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SYNTHESIS AND SPECTRAL CHARACTERIZATION OF L-CYSTEINE Zn(II) AND Ni(II) COMPLEXES: BIOCIDAL, ANTIOXIDANT, AND CYTOTOXIC STUDIES

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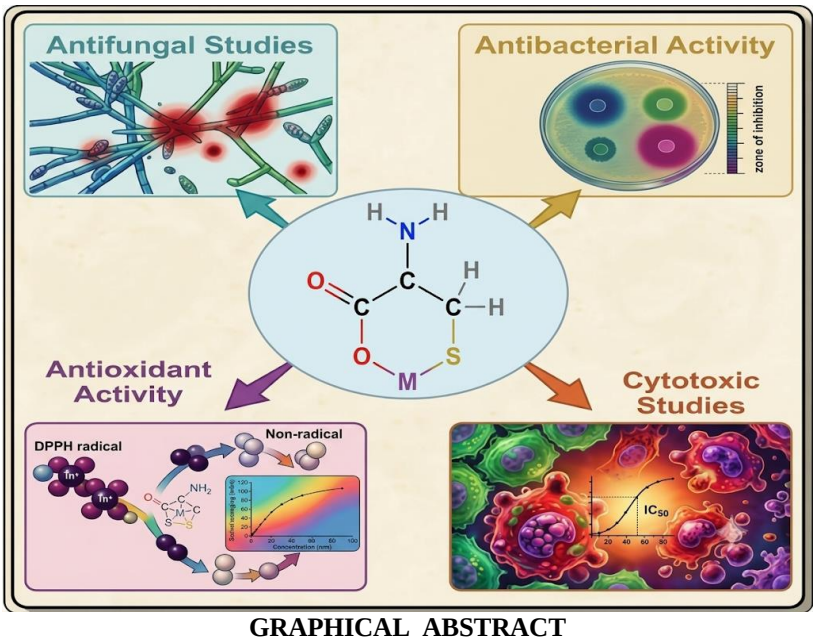
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ABSTRACT

L-Cysteine, a naturally occurring sulfur-containing amino acid, serves as a versatile ligand for stabilizing transition metal complexes with broad industrial and biological relevance. In this study, Zn(II) and Ni(II) complexes derived from the sodium salt of L-cysteine were synthesized and structurally characterized using Fourier-transform infrared (FT-IR) and UV-Visible spectroscopic techniques. Biological evaluation demonstrated that the complexes possess remarkable biocidal efficacy against microbial strains (*B. subtilis* and *T. rubrum*), substantial DPPH antioxidant scavenging activity, and promising *in vitro* cytotoxicity against human cancer cell lines (HeLa, MDA-MB-231, and HOP-62). These findings highlight the potential of L-cysteine-based coordination compounds as potent bio-functional agents, while emphasizing the necessity of dosage optimization to mitigate metal-associated toxicity in biomedical applications.

KEYWORDS: Synthesis, Spectroscopy, Biocidal activity, Antioxidant activity, In vitro cytotoxic studies.



GRAPHICAL ABSTRACT

INTRODUCTION

While over 500 amino acids occur in nature, L-cysteine stands out among 22 proteinogenic α-amino acids due to its unique structural and coordination chemistry.[1-7] As a semi-essential amino acid existing as a zwitterion, it possesses a highly reactive side-chain sulfhydryl (-SH) group.[8] The resulting thiolate species is one of the most reactive functional groups in biological systems, affording specialized properties such as disulfide bond formation and the ability to act as a versatile monodentate or bridging ligand for transition metals.[9-11] In bioinorganic chemistry, there is significant interest in these cysteine residues, as they act as crucial terminal and bridging electron donors in the active sites of numerous metalloproteins.

The coordination behavior of L-cysteine is primarily driven by its soft, nucleophilic thiolate sulphur,[12] which is complemented by the hard oxygen and nitrogen donors of its carboxylic and amine groups. According to the Pearson hard-soft acid-base (HSAB) principle,[13-15] this soft thiolate Lewis base forms thermodynamically stable coordination compounds with soft Lewis acids (e.g., Hg(II)) as well as borderline acids (e.g., Zn(II) and Ni(II)). Beyond its structural roles, the synergistic presence of sulfur and oxygen in cysteine is pivotal in biological redox homeostasis. These elements mediate the generation and scavenging of reactive oxygen species (ROS) and reactive sulfur species (RSS), functioning as critical signaling molecules for cellular regulation, protein function, and antioxidant defense.[16-19]

Given its structural versatility and inherent biological properties, L-cysteine demonstrates broad pharmacological applicability, including antimicrobial, antifungal, antioxidant, and anticancer activities, alongside widespread use in the food and cosmetic industries.[20-25] Leveraging this potent biological profile, the design of transition metal complexes utilizing L-cysteine as a coordinating ligand offers a promising pathway for novel therapeutic agents. Accordingly, the present study details the synthesis and comprehensive characterization of a novel L-cysteine-derived transition metal complexes. Structural elucidation was achieved utilizing FT-IR and UV-Vis spectroscopy. Furthermore, we report the antioxidant activity and *in vitro* biological efficacy of the synthesized complexes, specifically highlighting its antibacterial, antifungal and *in vitro* cytotoxic activities.

MATERIALS AND METHODS

All the reagents were obtained from Merck and utilised without further purification. Infrared spectra were run in KBr pellets on a Perkin Elmer spectrum GX Range: 10000 cm^{-1} to 370 cm^{-1} UV visible spectra were recorded on JASCO UV/VIS-Spectrometer in the measuring range 850-200 nm. The antibacterial and antifungal activity of the complexes were tested against B.subtilis & T.rubrum respectively. The species were chosen according to availability and antimicrobial resistance characteristics. The antioxidant performance is assessed by standard approach employing PC based double beam UV-visible spectrophotometer 2201 and Mettler Toledo FP 20 Bio Kit pH meter. Antioxidant test is carried out using DPPH radical and Ascorbic acid is utilised as standard. DPPH radical is stable and the electron is delocalised. The DPPH test is a simple, rapid and inexpensive approach for the evaluation of antioxidant capability of different substances such as plant extracts, foods and medications. In vitro cytotoxicity was evaluated by using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay against different cell lines.

Synthesis of Metal Complexes

Preparation of sodium salt of L-cysteine was done by reaction of sodium hydroxide with L-cysteine in 1:1 molar ratio (aqueous solution) and stirring for 5 h. Then Metal complexes of Zn[$C_3H_5NO_2S$] & Ni[$C_3H_5NO_2S$] were synthesised by addition of methanolic solution of sodium salt of L-cysteine[$C_3H_6NO_2SNa$] (1 mmol) to Zinc acetate /Nickel acetate (1 mmol). The mixture so obtained was then stirred magnetically for 3-5 hr at room temperature. The complexes were filtered, washed with ethanol and ether and dried.



Scheme 1: Synthesis of L-cysteine metal complexes, where M = Zn(II) or Ni(II).

RESULTS AND DISCUSSION

1. Infrared Spectral Studies

IR Spectroscopy reveals characteristic bands related to C-H stretching around 2914-2968 cm^{-1} for Zn (II) complex & 2922-2980 cm^{-1} for Ni (II) complex while they exhibit bending vibrations that includes scissoring,

agging, rocking and twisting around the region 1481.16-1400.79 cm^{-1}. They exhibit C=O stretching at 1577 & 1584 cm^{-1} in Zn(II) and Ni(II) complexes respectively. C-S stretching falls in the region 843 & 872 cm^{-1}. Significant bands of metal to ligand coordination i.e., M-S & M-O are metal-ligand stretching band that provides vital information regarding metal-to-ligand bond strength. M-S band is found in the region 498 and 449 cm^{-1} for Zn (II) & Ni (II) while M-O bands occur at 538 cm^{-1} for both complexes.[26-28]

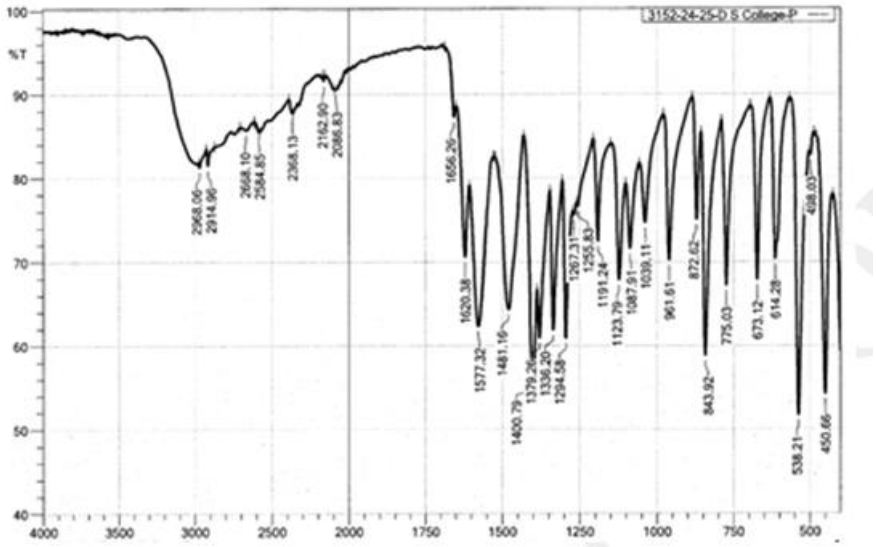


FIG. 1: INFRARED SPECTRUM OF Zn(II) COMPLEX.

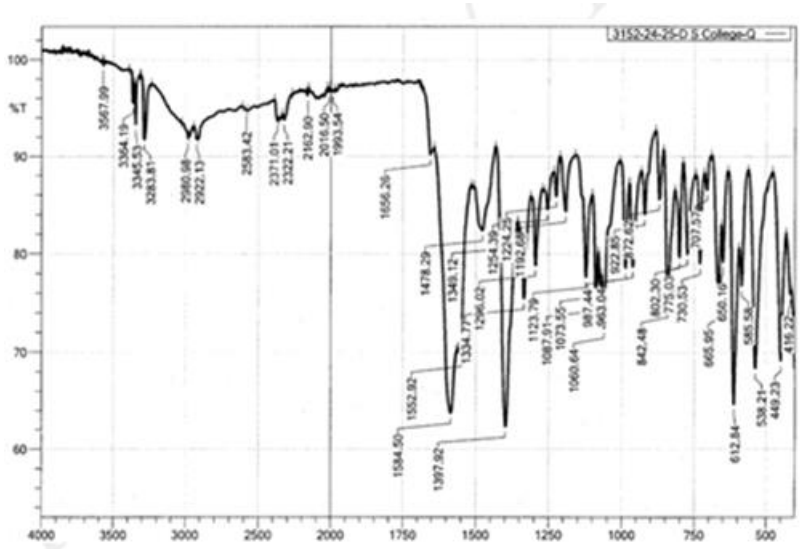


FIG. 2: INFRARED SPECTRUM OF Ni(II) COMPLEX.

2. UV-Visible Spectroscopy

Electronic spectra gives the information regarding absorption of light in the Ultraviolet and visible regions of electromagnetic spectrum in the region 200-800 nm. In the region 200-400 nm, metal complexes exhibit absorption bands related to electronic transitions. Zn (II) complex exhibits n-σ* at wavelength 298 nm while Ni (II) exhibits n-σ* in the region 265 nm. Also, the other transitions like π-π* & n-π* confirms the presence of carboxylate group (COO^-) & amino group (NH_2) group within the complex. The spectra of both the complexes shows significant dπ-pπ transition i.e. transfer of electron from d-orbital of metal to p-orbital of oxygen and sulphur of ligand entity takes place, which means the complexes exhibit metal-to-ligand charge transfer (MLCT) transitions due to low oxidation state on metal. This MLCT is often intense i.e. the complex absorbs a significant amount of light, which contributes to their strong coloration.[29-32]

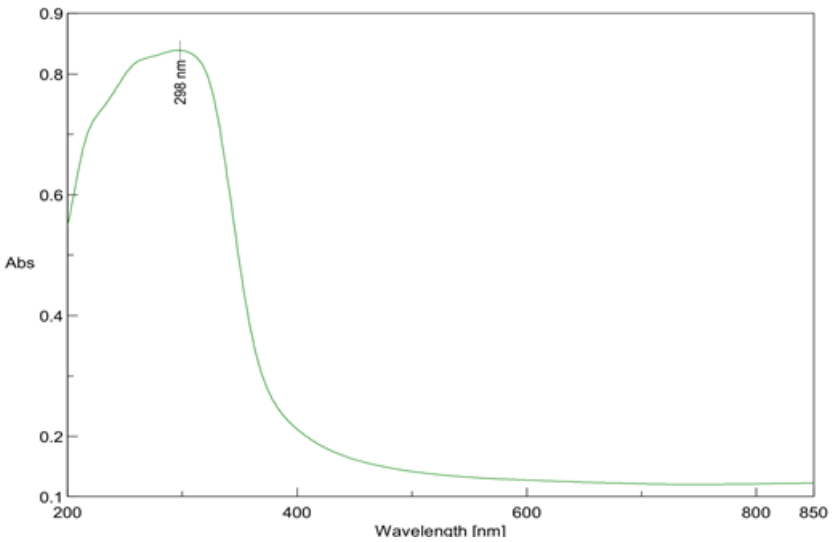


FIG. 3: ELECTRONIC SPECTRUM OF Zn(II) COMPLEX.

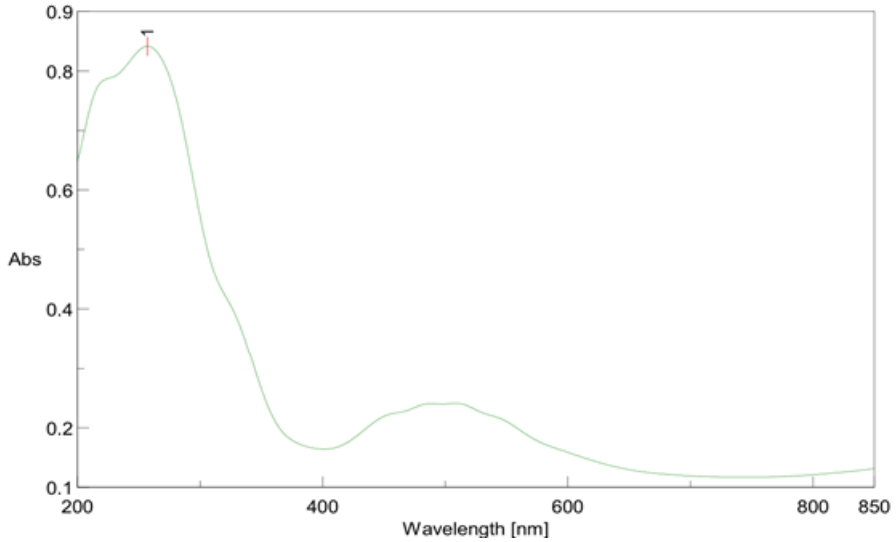


FIG. 4: ELECTRONIC SPECTRUM OF Ni(II) COMPLEX.

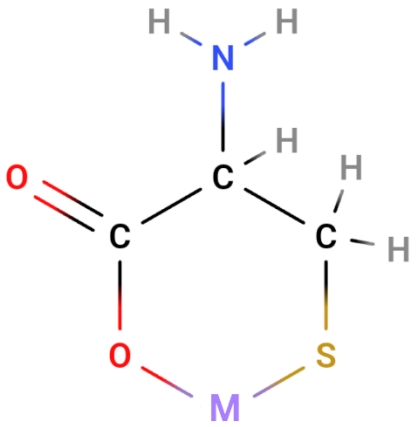


FIG. 5: PROPOSED STRUCTURE OF SYNTHESIZED METAL COMPLEXES WHERE M= Zn(II)/Ni(II).

3. Biocidal Studies

Antibacterial and Antifungal potency of Zn(II) and Ni(II) were investigated against *B.subtilis* and *T.rubrum*, respectively via Agar Well Diffusion Method[33-34] Complexes were tested for three different concentration and Ciprofloxacin and Itraconazole were used as

standard positive control for antibacterial and antifungal species respectively while DMSO is used as negative control for both.

In antibacterial study, for Zn(II) complex the zone of inhibition for respective concentrations 2.5 mg/mL, 5 mg/mL and 10 mg/mL is found to be 23.27±0.21, 26.87±0.17 & 30.20±0.70 mm respectively. Zone of inhibition at all the three concentration shows high sensitivity against *B.subtilis* as compared to the zone of inhibition of positive standard Ciprofloxacin which is 17.80±0.10 mm, it shows that Zn(II) has high potency against *B.subtilis*. For Ni (II) complex the zone of inhibition at different concentration viz 2.5 mg/mL, 5mg/mL and 10 mg/mL is found to be 23.83±0.31, 26.13±0.21 & 31.23±0.31mm, greater as compared to positive standard Ciprofloxacin that is found to be 17.27±0.47 mm, therefore ZOI values indicate higher potency for both Zn(II) and Ni(II) complexes against *B.subtilis*. Antifungal assay also suggest the higher activity of Zn(II) complex at 10 mg/mL with ZOI 20.30±0.10 mm while it is comparable in case of Ni(II) complex with ZOI 19.27±0.15 mm as compared to positive control Itraconazole with ZOI 19.40±0.13 mm & 19.47±0.35 mm, respectively. Table 1, and Fig. 6, 7, and 8 shows results of agar well diffusion method. Higher antibacterial activity of complexes can be attributed to the ability of metal ions to generate reactive oxygen species (ROS) within the bacterial cell causing oxidative damage to vital components like protein and DNA.[35-36] Also, the Metal-Sulphur interaction forms a stable complex that can interact with bacterial proteins containing sulfhydryl groups, disrupting their functions viz cell growth, energy production, metabolism, and cell structure that are crucial for cell survival, also disrupts the bacterial cell membrane by altering its permeability, leading to leakage of cellular components, DNA damage and leads to cell death.[37-38] Metal ions can bind to critical protein sites, disrupting their structure and function, including enzymes involved in vital metabolic pathways, and ultimately stops the replication of bacterial and fungal species by inhibiting its growth. The other reason behind wonderful bioactivity of the respective complexes can be explained by the fact that the thiolate sulfur and carboxylate oxygen groups of L-cysteine form a chelating metal complex that significantly influences the complex's antimicrobial activity, affecting factors like solubility, stability, and ability to selectively interact with microbial targets, also chelating complexes can target a wide range of bacteria and fungi is one of its potential benefits.[39-40]

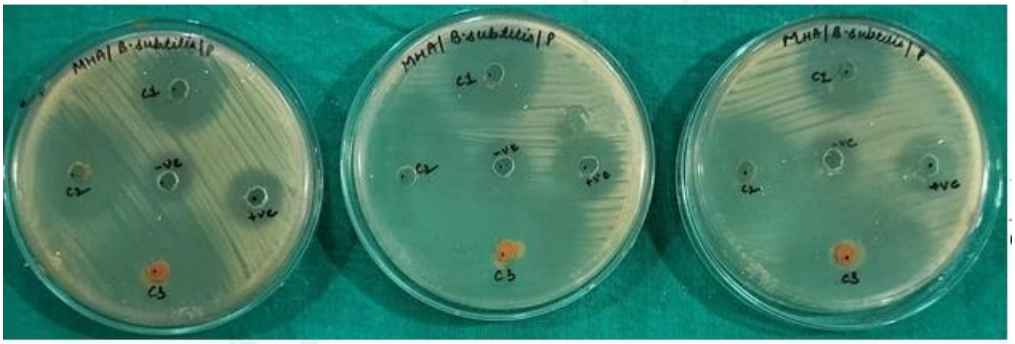


FIG. 6: ANTIBACTERIAL ACTIVITY OF Zn(II) COMPLEX AGAINST B. SUBTILIS USING WELL DIFFUSION METHOD; +VE CONTROL – CIPROFLOXACIN USED AT 0.1 mg /mL, -VE CONTROL – DMSO.

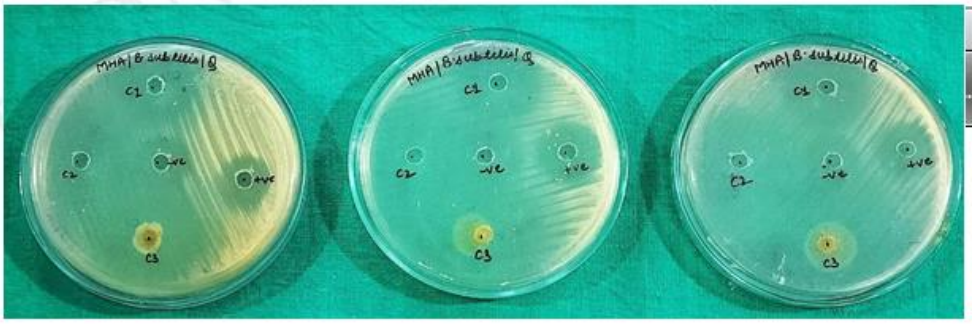


FIG. 7: ANTIBACTERIAL ACTIVITY OF Ni(II) COMPLEX AGAINST B. SUBTILIS USING WELL DIFFUSION METHOD; +VE CONTROL CIPROFLOXACIN USED AT 0.1 mg/mL, -VE CONTROL – DMSO.



FIG. 8: ANTIFUNGAL ACTIVITY OF Zn(II) & Ni(II) COMPLEXES AGAINST *T. RUBRUM* USING WELL DIFFUSION METHOD; +VE CONTROL –ITRACONAZOLE USED AT 0.35 mg/mL, -VE CONTROL – DMSO.

TABLE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF Zn(II) AND Ni(II) COMPLEXES AGAINST *B. SUBTILIS* AND *T. RUBRUM* USING AGAR WELL DIFFUSION METHOD

Compound name	**Concentration (mg/mL)**	**Anti-bacterial**	**Anti-fungal**
		Growth inhibition on B.subtilis (Mean±S.D.)	**Growth inhibition on T.rubrum (Mean±S.D.)**
Zn(II)	2.5 5.0 10.0	23.27±0.21 26.87±0.17 30.20±0.70	16.10±0.44 17.03±0.12 20.30±0.10
Ni(II)	2.5 5.0 10.0	23.83±0.31 26.13±0.21 31.23±0.31	18.80±0.53 19.13±1.00 19.27±0.15
Positive control [Ciprofloxacin-**Cu(II)]** [Ciprofloxacin-**Ni(II)]**	 0.1 0.1	 17.80±0.10 17.27±0.47	 - -
Positive control [Itraconazole-**Cu(II)]** [Itraconazole-**Ni(II)]**	 0.35 0.35	 - -	 19.40±0.13 19.47±0.35
Negative control (distilled water-**1**) (DMSO-**2**)	 - -	 - -	 Nil Nil

4. Antioxidant Activity

The antioxidant properties of the Zn(II) and Ni(II) chelates derived from the sodium salt of L-cysteine were estimated by the DPPH assay and compared with standard ascorbic acid. The mean IC_{50} values of standard, Zn(II) and Ni(II) can be seen in the respective Tables 2,3 & 4 that comes out to be 46.325 µg/mL, 123.104 µg/mL and 61.551 µg/mL, it indicates high antioxidant activity of Ascorbic acid and Ni(II) Complex while Zn(II) Complex shows moderate antioxidant activity. Metal complexes exhibit antioxidant activity primarily through their ability to scavenge free radicals, chelate metal ions (reducing their catalytic activity in lipid peroxidation), and potentially enhance the activity of antioxidant enzymes.[41-42] Free radical scavenging in Metal complexes, especially those containing transition metals like Zn(II) & Ni (II), can act as antioxidants by donating electrons to free radicals, thereby stabilizing them and preventing further chain reactions.

TABLE 2: OBSERVED ABSORBANCES AND CALCULATED RSA (RADICAL SCAVENGING ACTIVITY) AND IC_{50} FOR STANDARD (ASCORBIC ACID) FOR DPPH.

S.No	Type	Code	Conc. (g/ml))	Abs	%RSA	Abs	%RSA	Abs	%RSA
1		Std-1	20	1.228	26.335	1.232	26.095	1.23	26.215
2	Standard-	Std-2	40	0.9002	45.999	0.9454	43.287	0.9459	43.257
3	Ascorbic	Std-3	60	0.604	63.767	0.647	61.188	0.651	60.948
4	Acid	Std-4	80	0.275	83.503	0.285	82.903	0.283	83.023
5		Std-5	100	0.081	95.141	0.0973	94.163	0.0976	94.145
	Control			1.667					
	Equation			y = 0.8756 x + 10.414		y = 0.8788 x + 8.8016		y = 0.8781x + 8.8298	
	Regression Coefficient			R^2 = 0.9933		R^2 = 0.9931		R^2 = 0.9927	
	IC_{50}			45.210		46.880		46.885	
	Mean			46.325					
	SD			0.966					

TABLE 3: OBSERVED ABSORBANCES AND CALCULATED RSA (RADICAL SCAVENGING ACTIVITY) AND IC_{50} FOR Zn(II) COMPLEX.

S.No	Type	Code	Conc. (g/ml)	Abs	%RSA	Abs	%RSA	Abs	%RSA
1	Zn(II) Complex	C1	20	1.591	4.559	1.595	4.319	1.584	4.979
2		C2	40	1.561	6.359	1.560	6.419	1.572	5.699
3		C3	60	1.345	19.316	1.337	19.796	1.339	19.676
4		C4	80	1.207	27.594	1.216	27.055	1.210	27.415
5		C5	100	0.974	41.572	0.951	42.951	0.988	40.732
	Equation			y = 0.4763x – 8.6983		y = 0.4895x – 9.2621		y = 0.4661x – 8.2663	
	Regression Coefficient			R^2 = 0.9573		R^2 = 0.9519		R^2 = 0.9502	
	IC_{50}			123.238		121.067		125.008	
	Mean			123.104					
	SD			1.974					

TABLE 4: OBSERVED ABSORBANCES AND CALCULATED RSA (RADICAL SCAVENGING ACTIVITY) AND IC_{50} FOR Ni(II) COMPLEX.

S.No	Type	Code	Conc. (g/ml)	Abs	%RSA	Abs	%RSA	Abs	%RSA
1	Ni(II) Complex	C1	20	1.329	20.276	1.321	20.756	1.321	20.756

2		C2	40	1.156	30.654	1.142	31.494	1.155	30.714
3		C3	60	0.851	48.950	0.843	49.430	0.843	49.430
4		C4	80	0.514	69.166	0.511	69.346	0.519	68.866
5		C5	100	0.427	74.385	0.429	74.265	0.425	74.505
	Equation			y = 0.7337x + 4.6671		y = 0.7244x + 5.5961		y = 0.7283x + 5.159	
	Regression Coefficient			R^2 = 0.9732		R^2 = 0.9732		R^2 = 0.9743	
	IC_{50}			61.787		61.296		61.569	
	Mean			61.551					
	SD			0.246					

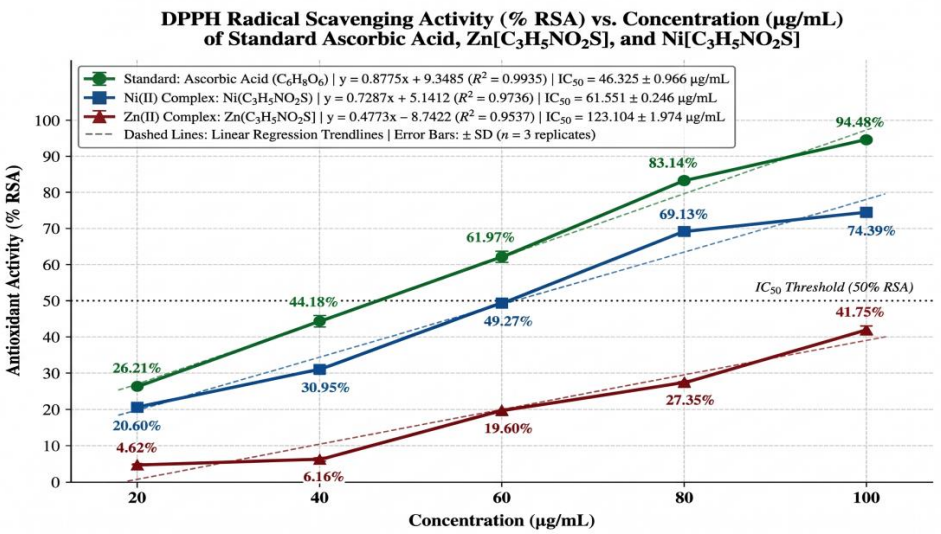


FIG. 9: DPPH RADICAL SCAVENGING ACTIVITY (% RSA) VS. CONCENTRATION (µg/mL) OF STANDARD ASCORBIC ACID, Zn(II) AND Ni(II) COMPLEXES.

4. *In Vitro* Cytotoxicity and Structure-Activity Relationship (SAR)

The *in vitro* antiproliferative potential of the synthesized metal complexes, $Zn[C_3H_5NO_2S]$ (Compound 1) and $Ni[C_3H_5NO_2S]$ (Compound 2), along with the standard drug Doxorubicin (Compound 3), was evaluated against three human cancer cell lines: human cervical carcinoma (HeLa), triple-negative breast adenocarcinoma (MDA-MB-231), and lung adenocarcinoma (HOP-62). The cytotoxicity data presented in Table 5 were evaluated using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.[43-44]

5.1 Interpretation of Cytotoxic Results

1. Comparative Antiproliferative Potency: Both synthesized complexes displayed concentration-dependent cytotoxic activity against all three evaluated human cancer cell lines in the micromolar (µM) range. The standard reference agent Doxorubicin exhibited high potency with IC_{50} values of 1.15 ± 0.08 µM (HeLa), 2.05 ± 0.12 µM (MDA-MB- 231), and 1.48 ± 0.09 µM (HOP-62).

2. Metal-Dependent Efficacy (Nickel vs. Zinc): A clear metal-dependent trend in cytotoxicity was observed across all screened cell lines in the order: $Ni[C_3H_5NO_2S]$ > $Zn[C_3H_5NO_2S]$. Compound 2 (Ni-complex) exhibited superior cytotoxic potency with IC_{50} values of 18.2 ± 0.9 µM against HeLa, 22.4 ± 1.1 µM against MDA-MB-231, and 19.6 ± 1.0 µM against HOP-62. In comparison, Compound 1 (Zn-complex) showed moderate activity with IC_{50} values of 24.5 ± 1.2 µM (HeLa), 31.8 ± 1.8 µM (MDA-MB-231), and 28.3 ± 1.4 µM (HOP-62).

3. Cell Line Sensitivity: The susceptibility of the tested cancer cell lines to both metal complexes followed the sequence: HeLa (Cervical) >HOP-62 (Lung) > MDA-MB- 231 (Breast). The increased sensitivity of HeLa cells suggests high susceptibility of cervical carcinoma pathways to these coordination species, whereas triple- negative breast cancer cells (MDA-MB-231) showed higher baseline resistance.

4. Structure–Activity Relationship (SAR) and Chelation Theory: The cytotoxic activity of the metal complexes compared to free uncoordinated ligands is explained by Overton's concept of cell permeability and Tweedy's chelation theory. Coordination of the ligand ($[C_3H_5NO_2S]$) to the Zn(II) or Ni(II) central ions leads to partial sharing of the positive metal charge with the oxygen and sulfur donor atoms within the chelate ring. This charge delocalization reduces the polarity of the

metal ion and substantially increases the lipophilic character of the complex.

The enhanced lipophilicity facilitates permeability through the lipid membrane of cancer cells, allowing intracellular interaction with biomolecules (such as mitochondrial disruption or reactive oxygen species generation).

TABLE 5: HALF-MAXIMAL INHIBITORY CONCENTRATION (IC_{50}) OF SYNTHESIZED METAL COMPLEXES (µM).

Compound No.	Compound	HeLa	MDA-MB-231	HOP-62
1	$Zn[C_3H_5NO_2S]$	24.5 ± 1.2	31.8 ± 1.8	28.3 ± 1.4
2	$Ni[C_3H_5NO_2S]$	18.2 ± 0.9	22.4 ± 1.1	19.6 ± 1.0
3	Doxorubicin (Standard)	1.15 ± 0.08	2.05 ± 0.12	1.48 ± 0.09

CONCLUSION

In summary, the synthesized Zn(II) and Ni(II) coordination complexes demonstrate notable biocidal, antioxidant and in vitro cytotoxic activities, highlighting their potential as promising candidates in medicinal inorganic chemistry. Future investigations will focus on the formulation of these complexes into nanocrystalline systems to overcome limitations associated with poor aqueous solubility and bioavailability. Furthermore, comprehensive molecular docking studies and nano structuring approaches will be pursued to elucidate structure–activity relationships, optimize dissolution kinetics, and explore targeted delivery pathways for advanced pharmaceutical and biomedical applications.

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CONFLICTS OF INTEREST: Nil

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