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Journal of Biological Research and Biotechnology

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## Evaluation of Radical Scavenging activity of Br-Substituted Schiff Base metal complexes: Investigating the Inhibitory Role of Inductive Electron Withdrawal

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### Abstract

The persistent search in medicinal chemistry for active synthetic antioxidants has the goal of relieving oxidative stress-related diseases. In this study, Schiff base ligands and their corresponding metal complexes using copper, nickel, and cobalt were synthesized, with the ligands substituted with bromine (INHBrS) or unsubstituted (INH-5H). The compounds were identified using FTIR, NMR, elemental analysis and AAS (for metal complexes only). In determining the antioxidant capacity of the ligands and their corresponding metal complexes, the compounds were subjected to the DPPH, FRAP, Nitric Oxide Radical Scavenging Assay, and Total Antioxidant Capacity tests using ascorbic acid as the standard control. The study demonstrated the critical role of the nature of the substituent in the scavenging capacity of the compounds. In INH-5BrS compound, the bromine is an electron-withdrawing group due to the inductive effect (-I), resulting in a decrease in the electron density on the aromatic ring, thereby showing less antioxidant potential compared to 1NH-5H. In the 1NH-5H series, the nickel complex 1NH-5HNi has the best antioxidant capacity in the DPPH Inhibition Assay (92.345%) and the Total Antioxidant Capacity (97.4%) tests, surpassing ascorbic acid. This study provides critical insights into the structure-activity relationship (SAR) regarding inductive effects in phenolic antioxidant systems.

**Keywords:** Schiff base-metal complex, Antioxidant, Electron withdrawingReceived: 1<sup>ST</sup> March 2026 / Accepted: 11<sup>th</sup> April 2026 / **Published online:**30<sup>th</sup> April 2026

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DOI: <https://dx.doi.org/10.4314/br.v24i1.25>

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## INTRODUCTION

Reactive species are constantly produced in biological systems as a result of routine metabolic processes as well as due to various environmental factors. These reactive species, particularly ROS, can cause oxidative stress when their production exceeds the body's defense capabilities (Juan et al., 2021; Pruteanu et al., 2023). Oxidative stress is considered to cause various pathological disorders such as neurodegenerative diseases, heart problems, cancer, and inflammation (Ansari et al., 2025; Sies et al., 2022; Checa & Aran, 2020; Phaniendra et al., 2015). Antioxidants have been found to mitigate oxidative stress by scavenging free radicals or by inhibiting their formation (Chaudhary et al., 2023; Krishnamurthy et al., 2024; Chandimali et al., 2025; Mukherjee et al., 2025). While much emphasis is given to the study of natural antioxidants obtained from plants (Akeghware et al., 2026; Bashir, Shehu & Isah, 2026) as well as other biological sources, their availability in large quantities is limited (Anwar et al., 2018; Lourenco et al., 2019; Rasheed & Fathima, 2019; Parveen et al., 2025). Besides this, it is also found that these antioxidants do not suffice to meet the ever-increasing need for effective scavengers of free radicals (Flieyer et al., 2021; Usoro 2024). Consequently, the development of synthetic antioxidant compounds has gained significant attention. In this regard, Schiff bases and their metal complexes have proven to be potent antioxidant agents. This is due to their ease of synthesis and the fact that these compounds can be easily modified to have desired electronic properties (Racheal et al., 2023; Panghal et al., 2023; Dag et al., 2023; Lawan et al., 2024). In addition, the antioxidant activity of phenolic Schiff bases can be affected by the ease with which the phenolic O-H bond can be broken during Hydrogen Atom Transfer (HAT) (Kumar & Goel 2019). This can be significantly affected by the substituents on the phenyl ring. This study focuses on the Inductive Effect of Bromine, a halogen, versus the unsubstituted-based scaffold (1NH-5H). Halogens are used as substituents to enhance lipophilicity. However, halogens have the disadvantage of being electron withdrawing. This may result in the deactivation of the antioxidant compound by strengthening the O-H bond. This study aims to examine the antioxidant activity of *N*-(2-hydroxybenzylidene)isonicotinohydrazide based on their electron-withdrawing properties and their Cu(II), Ni(II) and Co(II) complexes. This will be useful in understanding the type of structures that can be used to enhance the antioxidant activity of these compounds thereby guiding future research toward more effective compounds while reducing the time and cost associated with extensive experimental screening (Silverman & Holladay 2014).

## Materials and methods

### Materials

All chemicals and solvents were used as received from Sigma-Aldrich (Germany). No further purification of chemicals was carried out. Melting Point was recorded using a Stuart SMP3 instrument. The values were recorded without correction. FT-IR spectra were recorded using an

FTS 7000 Digilab Win-IR Pro instrument coupled with an ATR-Diamond accessory. NMR spectra were recorded using a Varian 300 MHz instrument. The spectra were recorded in DMSO- $d_6$  solvent. Tetramethylsilane was used as an internal standard. Elemental analysis was carried out using a Perkin-Elmer 2400 instrument. Metal content was recorded using an Analyst 200 instrument.

### Typical synthesis of Schiff bases

A hot solution of isonicotinic acid hydrazide (13.70 mg, 0.10 mmol) in absolute ethanol (20 mL) in a round-bottom flask was added 2-hydroxybenzaldehyde (INH-5H) (12.20 mg, 0.10 mmol) and 5-bromo-2-hydroxybenzaldehyde (INH-5BrS) (2.02 mg, 0.10 mmol) in absolute ethanol respectively. Glacial acetic acid (0.3 mL) was added, and the mixture was stirred at room temperature for 1 h, then refluxed at 60 °C for 6 h. Upon cooling, white precipitate was collected by filtration, recrystallized from ethanol:hexane (1:1), and dried over silica gel.

### *N*-(2-hydroxybenzylidene)isonicotinohydrazide (INH-5H)

A final yield of 92% was realized, and this corresponded to 22.20 mg. The properties of the compound include a melting point of 256-257 °C and an  $R_f$  value of 0.75. The compound's absorption bands in the IR spectroscopy correspond to 3308, 3002, 1675, 1611, 1567, 1489, 1407, 1353, 1289, 1272, 1159, 1067, 1034, 971, 949, 929, 891, and 871  $cm^{-1}$ . The compound's signals in  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ) appear at  $\delta$  117, 119, 120, 122, 130, 132, 141, 150, 151, 158, and 162. The signals for  $^1H$  NMR (300 MHz, DMSO- $d_6$ ) appear at  $\delta$  6.65 (multiplet, 2H), 7.34 (triplet,  $J = 15.3$  Hz, 1H), 7.65 (doublet of doublets,  $J = 18$  Hz, 1H), 7.87 (doublet,  $J = 6$  Hz, 2H), 8.69 (singlet, 1H), 8.81 (doublet,  $J = 4.5$  Hz, 2H), 11.11 (singlet, 1H), 12.31 (singlet, 1H).  $C_{13}H_{11}N_3O_2$  was Found (calculated) as follows: C, 64.72 64.89 (64.72); H, 4.49 (4.60); N, 17.83 (17.42).

### *N*-(5-bromo-2-hydroxybenzylidene)isonicotinohydrazide (INH-5BrS)

A yield of 95% (9.13 mg) (95%). The properties of the compound include a melting point of 261-264°C and  $R_f$ : 0.78. Absorption bands in the IR spectroscopy correspond to 3252, 3068, 2957, 1669, 1616, 1550, 1473, 1407, 1376, 1339, 1286, 1264, 1194, 1158, 1062, 997, 926, 853, 833, 795  $cm^{-1}$ . The compound's signals in  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ ) appear at  $\delta_c$  111.09, 119.23, 121.84, 122.07, 130.60, 134.44, 140.41, 146.81, 150.91, 156.98, 162.02. The signals for  $^1H$  NMR (300 MHz, DMSO- $d_6$ ) appear at  $\delta_H$  6.93 (doublet,  $J$  8.7Hz, 1H), 7.45 (doublet of doublet,  $J$  11.7Hz, 1H), 7.85 (multiplet, 3H), 8.65 (singlet, 1H), 8.81 (doublet,  $J$  5.4Hz, 2H), 11.14 (singlet, 1H); 12.36 (singlet, 1H).  $C_{13}H_{10}BrN_3O_2$  was found (calculated) as follows: C, 48.7(48.77), H, 2.79 (3.15), N, 13.15 (13.13).

### Typical synthesis of metal complexes

An ethanolic solution of 20 mL containing 0.01 mol of the appropriate metal salt, such as copper(II) chloride dihydrate, cobalt(II) chloride hexahydrate, or nickel(II)

chloride hexahydrate, was added to a solution of the ligands INH-5H and INH-5BrS. The reaction mixture was then refluxed for 2-3 hours, during which a colored precipitate was formed, indicating that the complex formation reaction had occurred. After this, the mixture was left to cool at room temperature, and the complex thus formed was filtered off.

**INH-5HCu:**  $C_{26}H_{20}CuN_6O_9$  was found (calculated) as follows: C, 49.79 (49.25), H, 4.74 (4.77), N, 13.08 (13.25), Cu, 10.85 (10.02). Yield: 3.42 mg (54%). Melting point: >349 °C. Infrared: 3000, 1602, 1544, 1506, 1437, 1300, 1200, 1152, 1061, 892, 846, 498, 466  $cm^{-1}$ .

**INH-5HNI:**  $C_{13}H_{20}ClN_3NiO_7$  was found (calculated) as follows: C, 37.21 (36.79), H, 4.15 (4.75), N, 10.07 (9.90), Ni, 11.57 (13.88). Yield: 2.58 mg (61%). Melting point: >349 °C. Infrared ( $cm^{-1}$ ): 3366, 1595, 1544, 1441, 1277, 1197, 1151, 1068, 889, 846, 750, 674, 495. 463  $cm^{-1}$ .

**INH-5HCo:**  $C_{13}H_{18}ClCoN_3O_6$  was found (calculated) as follows: C, 38.11 (38.39), H, 3.71 (4.46), N, 10.28 (10.33), Co, 14.09 (14.49). Yield: 2.56 mg (63%). Melting point: >349.1 °C. Infrared: 3013, 1738, 1592, 1539, 1285, 1200, 1154, 1063, 585, 508, 464  $cm^{-1}$ .

**INH-5BrSCu:**  $C_{26}H_{26}Br_2CuN_6O_8$  was found (calculated) as follows: C, 40.35 (40.00), H, 3.39 (2.43), N, 10.86 (10.57), Cu, 8.21 (8.81). Yield: 4.49 mg (58%). Melting point: 313-317 °C. Infrared: 3049, 1597, 1499, 1448, 1415, 1379, 1352, 1285, 1176, 1030, 856, 816, 727, 690, 663, 645, 591, 541, 483  $cm^{-1}$ .

**INH-5BrSNI:**  $C_{13}H_{13}BrClN_3NiO_4$ : C, 34.75 (35.95), H, 2.92 (3.29), N, 9.35 (9.45), Ni, 13.06 (13.27). Yield: 2.29 mg (51%). Melting point: 307-309 °C. Infrared: 3320, 2359, 1592, 1543, 1499, 1442, 1420, 1381, 1316, 1272, 1152, 889, 761, 726, 622, 495  $cm^{-1}$ .

**INH-5BrSCo:**  $C_{26}H_{18}Br_2CoN_6O_4$ : C, 45.69 (45.70), H, 3.54 (3.12), N, 16.40 (16.00), Co, 8.62 (8.72). Yield: 4.16 mg (60 %). Melting point: 254-257 °C. Infrared: 3015, 1598, 1539, 1436, 1419, 1288, 1201, 1153, 1066, 713, 688, 585, 508, 464  $cm^{-1}$ .

## Evaluation of Antioxidant Activities

### DPPH radical scavenging assay

The ability of the synthesized compounds to scavenge free radicals was measured using a reduction in the absorbance of DPPH with slight changes in the methods of [Mensor et al, \(2001\)](#) and [Gbasemi et al, \(2009\)](#). The samples' serial solutions (25–100  $\mu g/mL$ ) were made with distilled water. 1 mL of a methanolic solution of DPPH (0.01 mM) was mixed with 4 mL of each solution. After shaking the combination, it was left in a dark closet for 30 minutes. Spectrophotometry was used to detect absorbance at 517 nm. Serially diluted ascorbic acid solutions was utilized as the standard and methanol as the blank. Every test was

carried out in triplicate. The samples' scavenging effect was shown as a percentage of inhibition. Scavenging Effect (%) =  $\frac{D_0 - D_1}{D_0} \times 100$ ; where  $D_1$  is the absorbance when the extract and standard sample are present, and  $D_0$  is the absorbance of the control.

### Nitric oxide scavenging activity

With few modifications, the nitric oxide scavenging activity was measured using spectrophotometry as described by [Green et al, \(1982\)](#). It has been shown that nitrite ions are produced by aqueous sodium nitroprusside at physiological pH ([Ebrahimzadeh, et al., 2010](#)). A pH 7.4 phosphate buffered saline was made using sodium nitroprusside (5 mM L<sup>-1</sup>). The samples were serially diluted to a concentration of 25–100  $\mu g/mL$ . 50  $\mu L$  of each sample solutions were combined with the buffer. For 30 minutes, the mixture was incubated at 25°C. After that, 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride) was added to 1.5 mL of the incubated mixture. At 546 nm, absorbance was measured. The positive standard was ascorbic acid, while the negative control was phosphate buffered saline and 10 mM sodium nitroprusside.

### Ferric reducing antioxidant Power (FRAP)

The modified approach of [Yen & Chen \(1995\)](#) was used to determine the samples' reducing power. Phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [ $K_3Fe(CN)_6$ ] (2.5 mL, 1%) were combined with an aliquot of the samples (1.0 mL) at different serially diluted values (25, 50, 75, and 100  $\mu g/mL$ ). For 20 minutes, the mixtures were incubated at 50 °C. Each combination was mixed with 2.5 mL of 10% trichloroacetic acid and centrifuged for 15 minutes at 1000 rpm. Each of the resultant supernatants was combined with 2.5 mL of distilled water and 0.1% of freshly made iron (III) chloride (0.5 mL). Methanol was used as the blank when measuring absorbance at 700 nm. The reducing power of the samples or substance is indicated by an increasing absorbance value. Ascorbic acid (vitamin C), which was likewise synthesized in serial form, served as the study's reference medication. Every experiment was conducted in triplicate.

### Total antioxidant capacity

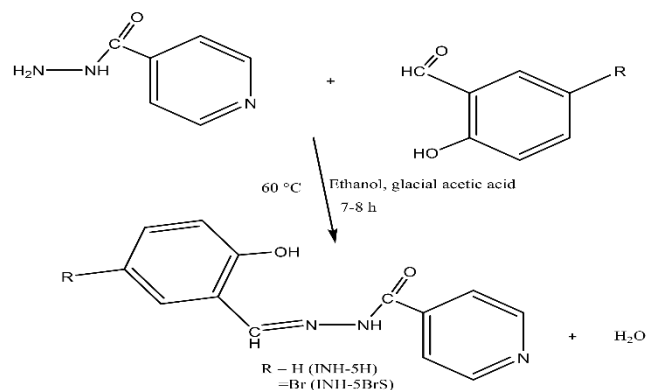
Using modified method ([Prieto et al., 1999](#)), the samples' total antioxidant capacity was measured. Each sample was serially diluted with ethanol/methanol at 25, 50, 75, and 100  $\mu g/mL$ . An aliquot (0.4 mL) of the diluted sample solution was combined with 4 mL of a reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) in a vial. For ninety minutes, the vial was incubated at 95 °C in a water bath. The absorbance was measured at 695 nm against ethanol/methanol as a blank once the vial had cooled to room temperature. Every sample's serially diluted solution was tested in triplicate.

## Results and discussion

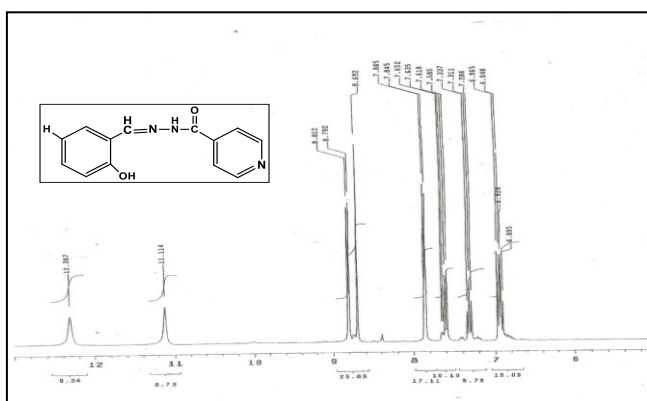
### Synthesis

The Schiff bases INH-5H and INH-5BrS were obtained in moderate yields from the condensation of INH with the

aldehydes namely 2-hydroxybenzaldehyde (INH-5H) and 5-bromo-2-hydroxybenzaldehyde (INH-5BrS) (Scheme 1). The compounds were purified by recrystallization from ethanol.



The Schiff bases and their corresponding complexes with metals were synthesized with good to moderate yields, which means that the process used in the synthesis was effective. The compounds yields were also acceptable. The chemical formula of the compounds was ascertained according to their elemental (CHNM) composition, which was consistent with the theoretical prediction. It is worth mentioning that the melting points of all the synthesized Schiff bases and metal complexes were sharp, indicating good quality and purity of the products



**Table 1:** Physical and analytical data of Schiff bases and their metal complexes

Code	M.wt.(g/mol)	mp (°C)	Yield(%)	Microanalysis: % Calculated (Found)			
				C	H	N	M
1NH-5H	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub> (241)	256-257	92	64.72 (64.89)	4.60 (4.49)	17.42 (17.83)	-
INH-5HCu	C <sub>26</sub> H <sub>20</sub> CuN <sub>6</sub> O <sub>9</sub> (633)	>349	54	49.25 (49.79)	4.77 (4.74)	13.25 (13.08)	10.02 (10.85)
INH-5HNi	C <sub>13</sub> H <sub>20</sub> ClN <sub>3</sub> NiO <sub>7</sub> (423)	>349	61	36.79 (37.21)	4.75 (4.15)	9.90 (10.07)	13.88 (11.57)
INH-5HCo	C <sub>13</sub> H <sub>18</sub> ClCoN <sub>3</sub> O <sub>6</sub> (406)	>349	63	38.39 (38.11)	4.46 (3.71)	10.33 (10.28)	14.49 (14.09)
1NH-5BrS	C <sub>13</sub> H <sub>10</sub> BrN <sub>3</sub> O <sub>2</sub> (320)	261-264	95	48.77 (48.75)	3.15 (2.79)	13.13 (13.15)	-
INH-BrSCu	C <sub>26</sub> H <sub>26</sub> Br <sub>2</sub> CuN <sub>6</sub> O <sub>8</sub> (774)	313-317	58	40.35 (40.00)	3.39 (2.43)	10.86 (10.57)	8.21 (8.81)
INH-5BrNi	C <sub>13</sub> H <sub>13</sub> BrClN <sub>3</sub> NiO <sub>4</sub> (449)	307-309	51	34.75 (35.95)	2.92 (3.29)	9.35 (9.45)	13.06 (13.27)
INH-5BrSSCo	C <sub>26</sub> H <sub>18</sub> Br <sub>2</sub> CoN <sub>6</sub> O <sub>4</sub> (697)	254-257	60	44.79 (45.63)	2.60 (3.57)	12.05 (12.54)	8.45 (8.51)

M.wt = molecular weight; mp = Melting point

**Table 2:** Characteristic IR (cm<sup>-1</sup>) and NMR bands of Schiff bases and their metal complexes

Code	IR bands (cm <sup>-1</sup> )					Chemical shift (ppm)			
	νOH	νC=N	νC=O	ν(M-O)	ν(M-N)	HC=N		C-OH	
						δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>
1NH-5H	3308	1611	1675	-	-	8.69	140.46	12.31	158.01
INH-5HCu	-	1602	-	498	466	-	-	-	-
INH-5HNi	3366 (νH <sub>2</sub> O)	1595	-	495	463	-	-	-	-
INH-5HCo	-	1598	1738	508	464	-	-	-	-
1NH-5BrS	3252	1616	1669	-	-	8.65	140.41	12.36	156.98
INH-BrSCu	-	1597	-	541	483	-	-	-	-
INH-5BrSNi	3320 (νH <sub>2</sub> O)	1592	-	622	495	-	-	-	-
INH-5BrSCo	-	1598	-	508	464	-	-	-	-

**Table 3: DPPH Scavenging Activity (% Inhibition)**

Sample	µg/mL			
	25	50	75	100
Code	25	50	75	100
1NH-5H	45.24	54.76	74.46	80.14
INH-5HCu	45.29	55.31	73.81	80.12
INH-5HNi	45.02	54.43	77.71	92.35
INH-5HCo	37.30	45.29	55.25	70.29
1NH-5BrS	35.67	42.71	54.53	68.22
INH-BrSCu	44.77	54.87	69.86	80.35
INH-5BrSNi	42.37	54.41	74.21	79.35
INH-5BrSCo	42.68	51.36	69.86	76.24
Ascorbic Acid	45.67	54.71	78.53	92.22

The Comparison of the IR spectra of the complexes with those of the free ligands was done with the aim of establishing the nature of interaction of the Schiff bases with the metal ions in the complexes. The absorption bands due to the ligands, which appeared at 3308, 1611, and 1675 cm<sup>-1</sup> for 1NH-5H and 3252, 1616, 1669 for 1NH-5BrS respectively were shifted by ± 3-20 cm<sup>-1</sup> upon complex

formation, suggesting coordination through the OH bond, azomethine nitrogen and carbonyl oxygen. The coordination of the ligand's phenolic oxygen with the metal ion caused the C-O band to appear at higher frequencies. It is inferred that the Schiff bases are tridentate ligands coordinating with the phenolic oxygen, azomethine nitrogen and the carbonyl oxygen (Aggoun et al., 2020).

#### Antioxidant Assay

The antioxidant effects of the synthetic test antioxidants were assessed using the antioxidant power in DPPH assay, nitric oxide scavenging activity, ferric reducing antioxidant power, and total antioxidant capacity methods.

#### DPPH Radical Scavenging Activity

The DPPH test also proved that all the compounds fought the radical in direct proportion to the concentration, and there were differences between the bromine-substituted compound (INH-5BrS) and the unsubstituted ligand (INH-5H).

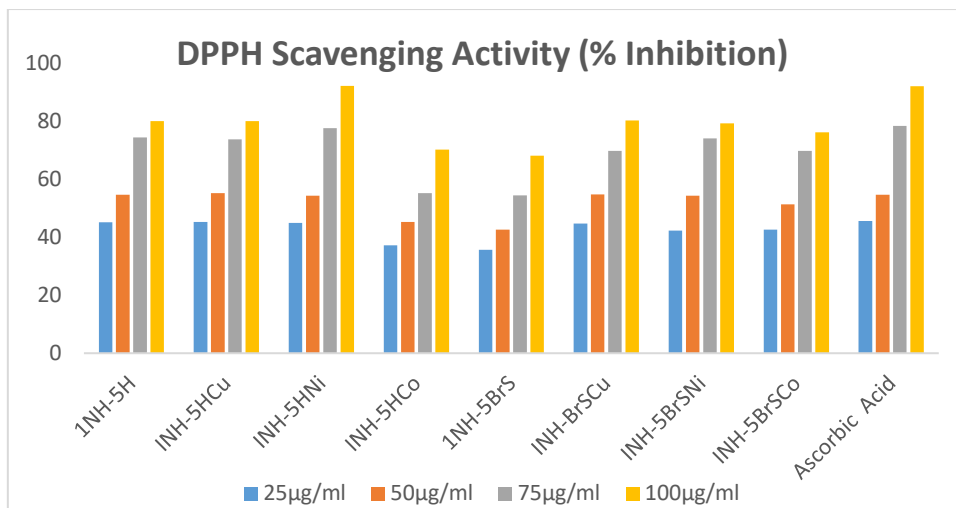


Figure 2: DPPH Scavenging Activity of the Ligands and their Metal Complexes

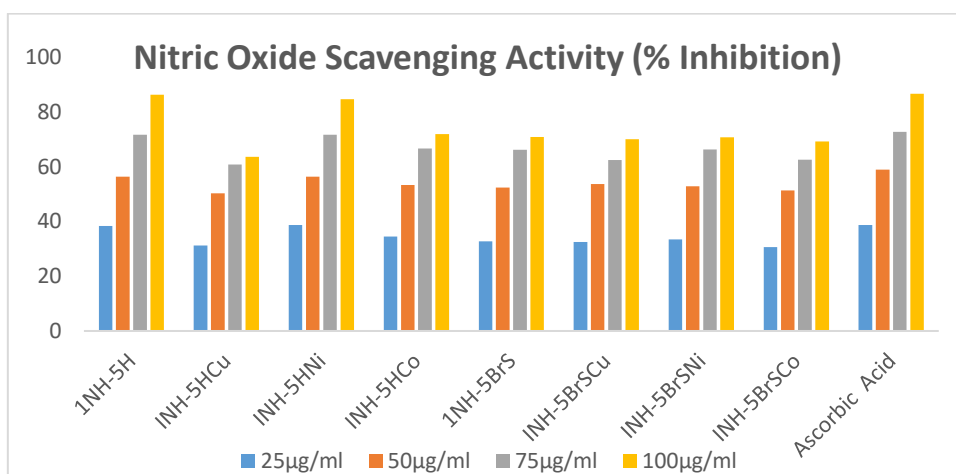


Figure 3: NO Scavenging Activity of the Ligands and their Metal Complexes

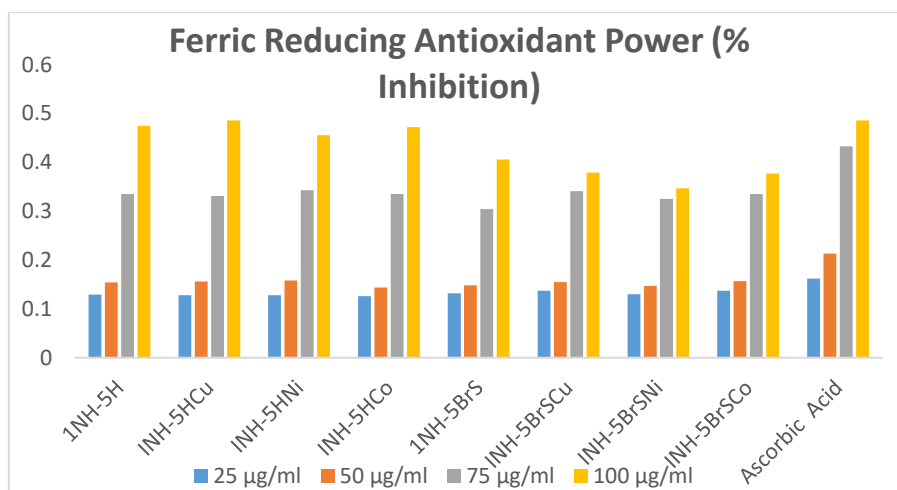


Figure 4: FRAP of the Ligands and their Metal Complexes

The unsubstituted compounds (1NH-5H showed the highest radical scavenging activity at 100 µg/mL with 80.14% radical scavenging capacity whereas 1NH-BrS lagged behind with 68.22%. When the compounds are in the complex form with the nickel ion, the antioxidant capacity increases. In this case, 1NH-5HNi reaches 92.35%, close to the standard ascorbic acid, which reaches 92.22%.

The compound with the bromine substitution, 1NH-BrSNi, reaches 79.35%, whereas the copper complex reaches 80.35%. These results suggest that bromine substitution reduces radical scavenging, while metal complexation generally improves activity, especially for unsubstituted ligands.

### Nitric Oxide (NO) Scavenging Radical

The nitric oxide scavenging assay (Table 2) also showed a similar trend to that of the DPPH assay. The unsubstituted compound 1NH-5H showed high activity with 86.39% inhibition at a concentration of 100 µg/mL compared to the substituted compound 1NH-BrS, which showed lower activity of 70.87%. This is due to the electron withdrawing effect (-I effect) of the bromine atom that likely reduces the scavenging ability.

**Table 4:** Nitric Oxide Scavenging Activity (% Inhibition)

Sample Code	µg/mL			
	25	50	75	100
1NH-5H	38.37	56.33	71.66	86.39
1NH-5HCu	31.27	50.30	60.82	63.67
1NH-5HNi	38.77	56.38	71.71	84.70
1NH-5HCo	34.49	53.36	66.63	71.99
1NH-5BrS	32.72	52.44	66.26	70.87
1NH-5BrSCu	32.50	53.69	62.47	70.10
1NH-5BrSNi	33.45	52.84	66.36	70.75
1NH-5BrSCo	30.67	51.36	62.53	69.22
Ascorbic Acid	38.77	58.93	72.71	86.70

### Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power assay indicates that the compounds with bromine substitutions, like 1NH-BrNi with 0.347 at 100 µg/mL, have lower absorbance than 1NH-5H with 0.4745. This demonstrates that the bromine substitutions have reduced the capacity of the compounds to donate electrons and act as reducing agents, whereas the incorporation of metals ion enhances the ferric reducing antioxidant power.

**Table 5:** Ferric Reducing Antioxidant Power (% Inhibition)

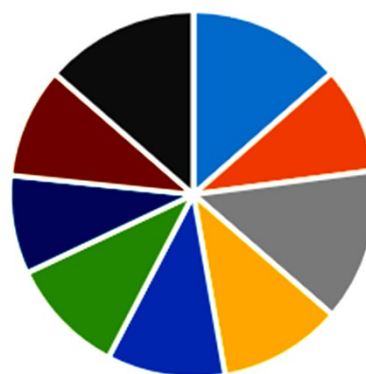
Sample Code	µg/mL			
	25	50	75	100
1NH-5H	0.129	0.154	0.335	0.475
1NH-5HCu	0.128	0.156	0.331	0.486
1NH-5HNi	0.128	0.158	0.343	0.456
1NH-5HCo	0.126	0.144	0.335	0.472
1NH-5BrS	0.132	0.148	0.304	0.406
1NH-5BrSCu	0.137	0.155	0.341	0.379
1NH-5BrSNi	0.130	0.147	0.325	0.347
1NH-5BrSCo	0.137	0.157	0.335	0.377
Ascorbic Acid	0.162	0.213	0.433	0.486

### Total Antioxidant Capacity

The TAC further supported the trend in the antioxidant capacity of the synthesized compounds. The unsubstituted 1NH-5H compound and its nickel complex 1NH-5HNi, had high antioxidant activity: 97.85% and 97.4%, respectively. However, the bromo-substituted 1NH-5BrS was reduced to 75.39%. In other words, introduction of halogen such as with bromine reduces electron donor capabilities as well as reducing power.

**Table 5:** Total Antioxidant Capacity (% Inhibition)

Sample Code	% Inhibition
1NH-5H	97.85
1NH-5HCu	67.67
1NH-5HNi	97.40
1NH-5HCo	78.45
1NH-5BrS	75.39
1NH-5BrSCu	74.77
1NH-5BrSNi	62.67
1NH-5BrSCo	70.42
Ascorbic Acid	98.24



■ 1NH-5H ■ 1NH-5HCu ■ 1NH-5HNi

**Figure 5:** Total Antioxidant Capacity (% Inhibition) TAC of the Ligands and their Metal Complexes

### Structure-Activity Relationship (SAR) Analysis

The study reveals the adverse effect of bromine substitution on the antioxidant activity of the synthesized compounds and their metal complexes. This is demonstrated in their reduced scavenging capacity of the compounds, total antioxidant potential and reducing power. It has been found by researchers (Rana et al., 2024) that the type of substituents used, either withdrawing or donating electrons, significantly affects the activity of a compound. Bromine is more electronegative than carbon. This causes a strong electron-withdrawing inductive effect (-I) across the entire aromatic ring's σ-bond system. This leads to a reduction of the electron density at the phenolic oxygen, which is the principal center for the donation of hydrogen atoms to neutralize free radicals like DPPH or nitric oxide. The phenolic oxygen becomes more positive due to the reduction of its electron density leading to the strengthening of the O-H bond. This makes the process of breaking the bond more energetically demanding which in turn, makes the process of hydrogen atom transfer thermodynamically less favorable and reduces the capacity of the ligand substituted with bromine to stabilize free radicals compared to the unsubstituted counterparts. After the hydrogen transfer process, a phenoxy radical is formed. The electron-withdrawing nature of the bromine does not provide adequate electrons to delocalize and stabilize the unpaired electron. This agrees with the work done previously (Thanuja et al., 2022), which showed that ligands containing an electron-donating group on the phenolic ring have better antioxidant activities due to the ability to stabilize the radical formed compared to those

containing an electron-withdrawing group that hinders the antioxidant activities. The unsubstituted ligands have better stability due to the ability to delocalize electrons. From all the results combined, it was observed that compound containing bromine group is detrimental to antioxidant ability. This is because it makes the O-H bond less accessible for cleaving and destabilizes the radical that is formed. On the other hand, forming a complex with transition metals such as nickel can reduce this disadvantage. This is because coordination with metals increases the ability to scavenge for radicals and increases reduction potential to facilitate hydrogen transfer.

## Conclusion

A comparative study of 1NH-5H and 1NH-5BrS derivatives showed that the presence of a Br atom greatly reduced antioxidant activity due to the inductive effect of Br, which increases the strength of the O-H bond in the phenolic compound and thereby interferes with the hydrogen atom transfer mechanism. However, 1NH-5H derivatives, especially the nickel complex, exhibited antioxidant activity as a potential lead compound similar to that of ascorbic acid. In designing new molecular structures for antioxidant capacity, this effect should be avoided.

## Ethical Approval

This study did not involve human participants or animal experimentation and therefore did not require formal ethical approval. All procedures involving data handling and analysis were conducted responsibly and in accordance with standard academic and institutional guidelines.

## Declaration of conflict of interest

The authors declare that no known conflict of interest or personal relationships that could have influence the work reported in this paper.

## Funding

No Source of Funding

## Declaration of Data Availability

Data are available upon request from the corresponding author.

## Author's Contribution

CUD carried out and wrote the synthetic and characterization part of the work, TMF supervised the project and JIO interpreted and wrote the radical scavenging activity part of the work.

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