons, methoxy proton 3.79 (3H, s) ppm, methylene protons at the range δ 2.51-3.18 (multiplets), methyl protons at δ 2.09 (3H, d), δ 1.10 (3H, t) ppm. The ^{13}C spectrum reveals the presence of ketonic carbon at δ 188.4 ppm, the signal of δ 162.2 ppm is due to the phenolic carbon, amide carbon at δ 161.4 ppm, δ 151.9 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 145.5, 130.0, 128.9, 124.3, 114.1, 112.3 ppm, methoxy carbon at 95.1 ppm, methylene carbon at δ 55.9 and 25.8 ppm, methyl carbon at δ 13.14 ppm [4, 14, 17, 21].

The ^{1}H NMR spectrum of the complex: Mg(acac)(L^2) exhibited singlet signals at δ 11.41 ppm which could be attributed to the proton of an amide nitrogen, azomethine proton signal at δ 8.30 ppm (1H, s), aromatic protons at 7.90 (1H, s), δ 7.88 (1H, s), δ 7.54 (1H, s), δ 7.52 (1H, s), signals appearing as multiplets in the δ 6.75-7.05 ppm region are due to the aromatic protons, methoxy proton 3.83 (3H, s) ppm, methylene protons at the range δ 2.50-3.33 (multiplets), methyl protons at δ 1.71 (3H, d), δ 1.22 (3H, t) ppm. The ^{13}C spectrum shows the presence of ketonic carbon at δ 188.67 ppm, the signal of δ 162.58 ppm is due to the phenolic carbon, amide carbon at δ 162.26 ppm, δ 151.92 ppm (C=N) is due to carbon atom in the azomethine groups, aromatic carbons at δ 148.54, 129.82, 128.8, 126.33, 122.26, 112.30 ppm, methoxy carbon at 99.0 ppm, methylene carbon at δ 55.87 and 28.17 ppm, methyl carbon at δ 15.7 ppm [4, 14, 17, 21].

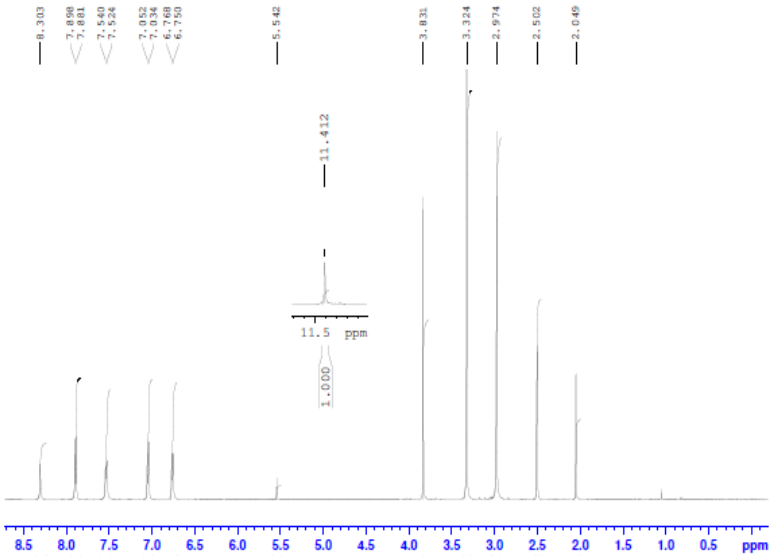


Fig. 9: Proton NMR for Co(acac)(L²): cobalt acetylacetonato(*N′*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide)

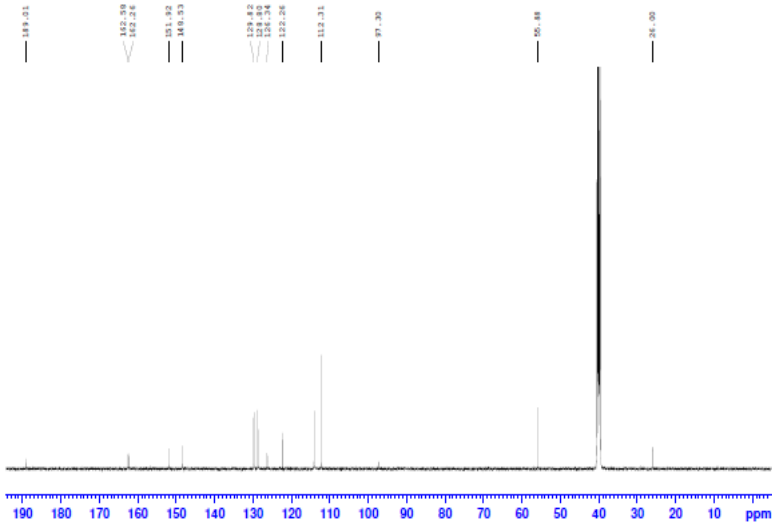


Fig. 10: Carbon-13 NMR for Co(acac)(L²): cobalt acetylacetonato(*N′*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide)

Biological evaluation

The as-synthesized compounds were evaluated for *in vitro* antimicrobial activity. The activity was tested against two Gram-positive bacteria, including *Staphylococcus aureus* and *Enterococcus faecalis*, using established antimicrobial agents as standards. The well-diffusion and disc diffusion methods were employed in screening the Schiff base ligands and their complexes for their sensitivity and minimum inhibitory concentration (MIC) respectively. Standard antibacterial compounds: ciprofloxacin and ampicillin were used as positive control. Many of the complexes and the ligands had no antimicrobial effects on both the environmental and control bacterial strains. The results of the biological studies are displayed in fig. 11 and 12.

The complexes showed more antibacterial activity than the free ligands. The screening data (zone of Inhibition) indicated that Mn(acac)(L²), with a 4-diethylamino and 4-methoxy group on the benzaldehyde and benzohydrazide rings respectively, showed the best antibacterial activity against *E. faecalis* and *S. aureus* among the synthesised Schiff base complexes (fig. 11 and 12). The compound: Mn(acac)(L²) effectiveness distinction is dependent either on the cell microbes' permeability or differences in the cells ribosomes [14]. The compound activity against all the species of the studied bacterial strains can be explained by Overtone's concept and Tweedy's chelation theory [4, 14]. With respect to Overtone's concept of cell permeability, the lipid materials surrounding the cell allow the passage of only the lipidsoluble materials leading to liposolubility and permeability through the lipid layer of cell membranes as a significant feature of antimicrobial activity [9, 13, 22]. Tweedy chelation theory [9, 12] supports the reduction of metal ion polarity to a considerable extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Chelation increases the delocalization of π-electrons within the chelate ring and enhances the lipophilicity of the complexes. This amplified lipophilicity enhances the penetration of the complex into lipid membranes, thereby, blocking of the metal binding sites in the enzymes of microorganisms. The compound also disturbs the respiratory process of the cell, thereby, hindering the synthesis of proteins, and restricts further organism growth [4, 9, 13].

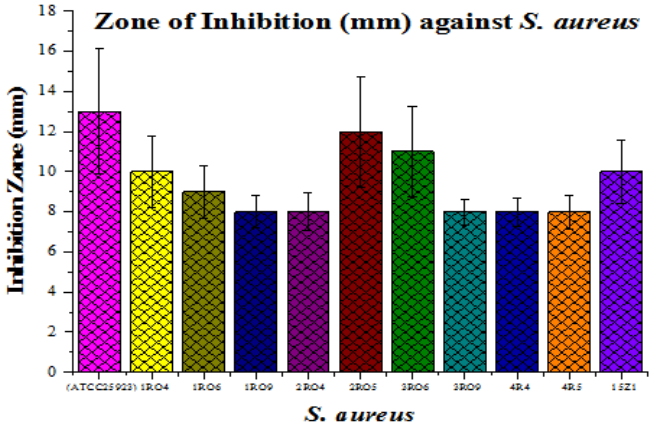


Fig. 11: Zone of Inhibition of manganese complex against *Staphylococcus aureus,* Mn(acac)(L²)-Manganese acetylacetonato(*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide); mm-millimetre; *S. aureus-Staphylococcus aureus*; (ATCC 25923)-*S. aureus*; (1RO4)-*S. aureus*; (1RO6)-*S. aureus*; (1RO9)-*S. aureus*; (2RO4)-*S. aureus*; (2RO5)-*S. aureus*; (3RO6)-S. aureus; (3RO9)-*S. aureus*; (4R4)-*S. aureus*; (4R5)-*S. aureus* and (15Z1)-*S. aureus*, n = 3, X±SEM

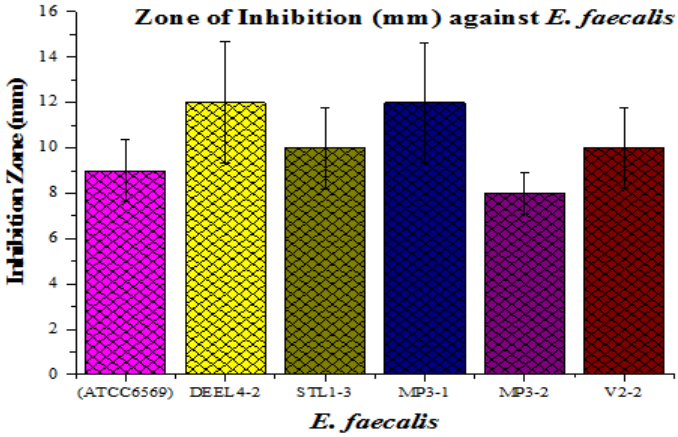


Fig. 12: Zone of Inhibition of manganese complex against *Enterococcus faecalis,* Mn(acac)(L²)-Manganese acetylacetonato(*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide); mm-millimetre; *E. faecalis-Enterococcus faecalis*; (ATCC 6569)-*E. faecalis*; (DEEL4-2)-*E. faecalis*; (STL1-3)-*E. faecalis*; (MP3-1)-*E. faecalis*; (MP3-2)-*E. faecalis* and (V2-2)-*E. faecalis*, n = 3, X±SEM

In addition, the antibacterial activities of the discussed compounds depends on the kind of substituent on the benzo hydrazide rings at the para position. The compound with electron donating (methoxy) group on the benzo hydrazide rings at the para position showed the higher activity than the one with electron withdrawing (nitro) [23]. Schiff base complex Mn(acac)(L²) exhibited antimicrobial activities against both *E. faecalis* and *S. aureus* bacteria tested with a MIC of 398.0 µg/ml (table 4). However, Mn(acac)(L²) show lower antibacterial activity when compared to ciprofloxacin and ampicillin as standard drugs but their activities on preventing the growth of *E. faecalis* and *S. aureus* bacteria is satisfactory making them potential anti-Staphylococcus and anti-Enterococcus lead compounds.

Table 4: Antibacterial activity of manganese complex and standard agents

S. aureus	MnL²	Amp.*	Cipro.*	*E. faecalis*	MnL²	Amp.*	Cipro.*
	(MIC, µg/ml)				(MIC, µg/ml)		
(ATCC25923)	398.0	247.2	247.2	(ATCC6569)	398.0	247.2	305.3
1RO4	398.0	247.2	247.2	DEEL4-2	398.0	247.2	305.3
1RO6	398.0	247.2	247.2	STL1-3	398.0	247.2	305.3
1RO9	398.0	247.2	247.2	MP3-1	398.0	247.2	305.3
2RO4	398.0	247.2	247.2	MP3-2	398.0	247.2	305.3
2RO5	398.0	247.2	247.2	V2-2	398.0	247.2	305.3
3RO6	398.0	247.2	247.2				
3RO9	398.0	247.2	247.2				
4R4	398.0	247.2	247.2				
4R5	398.0	247.2	247.2				
15Z1	398.0	247.2	247.2				

Mn-Manganese; L²-*N'*-{(*E*)-[4-(diethylamino)-2-hydroxyphenyl]methylidene}-4-methoxybenzohydrazide; *S. aureus-Staphylococcus aureus*; (ATCC 25923)-*S. aureus*; (1RO4)-*S. aureus*, (1RO6)-*S. aureus*, (1RO9)-*S. aureus*, (2RO4)-*S. aureus*, (2RO5)-*S. aureus*, (3RO6)-S. aureus; (3RO9)-*S. aureus*; (4R4)-*S. aureus*; (4R5)-*S. aureus*; (15Z1)-*S. aureus*; *E. faecalis-Enterococcus faecalis*; (ATCC 6569)-*E. faecalis*; (DEEL4-2)-*E. faecalis*; (STL1-3)-*E. faecalis*; (MP3-1)-*E. faecalis*; (MP3-2)-*E. faecalis*; (V2-2)-*E. faecalis*; Amp.-ampicillin; Cipro.-ciprofloxacin; *-Standards; MIC-Minimum Inhibitory Concentrations; µg/ml-microgram/millimetre.

CONCLUSION

The Schiff bases (HL^1–HL^2), derived from the condensation reaction of 4-(diethylamino)-2-hydroxybenzaldehyde and 4-nitro-benzohydrazide/or 4-methoxybenzohydrazide. The Schiff base metal complexes synthesized via metal acetylacetonates with the Schiff base ligands reaction were characterized. From the spectra, it can be concluded that the ligands acted as bidentate moieties, binding to the metal ions: cobalt, manganese, and magnesium in the complexes through azomethine N and phenolic O. The biological activity revealed that Mn(acac)(L^2) complex exhibited good inhibition against all the *E. faecalis* and *S. aureus* strains used, but lower antibacterial activity when compared to the ciprofloxacin and ampicillin (standard antibiotic) used in this study. In addition, the antibacterial activity of the compound depends on the kind of substituent on the benzo hydrazide rings at the para position. The compound bearing the Schiff base with electron donating (methoxy) group on the benzo hydrazide rings at the para position showed the higher activity than the Schiff base with electron withdrawing (nitro). The obtained results suggest the compound as promising chemotherapeutic agents for further structural optimization.

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AUTHORS' CONTRIBUTION

Charity W. Dikio carried out the experiment and analysis. Ikechukwu P. Ejidike researched and wrote the article. Fanyana M. Mtunzi supervised the project and revision. Michael J. Klink provided support towards the project. Ezekiel D. Dikio provided the necessary guidance and critical review.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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