



Synthesis, Spectroscopic, Antibacterial and Free radical scavenging studies of Cu(II), Ni(II), Zn(II) and Co(II) complexes of 4,4'-{ethane-1,2-diybis[nitrilo(1*E*)eth-1-yl-1-ylidene]}dibenzene-1,3-diol Schiff base

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Abstract

The present study focuses on the synthesis and spectroscopic studies of metal (II) complexes of symmetrical tetradentate Schiff-base ligand (N_2O_2), evaluation of their antibacterial activities against pathogenic microorganisms and their antioxidant potentials.

Copper(II), cobalt(II), zinc(II) and nickel(II) complexes of 4,4'-{ethane-1,2-diybis[nitrilo(1*E*)eth-1-yl-1-ylidene]}dibenzene-1,3-diol (OpdH₂) obtained from the condensation reaction of 4-acetylresorcinol and ethylene-1,2-diamine were synthesized and characterized by elemental analysis, UV-visible, FT-IR, ¹H-NMR and molar conductivity measurements. The compounds were screened for their antimicrobial properties against six Gram-positive and Gram-negative bacteria by agar diffusion method, the DPPH and ABTS radical scavenging assays were also investigated.

The FTIR spectra revealed that the Schiff base acts as tetradentate chelating ligand via nitrogen of the azomethine and phenolic oxygen atoms and electronic spectra studies indicate that the Co(II) complex is in tetrahedral geometry while Ni(II) and Cu(II) complexes are in square planar geometry. The complexes showed appreciable antibacterial potentials against *S. aureus*, *B. cereus*, *S. faecalis*, *E. coli*. DPPH scavenging potential showed that copper complex was more active than rutin (standard) but lower than that of Vit C. with an IC₅₀ value of 1.92±1.07 μM.

The results suggest that the complexes displayed higher antimicrobial activities compare to free ligand and possess antioxidant properties with scavenging activity pattern: Zn(II) < Ni(II) < Co(II) < Cu(II) on both ABTS and DPPH radicals, hence, the compounds may be useful as therapeutic agents.

Keywords: 4-acetylresorcinol, Schiff base complexes, Spectral studies; Antibacterial, Radical scavengers.

INTRODUCTION:

Schiff bases have been widely studied in recent years due to its easy preparation procedures and potential applications [1, 2]. Schiff bases containing nitrogen and oxygen donor atoms generally acts as tetradentate chelating ligands to different metal ions [3, 4] and have shown potential as antibacterial, antiviral, antifungal and anticancer agents [5, 6]. Metal complexes containing Schiff base are of interest because of their electronic properties that can be modified by suitable functionalization with amine and cyclic substituents [7-10]. Also, tetradentate Schiff base complexes have shown high stability when coordinated to metal ion using the N_2O_2 donor atoms [11-14]. Hence, they are used as structural probes in chemistry of nucleic acid [15], as antimicrobial and anticancer agents [16-18]. Reactive oxygen species (ROS) or free radicals are products of the *in vivo* physiological and bio-chemical processes in the living cells [19] that could cause lipid peroxidation [19] and may lead to diseases such as cancer, cardiovascular diseases, immunodeficiency, liver injury and other infections [20]. In order to safeguard the human body against cell damage by oxidative species, antioxidant is of paramount importance [21, 22]. They protect different cells in human body, such as hepatocytes, myocytes, erythrocytes, endothelial cells, against oxidative damages

[23]. The present study report the synthesis of copper(II), zinc(II), cobalt(II) and nickel(II) complexes containing symmetrical tetradentate Schiff-base ligand (N_2O_2) obtained from the condensation reaction of 4-acetylresorcinol and ethylene-1,2-diamine. Characterized by elemental analysis, molar conductivity measurements, UV-visible spectrophotometry, Fourier transform infrared (FT-IR) spectroscopy, proton NMR spectroscopy. The ligand and its metal complexes were screened for their antimicrobial properties against some bacteria isolates and their DPPH and ABTS radical scavenging assays were potentials were also evaluated.

MATERIALS AND METHODS:

Chemicals and solvents were of analytical grade, and used as obtained without any further purification. Ascorbic acid, ethylenediamine and the metal salts were received from Merck, South Africa; 2',4'-dihydroxyacetophenone was purchased from Sigma Aldrich, South Africa; butylated hydroxytoluene (BHT), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), rutin hydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), were received from Sigma Chemical Co., USA. Elemental analyses were recorded on Perkin Elmer elemental analyser (2400 Series). Freshly prepared 10⁻³ M DMF solutions with Crison EC-

meter basic 30+ conductivity cell was used for the conductivity measurements. Perkin Elmer FT-IR spectrometer (Spectrum 2000) in the range 4000–400 cm^{-1} using KBr pellets was used for IR spectra data collection. ^1H NMR spectra were obtained with a Bruker Avance DPX-600 spectrophotometer in d_6 -DMSO and reported relative to TMS as internal standard. Absorption data were documented with Perkin Elmer Lambda-25 UV-visible spectrometer ranging from 200–800 nm. Stuart melting Point (SMP 11) was used for the melting points determination.

Preparation of the ligand: 4,4'-{ethane-1,2-diylbis[nitrilo(1E)eth-1-yl-1-ylidene]}dibenzene-1,3-diol (OpdH₂)

The ligand was prepared according to literature with little modification [3]. An ethanol solution (25 ml) of 1,2-ethanediamine (0.01 mol) was carefully added to a stirred pre-warmed ethanolic solution of 4-acetylresorcinol (0.02 mol), and then reflux for 3 h; it was further stirred for 8 h at room temperature after which it was filtered, washed with ethanol and air-dried to give the desired product as brownish-yellow solid (Yield = 76.52 %, 2.51 g).

Synthesis of metal Schiff base complexes

The metal complexes were obtained by adding 0.5 mmol of $\text{Co}(\text{acet})_2 \cdot 4\text{H}_2\text{O}$, $\text{Zn}(\text{acet})_2 \cdot 2\text{H}_2\text{O}$, $\text{Cu}(\text{acet})_2 \cdot 2\text{H}_2\text{O}$, $\text{Ni}(\text{acet})_2 \cdot 4\text{H}_2\text{O}$ dissolved in 25 ml of (4:6 v/v) methanol-water, into a warm methanolic solution (25 ml) of (0.5 mmol, 0.1642 g) OpdH₂. The solution was then reflux for 3 h and allowed to cool to room temperature. The filtered precipitate was carefully washed with methanol and diethyl ether, dried over dry calcium chloride (CaCl_2).

In vitro antimicrobial assay

The synthesized compounds were tested to assess their growth inhibitory activities against six bacteria strains by agar diffusion method [24]. *Staphylococcus aureus* (ATCC 25923), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702)-Gram-positive bacteria and *Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 25922) and *Shigella flexneri* (KZN)- Gram-negative bacteria, while ciprofloxacin and amoxicillin were used as standards. Sub-culturing of the bacteria isolates on nutrients agar (SAARCHEM, Gauteng SA) plates was done by incubating at 37 °C for 24 h. 50 ml of nutrient broth in a 250 ml flask was stirred vigorously to incubate the bacteria cells from the nutrient agar plates at 37 °C for 16 h. The culture was diluted with fresh media, after incubation to obtain $D_{600\text{nm}}$ of 0.1. After which, 100 μL of the culture cells were drop onto the plate and spread into a bacterial lawn with a sterile glass spreader. Agar dilution method as contained in NCCLS [25] was used for minimum inhibitory concentration (MIC) of the OpdH₂ and Cu(II), Co(II), Zn(II) and Ni(II) complexes as previously described [26].

The antioxidant assay

Scavenging prospects of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity of OpdH₂ and its synthesized metal complexes were determined using a stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent following a previous procedure [26]. DMF solutions (1 ml) of the compounds with various concentrations (100, 200, 300, 400, and 500 $\mu\text{g}/\text{ml}$) was mixed thoroughly with 1 ml of methanolic solution of 0.4 mM DPPH and allowed to interact for about 30 min in dark. Ascorbic acid and rutin hydrate were used as standards for control experiment. Reduction in absorption of the solutions was measured spectrophotometrically at 517 nm against the control. The equation below was used to obtain the percentage scavenged DPPH radical:

Percentage scavenging activity

$$= \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS radical scavenging potentials

Metal compounds and OpdH₂ ABTS scavenging ability was studied using previously described method [27]. Two stock solutions in equal amounts: 7 mM ABTS solution and 2.4 mM potassium persulfate solution was mixed to obtain the working solution and left in the dark for 12 h for complete reaction. In order to obtain an absorbance of 0.706 ± 0.001 units spectrophotometrically at 734 nm, 1 ml of ABTS* was diluted. The scavenging properties of the samples were determined alongside the standard drugs: butylated hydroxyl toluene (BHT) and rutin hydrate for the control experiment. Triplicates analysis was carried out and averaged the results. The ABTS percentage inhibition calculated was determined following the equation:

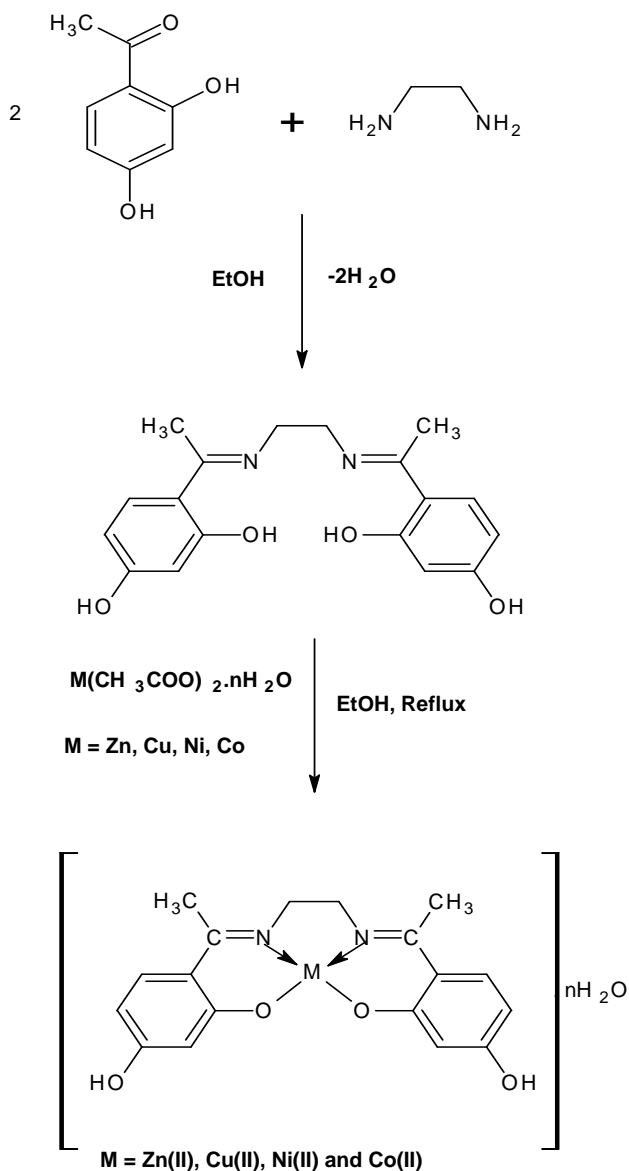
(%) ABTS Inhibition

$$= \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSIONS:

Synthesis and characterization of the compounds

Tetradentate Schiff base ligands bearing N_2O_2 donors have been described to act as a chelating compound exhibiting dianionic properties with transition metal moieties [3, 28]. The brownish-yellow tetradentate N_2O_2 Schiff base viz: 4,4'-{ethane-1,2-diylbis[nitrilo(1E)eth-1-yl-1-ylidene]}dibenzene-1,3-diol was synthesised by the condensation reaction of 4-acetylresorcinol and ethylene-1,2-diamine. The metal complexes were obtained by reaction the metal acetate in methanol with the ligand in a 1:1 mole ratio as shown in Scheme 1. The elemental composition and physico-chemical properties of the compounds are presented in Table 1. The complexes analytical data disclose metal: ligand molar ratio (1:1) for all systems (Scheme 1). The complexes were found soluble in DMF, DMSO and insoluble in other organic medium, with molar conductivity measured in DMF solution indicating a non-electrolyte character.



Where $n = 2$ for Cu(II), Co(II) and Ni(II); $n = 1$ for Zn(II)

Scheme 1: Syntheses and structures of Schiff base ligand (OpdH₂) and its metal complexes

Infrared spectra

The infrared spectra of the ligand were assigned on careful comparison and relevant FTIR data are presented in Table 2. The FTIR spectrum of the ligand showed a broad and weak band at 2678-2800 cm⁻¹ due to hydrogen-bonded O-

H in the free ligand [3, 6, 29]. A strong band at 3475 cm⁻¹ is assigned to $\nu(\text{OH})$ stretching vibrations in the free Schiff ligand. The complexes spectra showed broad bands at ~3420 cm⁻¹ which is due to the $\nu(\text{OH})$ stretching of the H₂O molecules associated with the complexes [17, 30, 31]. The intense band observed at 1616 cm⁻¹ in the free ligand assigned [-HC=N-] $\nu(\text{C}=\text{N})$ stretching vibrations [32-34] was shifted toward higher wavenumbers in the metal complexes spectra. This shifting towards higher wavenumbers around 1617-1620 cm⁻¹ regions is due to the coordination of the azomethine (>C=N) nitrogen atoms with the central metal ions [34, 35]. The disappearance of ligand stretching vibrations around 2678-2800 cm⁻¹ in the complexes indicates the deprotonation of phenolic OH and subsequent coordination to the metal centre. The stretching vibration of the phenolic $\nu(\text{C}-\text{O})$ observed at 1267 cm⁻¹ in the free Schiff base [30, 32] undergo hypochromic shift to 1334-1338 cm⁻¹ regions in the complexes upon complexation as listed in Table 2. This shifts further confirms the coordination of the phenolic oxygen leading to the formation of C-O-M bond [3, 28] (M = Ni, Co, Cu and Zn). There was consistency in the aromatic ring vibrations $\nu(\text{C}=\text{C})$ for all the compounds indicating it was not affected during complex formation [36]. Weak bands observed in the region 474-536 cm⁻¹ in the complexes are assigned to $\nu(\text{M}-\text{N})$ stretching vibrations while those in the region 407-437 cm⁻¹ are due to $\nu(\text{M}-\text{O})$ [17,30, 32]. The stretching vibrations around 842-850 cm⁻¹ are due to water rocking vibrational modes confirming that the water molecule is not directly bounded to the metal ions [33, 37, 38].

Proton NMR spectrum of the Schiff base ligand

The ¹H NMR data and assignments of 4,4'-{ethane-1,2-diylbis[nitrilo(1*E*)eth-1-yl-1-ylidene]}dibenzene-1,3-diol obtained in d₆-DMSO at room temperature, with tetramethylsilane (TMS) as internal standard. In the spectrum, the peaks at 2.34 ppm (s, 6H, N=C-CH₃) is due to methyl protons of the azomethine group in the Schiff base ligand. The aliphatic protons (s, 4H, aliphatic-CH₂-) in the ethylenediamine chain show singlet peak at 3.83 ppm, and the aromatic protons (m, 6H, aromatic-CH=CH-) appear as multiplet at ~ 6.37 -7.76 ppm. The O-H protons of the phenolic group were observed as singlets at 9.74 ppm (s, 2H, Ortho-OH), and 12.62 ppm (s, 2H, Para-OH) and were shifted downfield due to intramolecular hydrogen bonding [3, 33, 39].

Table 1. Physicochemical and analytical data of [OpdH₂] and its metal complexes

	Empirical formula	F. Wt. (g)	Colour	Yield (%)	% Found (Calcd.)			Dec. Tp, °C	Conduct. (μS cm ⁻¹)
					C	H	N		
OpdH ₂	C ₁₈ H ₂₀ N ₂ O ₄	328.36	Brownish-yellow	76.52	65.73(65.84)	6.28(6.14)	8.71(8.53)	244	-
[Zn(Opd)]·H ₂ O	C ₁₈ H ₂₀ N ₂ O ₅ Zn	409.75	Lemon-yellow	72.11	52.59(52.76)	5.08(4.92)	7.11(6.84)	242	1.70
[Cu(Opd)]·2H ₂ O	C ₁₈ H ₂₂ N ₂ O ₆ Cu	425.93	Dark-purple	74.11	50.93(50.76)	5.02(5.21)	6.74(6.58)	240	1.58
[Ni(Opd)]·2H ₂ O	C ₁₈ H ₂₂ N ₂ O ₆ Ni	421.07	Reddish-brown	65.94	51.16(51.34)	5.45(5.27)	6.39(6.65)	242	2.28
[Co(Opd)]·2H ₂ O	C ₁₈ H ₂₂ N ₂ O ₆ Co	421.32	Grey	63.86	51.48(51.31)	5.52(5.26)	6.46(6.65)	223	2.61

F. Wt. = Formula weight; Dec. Tp. = Decomposition Temperature; Conduct. = Conductance

Table 2. FT-IR spectra of the Schiff base [OpdH₂] and its metal complexes (cm⁻¹)

Compound	$\nu(\text{OH})\cdot\nu(\text{H}_2\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{C})$	$\nu(\text{C}-\text{O})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$
OpdH ₂	3475sb	1616vs	1588s, 1532s	1267s	-	-
[Zn(Opd)]·H ₂ O	3419sb	1617vs	1587s, 1533m	1334s	536m	437w
[Cu(Opd)]·2H ₂ O	3416sb	1620vs	1592s, 1539m	1337m	474m	424m
[Ni(Opd)]·2H ₂ O	3416sb	1618vs	1588m, 1541m	1337m	482m	441m
[Co(Opd)]·2H ₂ O	3416sb	1618vs	1542m, 1534m	1338m	474m	407w

Abbreviations: s = strong; b = broad; v = very; m = medium; w = weak

Absorption spectra studies

The UV-visible spectra of the free ligand (OpdH₂) and the metal complexes [metal = Zn(II), Cu(II), Ni(II) and Co(II)] are shown in Figure 1 and relevant data presented in Table 3. The free ligand spectra exhibits bands at 284 and 309 nm due to aromatic $\pi\text{-}\pi^*$ and imino $\pi\text{-}\pi^*$ transitions respectively [40, 41], while the band at 383 nm is attributed to the $n\text{-}\pi^*$ transitions. In the metal complexes, these absorption bands shifted to higher wavenumbers with increased intensity and this could be ascribed to ligand to metal charge transfer transitions (LMCT) [3, 38].

The Co(II) complex absorption spectrum in the visible region showed one absorption band around 621 nm assigned to $^4\text{A}_2(\text{F}) \rightarrow ^4\text{T}_1(\text{P})$ of a cobalt(II) in a tetrahedral environment [42]. Nickel(II) complexes have been reported to possess varying coordination geometries [30], with absorption spectra that are often characterized by complicated equilibria as a result of diverse structural types [43]. The absorption spectrum of Opd-Ni complex exhibit a band at 403 nm attributable to charge-transfer transitions $\text{L} \rightarrow \text{M}$ (LMCT) and two absorption bands around 434 and 560 nm due to $^1\text{A}_{1g} \rightarrow ^1\text{A}_{2g}$ and $^1\text{A}_{1g} \rightarrow ^1\text{B}_{1g}$ transitions of Ni(II) complex in a square planar geometry [38]. The

observed electronic transitions and reddish-brown colour of the complex further confirms square-planar geometry for Ni(II) complex [26]. Copper complex in the visible region displayed two absorption bands at 559 and 447 nm ascribed to $^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}$ and $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$ transitions of a square-planar copper(II) complex [42]. In generally, Zn(II) complexes possess completely filled d^{10} electronic configuration. Hence, no d-d electronic transition is expected but always shows prominent charge transfer transitions. The Zn(II) complex, showed an absorption band at 376 nm is due to the LMCT transitions of zinc complex in a tetrahedral geometry [44, 45].

Molar conductivity measurements

The conductivity values ($\Lambda\mu$) of the Zn(II), Co(II), Ni(II) and Cu(II) complexes in 10^{-3} DMF solution are listed in Table 1. The results showed molar conductance in the range 1.58–2.28 μScm^{-1} indicating that the compounds are non-electrolytes [46, 47]. The low molar conductivity of the complexes could be due to low ionic mobility of the anionic coordination sphere possessing the bulky size [41].

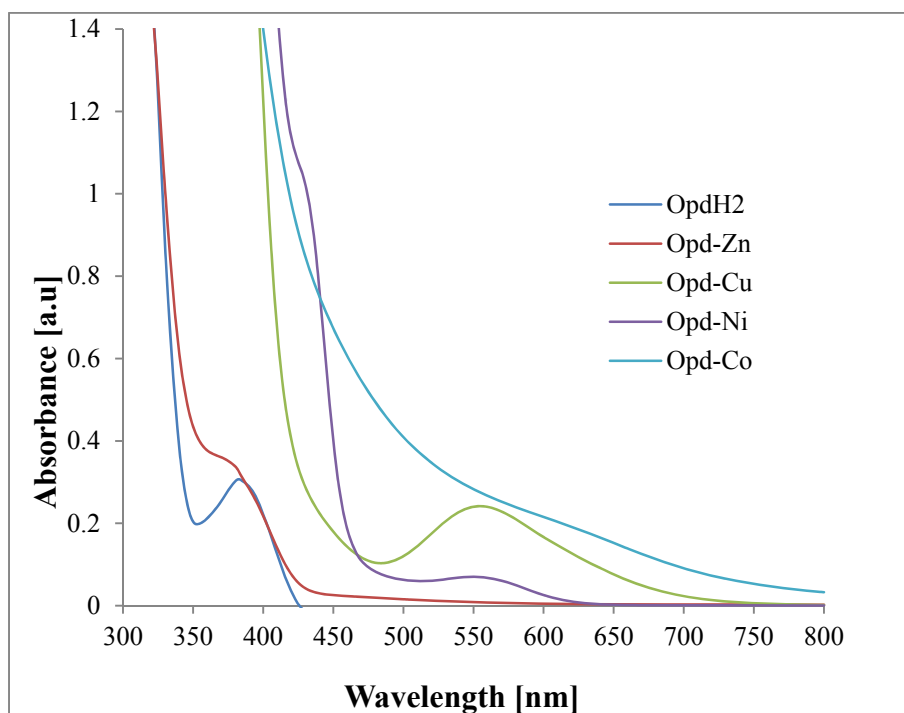


Figure 1. Electronic visible region spectra of Zn(II), Cu(II), Co(II) and Ni(II) complexes of OpdH₂ in 10^{-3} M DMF

Antimicrobial activity

The antimicrobial study of the Schiff base (OpdH₂), nickel(II), zinc(II), cobalt(II) and copper(II) complexes were compared with standards antibiotics, amoxicillin and ciprofloxacin against six bacterial strains: *Streptococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC

25923, *Bacillus cereus* (ATCC 10702)- Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582) and *Shigella flexneri* (KZN)- Gram-negative bacteria using agar diffusion method and DMSO as solvent.

Table 3. Electronic spectra of OpdH₂ ligand and its metal complexes

Compounds		Absorption transition, λ_{\max} (nm, DMF)				Band assignments
OpdH ₂	C ₁₈ H ₂₀ N ₂ O ₄	284,	309,	383		$\pi-\pi^*$, $\pi-\pi^*$, $n-\pi^*$
Zn(Opd)	C ₁₈ H ₂₀ N ₂ O ₅ Zn	285,	314,	381,	444	$\pi-\pi^*$, $\pi-\pi^*$, $n-\pi^*$, L → M (LMCT)
Cu(Opd)	C ₁₈ H ₂₂ N ₂ O ₆ Cu	315,	362,	389,	447,	L → M (LMCT), ${}^2B_{1g} \rightarrow {}^2E_g$, ${}^2B_{1g} \rightarrow {}^2A_{1g}$
Ni(Opd)	C ₁₈ H ₂₂ N ₂ O ₆ Ni	308,	338,	382,	434,	L → M (LMCT), ${}^1A_{1g} \rightarrow {}^1A_{2g}$, ${}^1A_{1g} \rightarrow {}^1B_{1g}$
Co(Opd)	C ₁₈ H ₂₂ N ₂ O ₆ Co	314,	374,	392,	621	L → M (LMCT), ${}^4A_2(F) \rightarrow {}^4T_1(P)$

Table 4. Schiff base [OpdH₂] and its metal complexes minimum inhibitory concentration (MIC) (mg/ml)

Compounds	Gram (+) bacteria			Gram (-) bacteria		
	<i>S. aureus</i>	<i>B. cereus</i>	<i>S. faecalis</i>	<i>P. aeruginosa</i>	<i>S. flexneri</i>	<i>E. coli</i>
OpdH ₂	>10	>10	>10	>10	>10	>10
Zn(Opd)	10	>10	10	>10	>10	>10
Co(Opd)	5	10	10	>10	10	10
Ni(Opd)	10	5	10	10	>10	>10
Cu(Opd)	5	10	5	5	10	5
Amoxicillin ^a	1.250	0.312	1.250	1.250	1.250	0.625
Ciprofloxacin ^a	0.312	0.312	0.312	0.312	0.312	0.312

(n = 3, mean±S.D), ^aStandards

Table 5. Influence of synthesised compounds and standard agents against ABTS* and DPPH* radicals

Compounds	DPPH radical scavenging activity		ABTS radical scavenging activity	
	IC ₅₀ (μM)	R ²	IC ₅₀ (μM)	R ²
OpdH ₂	5.99±1.37	0.953	1.81±1.59	0.843
Zn(Opd)	3.34±1.80	0.962	3.59±1.90	0.874
Cu(Opd)	2.14±1.39	0.979	1.92±1.11	0.920
Ni(Opd)	2.69±1.99	0.990	2.80±1.31	0.888
Co(Opd)	2.47±1.53	0.991	2.47±0.86	0.889
Vit. C*	1.92±1.07	0.978	-	-
Rutin*	2.52±1.60	0.798	2.83±1.84	0.983
BHT*	-	-	1.64±1.54	0.919

(n = 3, mean±SD), IC₅₀- Inhibitory concentration; shows the percent inhibition of the examined compound at 50 %, R² = correlation coefficient. *Standards

Table 4 shows the antimicrobial activities of OpdH₂ and its metal complexes; indicating that the complexes possess higher growth inhibition potential than the free Schiff base ligand. The metal complex activities were lower than that of the standard drugs: amoxicillin and ciprofloxacin. The complexes showed appreciable antibacterial potentials against *S. aureus*, *B. cereus*, *S. faecalis*, *E. coli*. The biological activity of the synthesized compounds are in the order: Cu(II) > Co(II) > Ni(II) > Zn(II) > OpdH₂ (Table 4). The compound efficacy variation is dependent either on the cell microbes' impermeability or differences in the cells ribosomes [48]. Toxicity activity of the metal complexes mechanism is increased and this could be elucidated on the basis of Overtone's theory of cell penetrability in which lipid materials that are soluble are allow to pass through cell lipid membrane as a significant feature of antimicrobial

activity [49] and Tweedy chelation theory [50] that supports the reduction of metal ion polarity to an extent in connection to the ligand orbital and metal ion positive charge partial sharing which enhances the complex lipophilicity [51, 52] as a result of π -electrons delocalization within the chelate ring, disturbing the respiratory activity of the cell and restricts further organism growth [53].

Free scavenging assay of OpdH₂ and complexes

The antioxidant activities of the compounds were evaluated using various concentrations in DMF as solvent, vitamin C, butylated hydroxytoluene (BHT), and rutin hydrate as standards. The results are presented in Table 5 as IC₅₀ values and displayed in Figures 2 and 3 as percentage inhibitions.

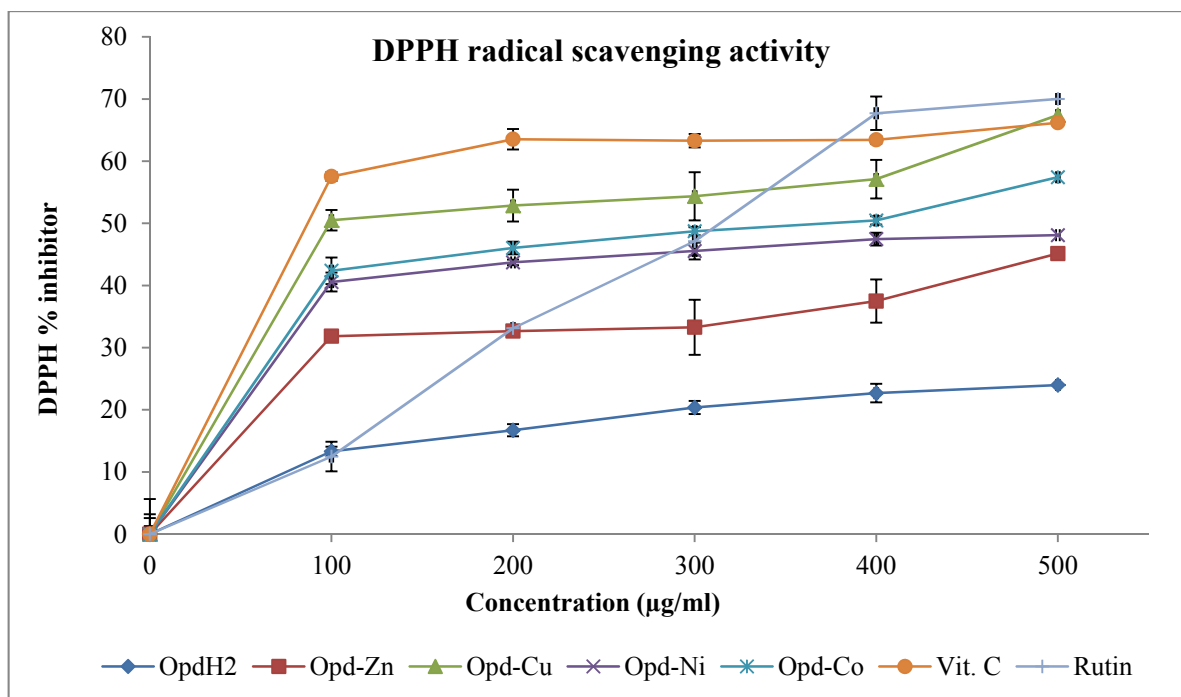


Figure 2. DPPH free radical scavenging assays of OpdH₂ and its metal(II) complexes (n = 3, mean±S.D)

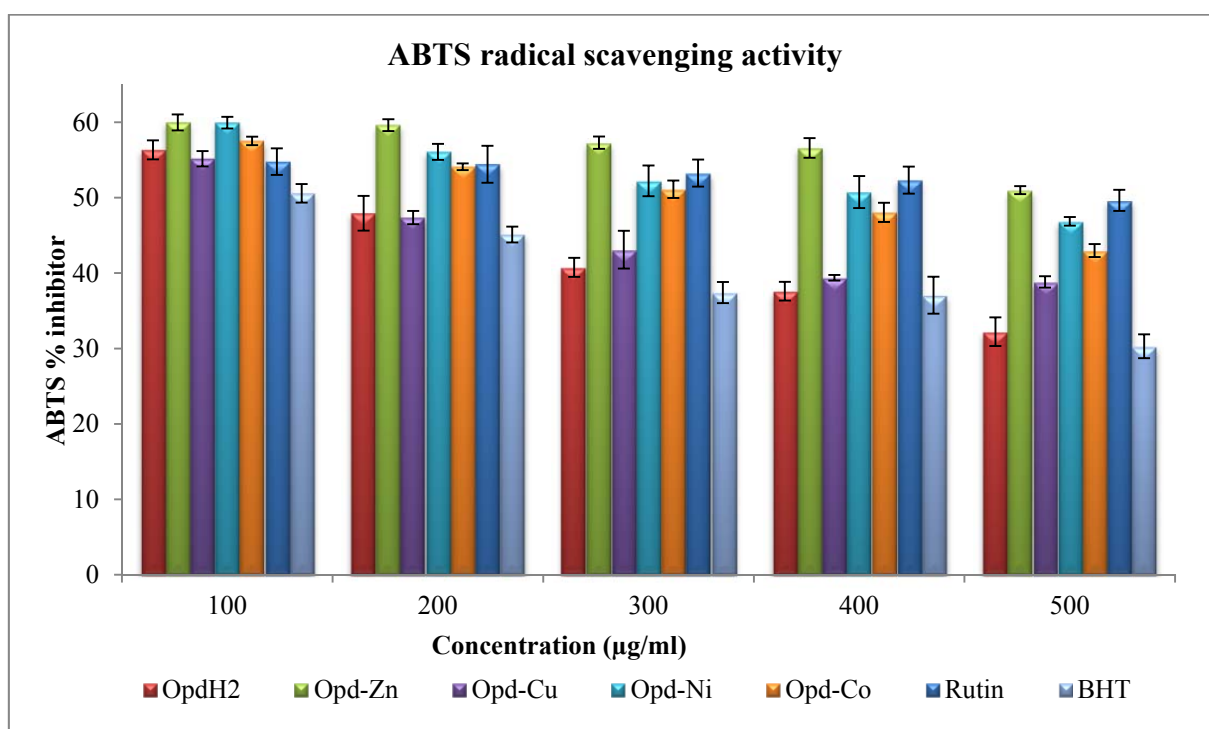


Figure 3. ABTS activities of OpdH₂, its metal(II) complexes and standard drugs (n = 3, mean±S.D)

2,2-diphenyl-1-picrylhydrazyl radical scavenging analysis

The DPPH scavenging effect is based on the absorbance decrease of alcoholic DPPH solution in the existence of proton releasing spicy [54]. The results are shown in Figure 2 and indicate that the ligand (OpdH₂) showed low DPPH activity but the metal complexes showed enhanced scavenging properties. The copper complex displayed the highest DPPH activity than the complexes of cobalt, nickel and zinc. IC₅₀ and its corresponding R² (correlation coefficient) values of the tested compounds are listed in

Table 5. IC₅₀ value of the free ligand is 5.99±1.37 µM whereas, [Opd-Cu], [Opd-Co], [Opd-Ni] and [Opd-Zn] shows an IC₅₀ values of 2.14±1.39, 2.47±1.53, 2.69±1.99 and 3.34±1.80 µM respectively. The results suggest that the metal complexes are better antioxidant than the Schiff base (OpdH₂) (Figure 2). Also, the IC₅₀ values revealed that the scavenging activity of [Opd-Cu] is higher than rutin (standard) but possess lower activity than that of Ascorbic acid with an IC₅₀ value of 1.92±1.07 µM.

ABTS scavenging property of OpdH₂ and Opd-M(II) complexes

To further confirm the synthesized OpdH₂ and Opd-M(II) complex anti-radical potential, we examined the ABTS assay in this study. The assay measures radical scavenging by electron donation. The outcome of OpdH₂ and Opd-M(II) activities on ABTS* radical are presented in Table 5 and displayed in Figure 3. At 734 nm, the absorbance of active ABTS* solution [55] obviously declined upon the addition of different concentrations of OpdH₂ and Opd-M(II) complex, same trend was also observed for the standard drugs: butylated hydroxytoluene (BHT) and rutin hydrate as displayed by the percentage inhibition. The effectiveness of the test samples in quenching ATBS* in the system was observed to be high at the lowest concentration of 100 µg/ml with both the ligand and the metal complexes exhibiting higher ABTS (%) inhibition than the standards. However, copper complex showed significantly higher ABTS scavenging activity with an IC₅₀ value of 1.92±1.11 µM while complexes of cobalt, nickel and zinc gave an IC₅₀ value of 2.47±0.86, 2.80±1.31, 3.59±1.90 µM respectively. The same scavenging activity pattern for the metal complexes was observed with both ABTS and DPPH radicals: [Opd-Cu > Opd-Co > Opd-Ni > Opd-Zn]. Categorically, the anti-radical studies showed that the synthesised compounds may be useful in developing therapeutic agent for averting cell oxidative damage, as various free radicals generated in the system often lead to cancer, aging and cardiovascular diseases [56].

CONCLUSION

Metal ions to ligand binding were confirmed by spectral analyses. Opd-Co complex was observed to possess tetrahedral geometry, square planar geometry assigned for Opd-Ni and Opd-Cu complexes. Mycological studies showed that the metal complexes displayed higher antimicrobial activities when compared to the free ligand. The compounds were also evaluated for their free radical scavenging property and observed varying antioxidant activities as compared to the standards; Opd-Zn, Opd-Cu, Opd-Ni, and Opd-Co showed higher DPPH and ABTS scavenging potentials than the free ligand. The multi-potential activities of the synthesised compounds support its usage for the development of chemotherapeutic agents useful for pathological radicals' related diseases treatment.

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