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Synthesis and characterization and biological evaluation of bivalent metal complexes with (E)-4-bromo-N'-(4-chlorobenzylidene) benzohydrazide ligand

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Abstract: New (E)-4-bromo-N'-(4-chlorobenzylidene)benzohydrazide (BrphOH) complexes of some bivalent metal ion complexes were synthesized and analyzed through microelemental investigation, infrared spectroscopy, conductivity measurements, and 1H NMR spectroscopy. The BrphOH was bonded with the M(II) as a chelating ligand through the oxygen atom of the carbonyl group and the nitrogen atom of the azomethine group to afford octahedral complexes of the formula $[MCl_2(BrphOH)(H_2O)_2]$ {MII = Co (1), Ni (2), and Cu (3)}, or as a square planar complex of the type $[PdCl_2(BrphOH)]$(4), or as tetrahedral complexes of the formula $[MCl_2(BrphOH)]$ (MII = Zn (5), Cd (6), and Hg (7)). The prepared complexes were screened against Chlamydia trachomatis, Bacillus subtilis, Nosocomiicoccus ampullae, and Escherichia coli. The Palladium (II) and Nickel (II) complexes presented more advanced microbial action than the other compounds.

Keywords: *Bacteria, Biological activity, Complexes, Isonicotinohydrazide, Schiff base.*

1. Introduction

Hydrazide are a class of organic compounds formed by the condensation of hydrazide with aldehydes or ketones. They contain the functional group -C=N-NH-CO-. Hydrazide complexes are formed when these hydrazide molecules act as ligands and coordinate with metal ions. These complexes are of significant interest refer to their varied range of in medicine chemistry applicants, catalysis, and materials science [1-7]. The presence of a C=N (azomethine) group and carbonyl group allows hydrazide molecules to act as ligands and can act as mono, bidentate or poly-dentate ligands, coordinating to metal ions through nitrogen atom, oxygen atom or both. They often form stable chelate complexes with a variety transition metal ion (like copper, nickel, cobalt, and zinc), rare earth metals, and even lanthanides [2-17]. The formation of hydrazide-metal complexes can lead to various geometries, including square planar, octahedral, and tetrahedral arrangements, dependent on the ions and the environment of the hydrazide compound [1-7].

Hydrazide complexes have shown significant biological activities, including antibacterial, antifungal, anticancer, and antiviral properties. The coordination to metals can enhance the biological activity of the hydrazide ligand, making these complexes potential therapeutic agents [6, 8, 12]. Further the hydrazide complexes are used as catalysts in organic reactions, such as oxidation, reduction, and C-C coupling reactions, due to their ability to stabilize various oxidation states of the metal center [18, 19]. Also, these compounds can be used in the development of advanced materials, such as sensors, due to

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their unique electronic and structural properties. According to the mentioned introduction, we didn't find any metal complexes synthesized from (E)-4-bromo-N'-(4-chlorobenzylidene) benzohydrazide. Consequently, this study focuses on synthesizing a variety of bivalent metal complexes with the hydrazone ligands and assessing how they function biologically against various pathogenic bacterial species.

2. Experiment Section

2.1. Apparatus

The NMR spectra were obtained at the King Saud University, Saudi, using a The ECZ Luminous™ (JNM-ECZL series) is an FT NMR spectrometer. Infrared spectra were obtained using a The JASCO FT/lR-6X FTIR spectrometer with KBr discs over the range of 400–4000 cm^{-1}. Measurements of digital molar electric conductivity were obtained using the CD-2005 conductivity analyzer. Elemental investigations were conducted at the King Saud University, Saudi. Melting points were determined using a Hanon MP360 automatic slip melting point apparatus and were unadjusted. The (E)-4-bromo-N'-(4-chlorobenzylidene)benzohydrazide ligand was prepared according to the literature [20].

2.2. Preparation of [$CoCl_2(BrphOH)(H_2O)_2$] (1)

A solution of the ligand (*E*)-4-bromo-N'-(4-chlorobenzylidene)benzohydrazide (BrphOH) (0.100g, 0.420mmol) in ethanol (10ml) was added to solution of $CoCl_2.6H_2O$ (0.141g, 0.420mmol) in distilled water(10ml) with stirring, the color was changed to an orange. The combination was stirred for 3 hours, throughout which an orange ppt. produced. The ppt. was filtered, washed with distilled water and ethanol, and dried under vacuum (Wt (g): 0.161 g; Yield 90%, m.p(°C): 261-262).The complexes [$NiCl_2(BrphOH)(H_2O)_2$] **(2)** , [$CuCl_2(BrphOH)(H_2O)_2$] **(3)**, [$PdCl_2(BrphOH)$] **(4),** [$ZnCl_2(BrphOH)$] **(5),** [$CdCl_2(BrphOH)$] **(6),** [$HgCl_2(BrphOH)$] **(7),** were synthesized by using similar method above.

2.3. Biological Activity

The standard agar diffusion method, initially detailed by Bauer, et al. [21] was employed to determine the activity index for all free hydrazone and its corresponding metal complexes. Tetracycline attended as the reference medication. The microbial activity was evaluated towards four pathogenic bacterial species. (*Chlamydia trachomatis*, Bacillus subtilis, *Nosocomiicoccus ampullaeand Escherichia coli.*), at 0.001 M of newly DMSO of the prepared compounds. The results are listed in Table 4.

3. Results and Discussion

3.1. Preparation

Treatment of two moles of ((*E*)-4-bromo-N'-(4-chlorobenzylidene) benzohydrazide (BrphOH) with one equivalent mole of metal salt chlorides gave a complex of the type [$MCl_2(BrphOH)(H_2O)_2$] {M^{II} = Co (1), Ni (2) and Cu (3)}, or complex of the type [$PdCl_2(BrphOH)$](4) or complexes of the type [$MCl_2(BrphOH)$] {M^{II} = Zn (5), Cd (6) and Hg (7)} as a lone produce in good yield (71 - 94%) (Scheme 1).

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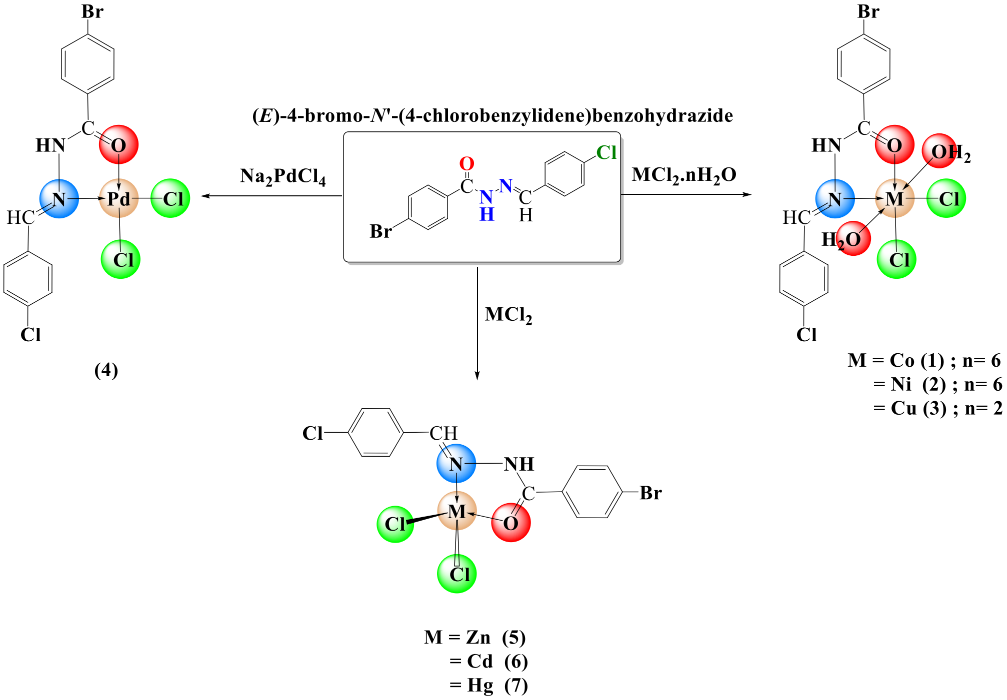

Figure 1.
Preparation pathway of the complexes (1-7).

The synthetic compounds exhibit stability and are soluble in dimethyl sulfoxide, and *N,N*-Dimethylformamide. The structures were subsequently analyzed by FT-IR, NMR, UV-Vis spectroscopy, molar conductivity, and magnetic susceptibility.

The ies measurements of the compounds in dimethyl sulfoxide solution (10^{-3} M at 25°C) are considerably low, indicating that prepared complexes are not electrolyte solution [22]. The CHN analysis and a few physical characteristics are enumerated in Table 1.

Table 1.
Physical properties, product percentage, conductivity and CHN analysis for the prepared compounds.

CHN analysis Found (cal.)%			**Produce %**	**m.p(C°)**	**Conductivity (ohm^{-1}. cm^2. mol^{-1})**	**Color**	**Compounds**	**Seq.**
N	**H**	**C**						
8.52	3.13	49.93	93	151-152	-	Off white	BrphOH	
-8.3	-2.99	-49.81						
5.72	2.75	33.42	90	261-263	9	Yellowish green	[CoCl$_2$(BrphOH)(H$_2$O)$_2$]	**1.**
-5.56	-2.8	-33.4						
5.69	2.93	33.59	78	206-208	4.7	Light green	[NiCl$_2$(BrphOH)(H$_2$O)$_2$]	**2.**
-5.57	-2.8	-33.42						
5.73	2.91	33.29	83	198-201	11.5	Blue	[CuCl$_2$(BrphOH)(H$_2$O)$_2$]	**3.**
-5.51	-2.78	-33.1						
5.6	2.09	32.69	94	231-232	3.9	Brown	[PdCl$_2$(BrphOH)]	**4.**
-5.44	-1.96	-32.66						
6.17	2.24	35.69	71	249-252	1.4	Off white	[ZnCl$_2$(BrphOH)]	**5.**
-5.91	-2.13	-35.48						
5.62	2.14	32.33	79	269-271	10.5	White	[CdCl$_2$(BrphOH)]	**6.**
-5.38	-1.94	-32.28						
5.37	2.22	31.89	87	216-217	5.7	White	[HgCl)$_2$BrphOH](	**7.**
-4.6	-1.65	-27.61						

3.2. Spectroscopic Studies

3.2.1. ^{1}H NMR Spectra

The ^{1}H-NMR spectrum of free BrphOH (Figure 2) showed two singlet peak, the first appeared at δH= 8.92ppm which was attributed to the proton of the CH=N group and the second peak showed at the δH= 12.85ppm due to the (NH) group, the integration of these peaks represents one proton for each. Also, the spectrum displayed four doublet peaks at δH= 8.92 ppm (2H), δH= 8.58 ppm (2H), δH= 7.71 ppm (2H), and δH= 7.35 ppm (2H), refer to the protons in position 3, 2, 4 and 1, respectively, with the coupling constant is $^3J_{H-H}$= 8.00Hz. Each of these peaks agrees to two protons.

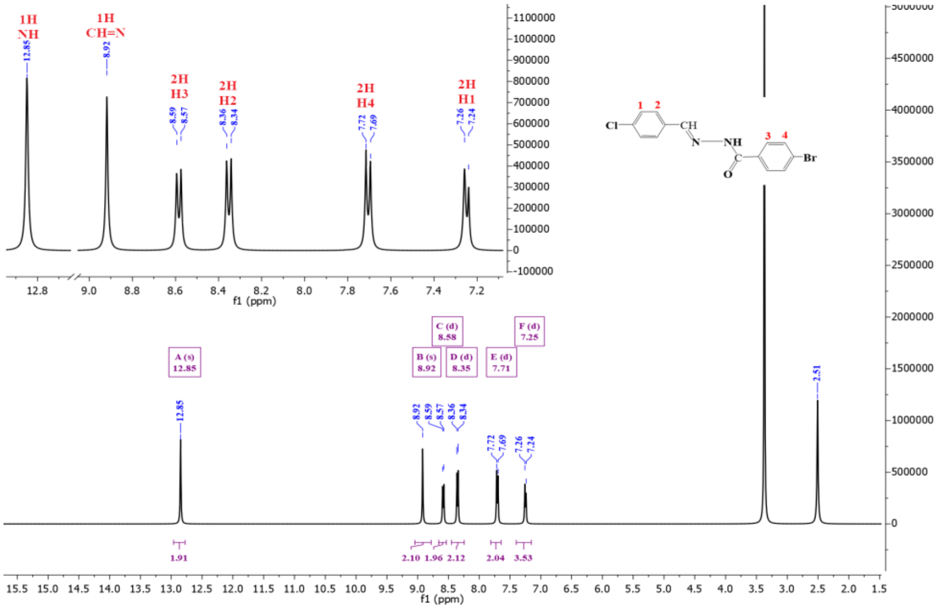

Figure 2.
^{1}H NMR spectrum of BrphOH ligand.

The ¹H-NMR spectrum of free BrphOH (Figure 2) showed two singlet peak, the first appeared at δH= 8.92ppm and 12.84 ppm due to the protons of C**H**=N and NH, respectively. In addition, the spectrum displayed four doublet peaks at δH= 8.76 ppm (2H), δH= 8.34 ppm (2H), δH= 7.54 ppm (2H), and δH= 7.45 ppm (2H), due to the protons in position 3, 2, 4 and 1, respectively. Each of these signals corresponds to two protons, as indicated by the integration values beneath each peak.

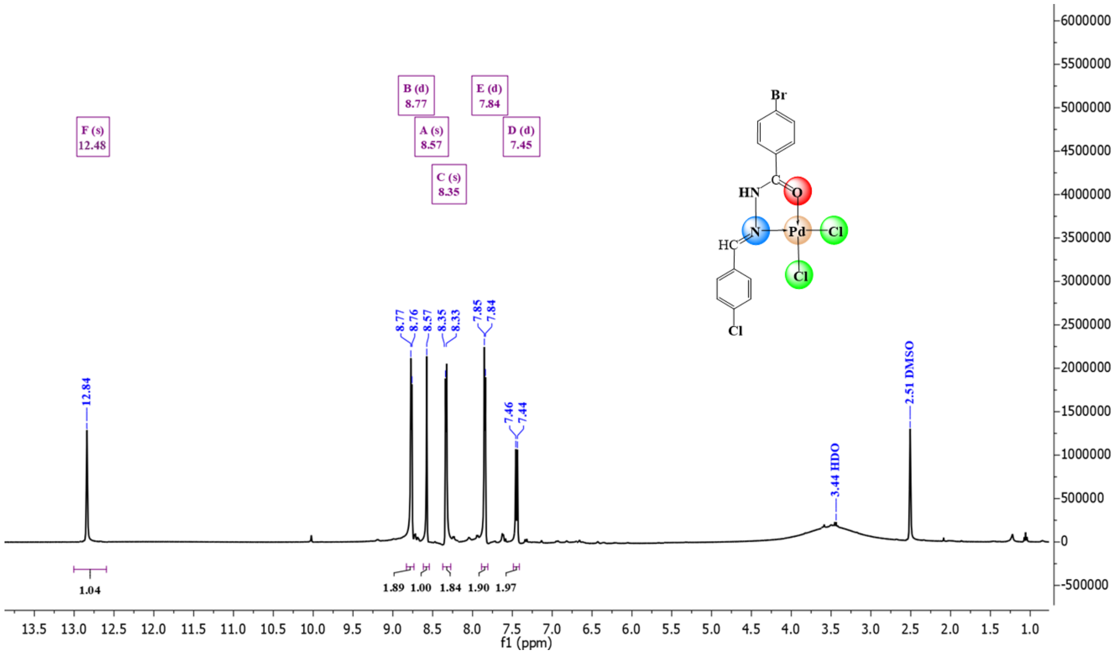

Figure 3.
¹H NMR spectrum of [$PdCl_2$(BrphOH)] complex.

In similar, the ¹H NMR spectrum of [MCl_2(BrphOH)] { M^{II} = Zn, Cd and Hg) complexes displayed the protons of the of the azometheine and NH groups as a singlet peaks with (δH= 8.61 – 8.70ppm) and (δH= 12.81 – 12.93ppm), each of these peaks agree to one proton as indicating from the integration values under each peak. Whereas the protons of the phenyl rings displayed in the aromatic region. All NMR results are listed in the Table 2.

Table 2.
¹H NMR chemical shifts for the prepared complexes (in ppm).

Complexes	**δH (ppm)**
BrphOH	12.85(s,1H, NH), 8.92 (s,1H, CH=N), 8.58 (d, 2H, H3), 8.35 (d, 2H, H2), 7.71 (d, 2H, H7), 7.25 (d, 2H, H8)
[$PdCl_2$(BrphOH)]	12.84(s,1H, NH), 8.76 (d, 2H, H3), 8.57 (s,1H, CH=N), 8.34 (d, 2H, H2), 7.54 (d, 2H, H7), 7.45 (d, 2H, H8)
[$ZnCl_2$(BrphOH)]	12.93(s,1H, NH), 8.81 (d, 2H, H3), 8.61 (s,1H, CH=N), 8.62 (d, 2H, H2), 7.76 (d, 2H, H7), 7.34 (d, 2H, H8)
[$CdCl_2$(BrphOH)]	12.81(s,1H, NH), 8.79 (d, 2H, H3), 8.70 (s,1H, CH=N), 8.59 (d, 2H, H2), 7.83 (d, 2H, H7), 7.43 (d, 2H, H8)
[$HgCl_2$(BrphOH)]	12.84(s,1H, NH), 8.83 (d, 2H, H3), 8.68 (s,1H, CH=N), 8.53 (d, 2H, H2), 7.70 (d, 2H, H7), 7.29 (d, 2H, H8)

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3.2.2. Infrared Spectra

The IR spectrum of the free (*E*)-4-bromo-N'-(4-chlorobenzylidene) benzohydrazide (BrphOH) ligand showed a four characteristic bands at 3176cm^{-1}, 1668 cm^{-1}, 1593cm^{-1}, 1085cm^{-1} and 688cm^{-1} due to the υN-H, υC=O, υC=N, υN-N, and ρC-H, respectively, some of these bands were change after complexation with the metal ions (**Figure 5** and **5**). The vibration stretching frequency of the carbonyl group ν(C=O) and azomethine group ν(C=N) were displayed as a strong bands within (1634-1670) cm^{-1} and (1548-1562)cm^{-1}range, respectively, the shifted towards lower frequencies than it was in the BrphOH ligand (showed at 1698 cm^{-1} and 1593 cm^{-1}) , designates that the BrphOH bonded as bidentate via the oxygen and nitrogen atoms of the ν(C=O) and ν(C=N) groups [23-25]. The spectra of the [$MCl_2(BrphOH)(H_2O)_2$] {M^{II} = Co (**1**), Ni (**2**) and Cu (**3**)} displayed the coordinate hydrate vibration frequency at (923-923)cm^{-1}, for the three complexes [26, 27]. Also, the spectra showed additional bands that were not present in spectrum of the free ligand, which are ν(M-O) and (M-N)ν which appeared at (467-490) cm^{-1} and (423-453) cm^{-1} respectively [24-29]. Additional bands are listed in Table 3.

Table 3.
Selected IR bands of the prepared complexes (in cm^{-1}).

Compounds	**νN-H**	**νC-H**		**νC=O**	**νC=N**	**νC=C**	**νN-N**	**ρC-H**	**νM-O**	**νM-N**
		Arom.	**Aliph.**							
BrphOH	3174m	3028w	2848w	1698vs	1593m	1552m	1085m	754s	-	-
[$CoCl_2(BrphOH)(H_2O)_2$]	3198m	3032w	2850w	1670vs	1558m	1546s	1078s	756s	467w	429w
[$NiCl_2(BrphOH)(H_2O)_2$]	3212m	3014w	2898w	1655vs	1558s	1456m	1079s	752s	489w	437w
[$CuCl_2(BrphOH)(H_2O)_2$]	3169w	3070w	2962w	1634vs	1569s	1550m	1087m	754s	484w	453w
[$PdCl_2(BrphOH)$]	3171m	3068m	2966m	1648vs	1565s	1548s	1087s	752s	478w	453m
[$ZnCl_2(BrphOH)$]	3209m	3056w	2923w	1652vs	1560s	1534s	1080s	745s	490w	438m
[$CdCl_2(BrphOH)$]	3189s	3038w	2876w	1644vs	1548s	1542m	1084s	762s	467w	423m
[$HgCl_2(BrphOH)$]	3166s	3062w	2989w	1656vs	1562s	1551s	1092s	748s	489w	441m

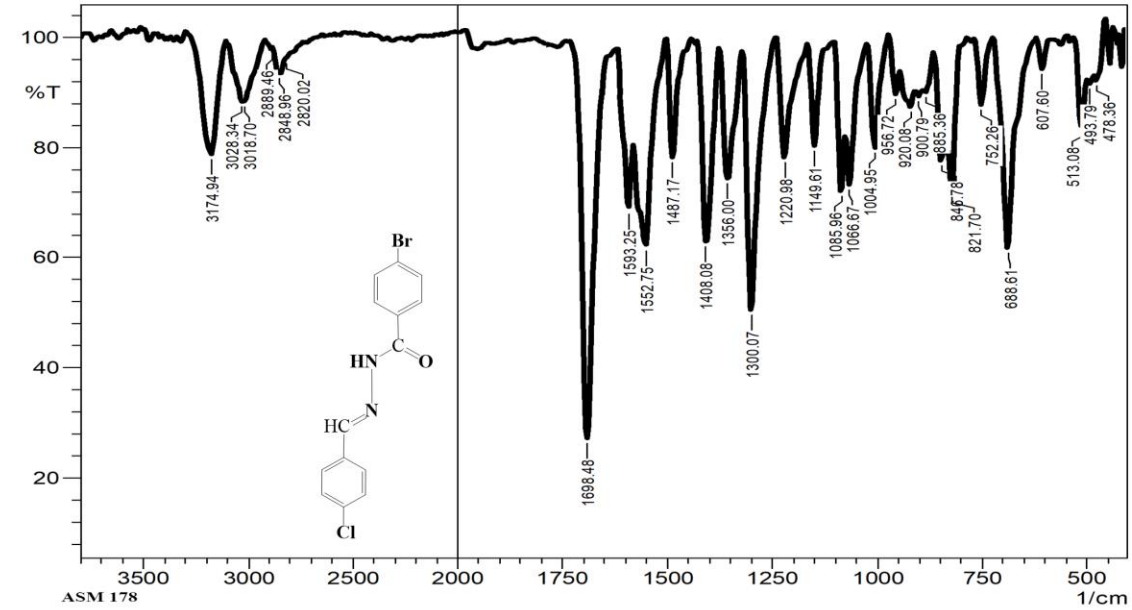


Figure 4.
IR spectrum of free ligand.

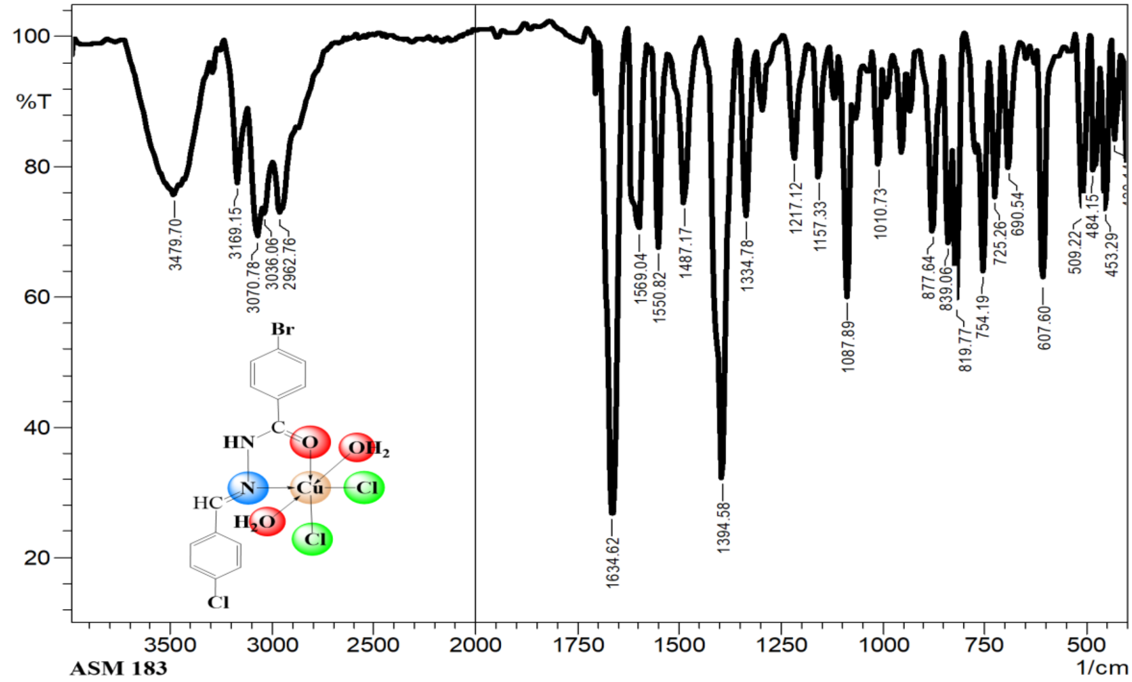


Figure 5.
IR spectrum of [$CuCl_2(BrphOH)(H_2O)_2$] complex.

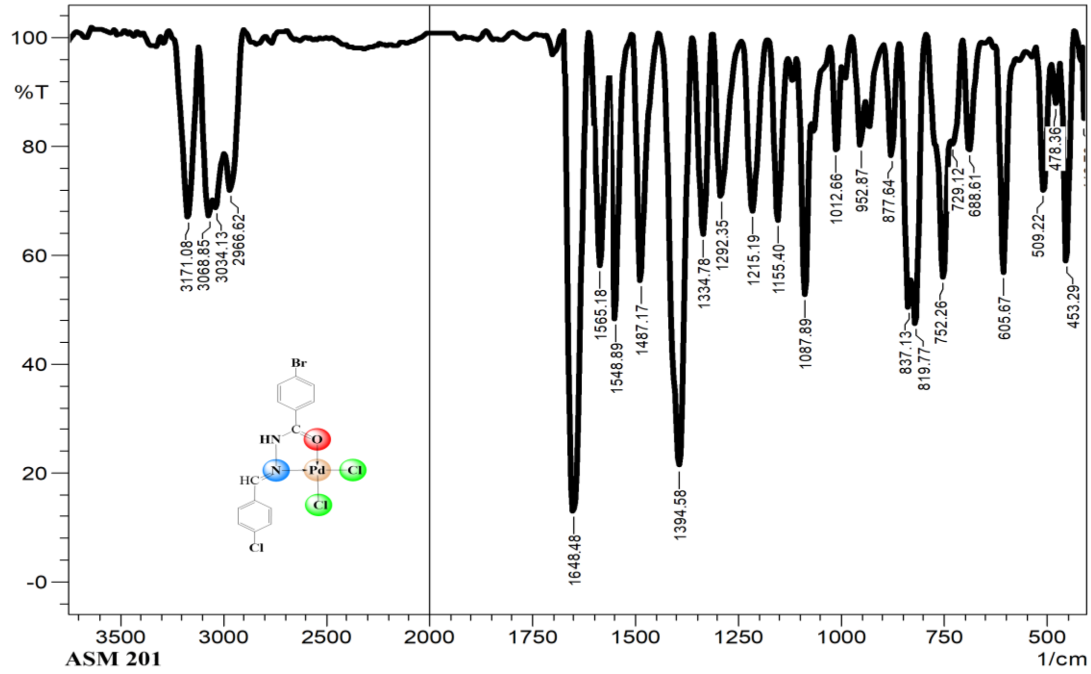


Figure 6.
IR spectrum of [$PdCl_2(BrphOH)$] complex.

3.2.3. *Magnetic Moments and Electronic Data*

The electronic data of the [$MCl_2(BrphOH)(H_2O)_2$] {M^{II} = Co (1), Ni (2) and Cu (3)} complexes displayed the characteristic bands for these complexes in the Uv-visible spectra which are listed in Table 4.

Table 4.
Electronic data and magnetic moments of the [$MCl_2(BrphOH)(H_2O)_2$] complexes.

Complexes	Bands	Transitions	State	Magnetic moment	Geometry
[$CoCl_2(BrphOH)(H_2O)_2$]	445 nm 734nm 1087nm	$^4T_1g \rightarrow {}^4T_1g(P)$ $^4T_1g \rightarrow {}^4A_2g$ $^4T_1g \rightarrow {}^4T_2g$	Paramagnetic	4.78 B.M	Octahedral
[$NiCl_2(BrphOH)(H_2O)_2$]	389nm 578nm 813nm	$^3A2g(F) \rightarrow {}^3T_2g(F)$ $^3A_2g(F) \rightarrow {}^3T_1g(F$ $^3A_2g(F) \rightarrow {}^3T_1g(P)$	Paramagnetic	2.98 B.M	Octahedral
[$CuCl_2(BrphOH)(H_2O)_2$]	515nm 620nm	d–d $^2B_{1g} \rightarrow {}^2A_{1g}$	Paramagnetic	2.04 B.M	Octahedral

The electronic spectra of the M(II) complexes exhibited bands absorption corresponding the octahedral geometry around the metal ions [30-33]. These results supporting by the magnetic moments of the metal ions.

3.3. *Antibacterial Studies*

The studies on the antibacterial activity of the free ligand and its synthesized complexes are presented in Table 5. The outcomes were obtained against four bacterial species: *Chlamydia trachomatis*, *Bacillus subtilis*, *Nosocomiicoccus ampullae*, and *Escherichia coli*. Figure 7 shows a summary of the results from the standard agar diffusion [21]. The size of the inhibiter region was contrasted to that of Tetracycline as a positive control. The comparative activity index (AI) percentage was computed as demonstrated below:

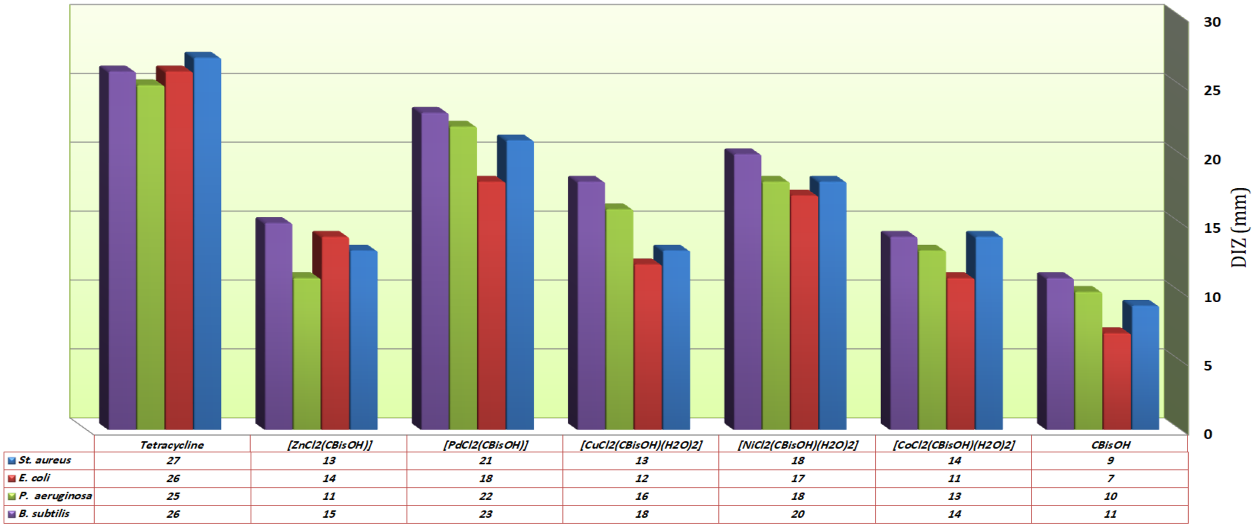
$$\%\ activity\ index = \frac{\text{Inhibition zone of the test compounds}}{\text{Inhibition zone of the standard drug}} * 100.$$

Table 5.
DIZ (mm) and AI (%) of prepared complexes against pathogenic bacteria.

Compounds	Diameter Inhibition zone (mm) and AI (%)							
	Nosocomiicoccus ampullae		E. coli		Chlamydia trachomatis		B. subtilis	
BrphOH	9	33	7	27	10	40	11	42
[$CoCl_2(BrphOH)(H_2O)_2$]	14	52	11	42	13	52	14	54
[$NiCl_2(BrphOH)(H_2O)_2$]	18	67	17	65	18	72	20	77
[$CuCl_2(BrphOH)(H_2O)_2$]	13	48	12	46	16	64	18	69
[$PdCl_2(BrphOH)$]	21	78	18	69	22	88	23	88
[$ZnCl_2(BrphOH)$]	13	48	14	54	11	44	15	58
Tetracycline	27	100	26	100	25	100	26	100

All compounds exhibited insignificant activity compared to Tetracycline, yet surpassed that of the BrphOH ligand. The Pd^{+2} and Ni^{+2} complexes exhibited superior activity relative to further compounds. is 21mm, 18mm, 22mm and 23 mm against *Chlamydia trachomatis*, Bacillus subtilis, *Nosocomiicoccus ampullae, and Escherichia coli*, respectively. Whereas the Co-complex has lowest activity with DIZ is 14mm, 11mm, 13mm and 14mm against *Chlamydia trachomatis*, Bacillus subtilis, *Nosocomiicoccus ampullae, and Escherichia coli*, respectively.

Two theories elucidate the heightened activity of complexes: (1) the overtone concept, which posits that the solubility of compounds in the cell membrane significantly influences antibacterial activity by permitting the translocation of only lipid-soluble materials, and (2) [34] chelation theory, which asserts that the polarity of a metal ion is markedly diminished due to ligand orbital overlap and the distribution of positive charge among the central metallic ion and the ligand's donor atoms [34, 35].



	Tetracycline	[ZnCl2(CBisOH)]	[PdCl2(CBisOH)]	[CuCl2(CBisOH)(H2O)2]	[NiCl2(CBisOH)(H2O)2]	[CoCl2(CBisOH)(H2O)2]	CBisOH
🟦 *St. aureus*	27	13	21	13	18	14	9
🟥 *E. coli*	26	14	18	12	17	11	7
🟩 *P. aeruginosa*	25	11	22	16	18	13	10
🟪 *B. subtilis*	26	15	23	18	20	14	11

Figure 7.
histogram representation of free ligand and their complexes activity.

4. Conclusion

The [$MCl_2(BrphOH)(H_2O)_2$] {M^{II} = Co (**1**), Ni (**2**) and Cu (**3**) }; [$PdCl_2(BrphOH)$](**4**) and [$MCl_2(BrphOH)$] {M^{II} = Zn (**5**), Cd (**6**) and Hg (**7**)} complexes were prepared and characterized by different physical and spectroscopic methods. The BrphOH ligand displayed a bidentate chelating ligand mode with the M(II) ion through oxygen and nitrogen atoms of carbonyl and azomethine groups respectively, to afford an octahedral geometry around the Co^{+2}, Ni^{+2} and Cu^{+2} ions or square planner around Pd^{+2} ion, and whereas gave a tetrahedral with the Zn^{+2}, Cd^{+2} and Hg^{+2} ions. Finally, the biological activity has been screened against four pathogenic. The Pd^{+2} and Ni^{+2} complexes exhibited superior activity relative to further compounds. is 21mm, 18mm, 22mm and 23 mm against *Chlamydia trachomatis*, Bacillus subtilis, *Nosocomiicoccus ampullae, and Escherichia coli*, respectively.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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