RJC

RASĀYAN ***J. Chem.***
Vol. 17 | No. 3 |945-956| July - September | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

STUDIES ON NOVEL METAL COMPLEXES OF COUMARIN SCHIFF BASE: SYNTHESIS, STABILITY CONSTANT, ANTIMICROBIAL AND ANTICANCER ACTIVITY

Vindhya R.[1], T. M. Sharanakumar[1], Veerendra Kumar A. Kalalbandi[1], K. N. Shashiraj[2], K. M. Nagarsha[3], H. M. Suresh Babu[4] and Suresh[1,✉]

[1]Department of Chemistry, Ballari Institute of Technology and Management, Bellary-583104, Karnataka, India
[2]P.G. Department of Botany, Karnataka University, Dharwad-580003, Karnataka, India
[3]Department of Chemistry, Sahyadri Science College, Shivamogga-577 203, Karnataka India
[4]Department of Chemistry, Rao Bahadur Y Mahabaleshwara Engineering College, Bellary-583103, Karnataka, India
✉Corresponding Author:drsuresh61@yahoo.com

ABSTRACT

A facile synthetic protocol was employed to synthesize new coumarin Schiff base (E)-3-(1-((2-hydroxy-5-methylphenyl)imino)ethyl)-2H-chromen-2-one] and their Co and Cu metal complexes. Synthesized molecules were characterized by various spectroscopic methods. The results of antibacterial studies demonstrated that the maximum level of growth inhibition for S. aureus and B. subtilis was shown by compound 5, with inhibition zones of 16±1 mm and 18.5±1.3 mm, respectively, while, Compound 4 exhibited 12.5±0.5 mm and 14±0.25 mm, respectively. *In-vitro* Cytotoxicity of Cu-complex 5 towards human hepatocellular adenocarcinoma cancer cells, at concentrations of 12.5, 25, 50, and 100 g/mL, displayed a reduction in the percentage of cell viability in a dose-dependent manner, with values of 72.45%, 60.12%, 48.69%, and 23.25%, respectively. The results revealed that Compound 5 has the potential to interact with the bacterial membrane surface, leading to the disruption of the same. Additionally, potentiometric analysis was used to estimate the proton-ligand dissociation constant of HL as well as its metal stability constants with Co^{2+} and Cu^{2+}.

Keywords: Coumarin, Anticancer, Antibacterial, Schiff Bases, Dissociation Constant.

RASĀYAN*J. Chem.*, Vol. 17, No.3, 2024

INTRODUCTION

Microbial infections and antibiotic resistance are currently the major problems endangering society's health. Worldwide, microbial infections cause millions of deaths each year. Infections were blamed for nearly 17% of all deaths in recent years.[1-3] The development of resistance has made the currently available antibacterial medications less efficient or even ineffective.[4] 4.95 million deaths in 2019 were attributed to antimicrobial resistance, and an additional 1.27 million deaths were reportedly caused directly by drug-resistant illnesses. Without coordinated action, this figure might increase to ten million and AMR could cost the global economy $100 trillion cumulatively by 2050. The World Health Organization (WHO) expressed concern in June 2020 that the COVID-19 pandemic's improper antibiotic use might contribute to the growing trend of antimicrobial resistance.[5] Further, as a result of recent increases in both the incidence and severity of both cancer and multi-resistant infections, both illnesses are already and will likely continue to be major causes of death. Furthermore, due to immune system instability that promotes the growth of cancer cells, persistent infections are one of the major causes of cancer. The World Health Organization (WHO) reports that cancer is the second most common cause of death worldwide, taking 9.6 million lives in 2018. The most prevalent types of cancer include lung, prostate, colon, stomach, liver, breast, cervical, and thyroid. To find alternatives to the side effects of chemotherapy and to get beyond the inherent resistance of cancer cells, the creation of anticancer medicines continues to be a crucial area of research. In the quest of finding newer molecules heterocycles and their metal complexes play a pivotal role. Amongst the heterocycles, coumarins are the large and

http://doi.org/10.31788/RJC.2024.1738886

well-researched class of chemical molecules,due to a wide range of biological characteristics they possess, including antibacterial[6], anticancer[7], antioxidant actions[7], antiviral[8], neuroprotective[9], and anti-inflammatory.[10] Coumarins, both natural and synthetic, furnish with a tremendous therapeutic potential. Consequently, it is possible to intend the coumarin skeleton as a preferred framework for the creation and synthesis of molecules with pharmacological activity.[11] The coumarin moiety can also be combined with other chemical species, such as Schiff bases, to improve their physicochemical characteristicsof coumarins and biological activity. Schiff bases with an imine or azomethine group (>C=N-) are important organic compounds due to their wide range of biological activities and applications in industry.[12] These compounds also act as the main class of ligands for coordination chemistry and as the building blocks for the synthesis of a number of heterocyclic compounds. They coordinate with metal ions via azomethine nitrogen.[13] Schiff base ligands have drawn a lot of attention in the field of coordination chemistry, mainly because of their easy synthesis, availability, and electrical properties. Transition metal complexes of Schiff bases with Cu(II), Ni(II), Zn(II),and Co(II) show exceptional biological activity.In addition, the Schiff bases and their metal complexes have the ability to interact with DNA and act as anti-cancer, anti-bacterial, antioxidant, and anti-fungal, and have many other applications such as being catalysts, dyes,and polymers.[14-16] Taking into account the biological significance of coumarin and from the view point of the antimicrobial potential of Schiff bases and their metal complexes, we have envisioned that the transition metal complexes derived from coumarin Schiff base could be a good starting point for the development of good antimicrobial and anticancer agents. Additionally, understanding stability constants is essential for interpreting many biological and analytical processes. The stability constants of the metal-ligand complexes that form are very useful in understanding the mechanisms by which medicines interact with metals. Nonetheless, the interplay between metals and ligands in complex media regulates the stability of metal complexes.[17-18] To determine stability constants rapid and efficient electroanalytical technique, particularly, the potentiometric titration method is the best choice. The benefits of potentiometric operation also include low cost, large dynamic ranges, low detection limit, fast reaction, simple instrumentation, and low detection limit. To maintain constant activity coefficients across all species in the experimental working environment, the potentiometry approach also requires continuous ionic power correction.[19-20] We report herein, a facile synthetic protocol for the synthesis of coumarin Schiff base and their Co and Cu metal complexes. Newly synthesized molecules were characterized by various spectroscopic methods. The proton-ligand dissociation constant of HL as well as its metal stability constants with Co^{2+} and Cu^{2+} were also discussed.Further, all the synthesized molecules were screened for their *in vitro* antimicrobial and anticancer activity.

EXPERIMENTAL

Materials and Methods

All chemical compounds, solvents, and reagents used were high-grade and used without purification (Sigma Aldrich). All the solutions were prepared with double-distilled water. Microbial strains, such as *Staphylococcus aureus* (MTCC 6908), *Bacillus subtilis* (MTCC 6633), *Escherichia coli* (MTCC 40), and *Shigella flexineri* (MTCC 1457) were obtained from IMTECH, Chandigarh. Ahuman hepatocellular adenocarcinoma (HepG2) cell line was obtained from NCCS, Pune, India. FTIR spectra have been recorded on a Perkin Elmer 1600 FT-IR spectrophotometer using KBr pellets. ^{1}H-NMR spectra were recorded by Varian Mercury 200 MHz spectrometer in $CDCl_3$, and Micromass Quatro LC/MSD was used to measure the Mass spectra. Elemental analysis for carbon, hydrogen, and nitrogen was done by Vario EL (III) CHNS analyzer, and Elico pH meter LI122 with combined electrode was used for the pH measurements. For each independent titration of perchloric acid with NaOH standard solutions was performed by maintaining identical experimental conditions of temperature and ionic strength.

Preparation of Standard Stock and Titration Solutions

The protonation constants of ligand and metal-ligand complexes were performed by the followingtitrations:

1. $HClO_4$ (5mL of 0.01 M), $NaClO_4$ (5mL of 1.0 M), and 47.5 ml 50% aq-alcohol taken in titration cell and titrated with standard NaOH (0.1 N).
2. $HClO_4$(5mL of 0.01 M), $NaClO_4$(5mL of 1.0 M), Ligand (5mL of 0.01M), and 42.5 mL 50% aq-

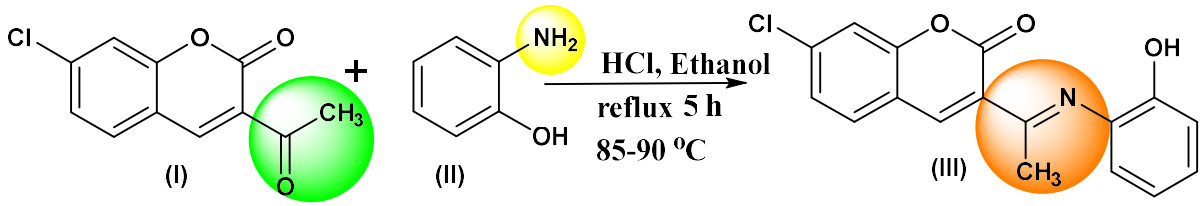
alcohol taken in titration cell and titrated with standard NaOH (0.1 N). (For the protonation constant of the Schiff base).

3. $HClO_4$ (5mL of 0.01 M), $NaClO_4$(5mL of 1.0 M), Ligand (5mL of 0.01M),metal (II) chloride (5mL of 0.01 M), and 37.5 ml 50% aq-alcohol taken in titration cell titrated with standard NaOH (0.1 N). (For the stability constant of the complexes)

Synthesis of Schiff base (E)-3-(1-((2-hydroxy-5-methylphenyl)imino)ethyl)-2H-chromen-2-one] Ligand (3)

The compound (E)-3-(1-((2-hydroxy-5-methylphenyl)imino)ethyl)-2H-chromen-2-one (3) was synthesized by the condensation of 7-chloro-3-acetyl coumarin (1) (0.01 M) with *o*-aminophenol (0.01M) in ethanol at reflux temperature for 5 h in acidic condition. The completion of the reaction was monitored by thin-layer chromatography (TLC) with a mobile phase of 25% ethyl acetate in pet ether. The contents of the reaction mixture were then transferred to crushed ice to obtain a brown-colored Schiff base. It was filtered, washed with alcohol, and dried in the oven for 30 minutes at 40-50 °C (Scheme-1).

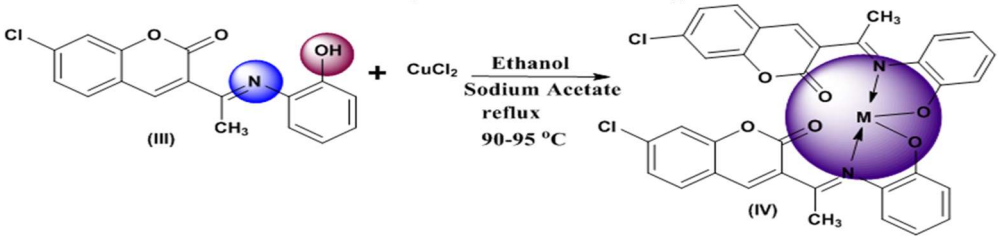
(E)-3-(1-((2-hydroxy-5-methylphenyl)imino)ethyl)-2H-chromen-2-one (3): Yield=70%. Melting point: 135-140°C; Brownsolid; Yield: 70 %;mp:135 °C. FT-IR (KBr, cm^{-1}) 3360 (-OH), 2926(Ar C-H), 1755 (C=O), 1603 (C=N).^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.46 (s, 3H, $-CH_3$), 8.45 (S, 1H, -C4 of Coumarin), 8.02 (d, *J* = 8 Hz, 1H, -C5 of Coumarin), 7.49-8.45 (Ar protons), 10.53 (s, 1H, -OH).MS (m/z): [M] 313.12.



Scheme-1: Synthesis of (E)-7-chloro-3-(1-((2-hydroxyphenyl)imino)ethyl)-2H-chromen-2-one(**3**)

Synthesis of (E)-7-chloro-3-(1-((2-hydroxyphenyl)imino)ethyl)-2H-chromen-2-onesubstituted metal complexes (4)

The metal complexes (4) were prepared according to Scheme-2. A mixture of Schiff base ligand (3) (0.02M) andmetal(II)chloride (0.01M) (Co and Cu), was dissolved in ethanol. To this, 2 g of sodium acetate was added and the contents of the reaction mixture were refluxed for 5-6 h at 80-90 °C. The completion of the reaction was monitored by thin-layer chromatography (TLC) with a mobile phase of 25% ethyl acetate in pet ether. Then the reaction mixture was poured into ice-cold water, the solid was separated, filtered, washed with water, and dried Yield=60%. Co and Cu complexes of (E)-7-chloro-3-(1-((2-hydroxyphenyl)imino)ethyl)-2H-chromen-2-one *(4 and 5)* (Scheme-2). Brown solid; Melting point: >300 °C;Yield: 60 %; FT-IR (KBr, cm^{-1}), 2928 (Ar C-H), 1756 (C=O).^{1}H-NMR (400 MHz, DMSO-d6) δ 2.66 (s, 3H, $-CH_3$), 8.00 (S, 1H, -C4 of Coumarin), 8.18 (d, *J* = 7.6 Hz, 1H, -C5 of Coumarin), 7.53-8.18 (Ar protons), MS (m/z): [M] 684.11 (Co complex), 689.02 (Cu complex).



Scheme-2: Synthesis of Schiff base Metal Complexes (M= 4) Co, 5) Cu)

Antibacterial Activity of (4 and 5)

Using the agar well diffusion method described by Aritonang et al. (2019), the antibacterial activity of (4 and5) against selected human pathogenic strains was evaluated. We chose *S. flexineri*, *E. coli*, *B. subtilis* and *S. aureus* for activity testing. Bacterial test strains were subcultured on the corresponding broth media. Using sterile cotton swabs, each culture strain was distributed uniformly over the surface of

nutrient agar plates. A gel-hole puncher was used to create the 6 mm wells on the 4 mm-thick agar plates. The wells were then loaded with (4 and 5) at concentrations ranging from 25 to 100 µg/µL. The plates were then placed in an incubator and incubated for twenty-four hours at 37 °C. As a positive control, streptomycin was used. At the conclusion of incubation, the inhibition zone formed around each well was measured in millimeters using a Hi-media antibiotic zone measuring scale.

In-vitro Anticancer Activity of(4 and5)by MTT Assay

The HepG2 cell line, derived from human hepatocellular adenocarcinoma cancer, was obtained from NCCS in Pune, India. The cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM) and incubated for 24 h at 37 ℃ in a 5% CO_2 atmosphere with 95 % humidity. To promote cell proliferation, 10 % fetal bovine serum (FBS) was added to the medium. Following the incubation period, the cells were introduced into 96-well microtiter plates at a concentration of 20,000 cells per well, with a total volume of 200 µL of complete culture medium. Subsequent to the initial step, HepG2 cells were seeded into wells that were preloaded with varying concentrations of (4 and 5) ranging from 12.5 to 200 µg/mL. Cell viability was assessed following incubation for specified durations in a humid incubator at 37 ℃. The experimental procedure incorporated doxorubicin at a concentration of 4 µM/mL as a positive control, while cells without any treatment were utilized as negative controls (4 and 5). Approximately 200 µL of recently prepared MTT solution was blended with each well, followed by an incubation period of 4 hours at 37 ℃, resulting in the observation of a purple color precipitate. Following the incubation period, the MTT solution was removed from the wells and replaced with 100 µL of buffered DMSO to homogenize the formazan crystals produced during the experiment. The plates were then vigorously agitated. Viable cells were quantified at a wavelength of 570 nm using a microtiter plate reader (ELX-800, BioTek, USA).[21-22] The IC_{50} value was utilized to express the final outcomes.

Proton Ligand Ionization Constants

The Metal-ligand stability constants with transition metal ions copper(II), and cobalt(II) in an aqueous alcohol medium have been evaluated the by Irving-Rosotti method at constant ionic strength.[23] The acid-base properties of these and their ability to form complexes with copper (II), and cobalt(II) in aqueous-alcohol (50:50% V/V) medium were quantitatively determined by potentiometric measurements at constant ionic strength. The formation constants of complex species with copper(II), and cobalt(II) were investigated potentiometrically in sodium perchlorate at 1 M at T = 25+1°C. The effects of substitution on ionization and stability of the complexes have also been studied.

RESULTS AND DISCUSSION

Synthesis and Characterization

The Schiff-base ligand-3 was prepared by acid-catalyzed condensation of 7-chloro-3-acetyl coumarin and *o*-aminophenol. The synthesized compound was crystallized in ethanol. The structures of newly prepared compounds were established by ^{1}H-NMR, Mass, FTIR, and C,H,N analysis.

Elemental Analysis of Compounds (3) and (4)

The Vario EL (III) C.H.N.S analyzer was used to perform elemental analysis for C, H, and N. By carefully evaporating and calcining a known quantity of the complexes with a mixture of H_2SO_4 and HNO_3, the compounds (3) and (4) were investigated Table-1.

Table-1: Elemental Analysis of the Compound (3) and (4)

Ligand / Complexes	Mol. Formula	Mol. Wt.	Color		Elemental Analysis (%) Calcd. (found)				
				C	H	N	O	Cl	M
Ligand	$C_{17}H_{12}ClNO_3$	313.73	Brown	65.08	3.86	4.46	15.30	11.30	-
				(65.05)	(3.82)	(4.44)	(15.26)	(11.28)	-
$[Co(L)_2]$	$C_{34}H_{22}N_2Cl_2O_6Co$	684.39	Dark Greay	59.67	3.24	4.09	14.03	10.36	8.61
				(59.64)	(3.20)	(4.04)	(14.00)	(10.34)	(8.60)
$[Cu(L)_2]$	$C_{34}H_{22}N_2Cl_2O_6Cu$	689.02	Dark Grey	59.27	3.22	4.07	13.93	10.29	9.22
				(59.24)	(3.20)	(4.05)	(13.91)	(10.26)	(9.20)

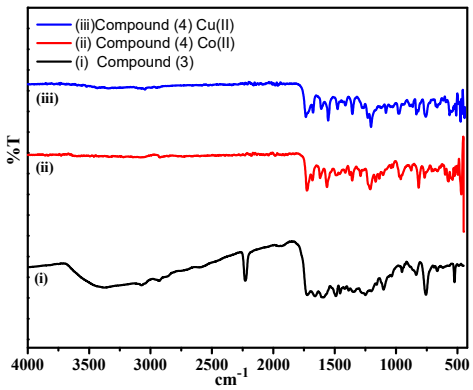


Fig.-1: FTIR Spectra of Compound 3, 4 and 5

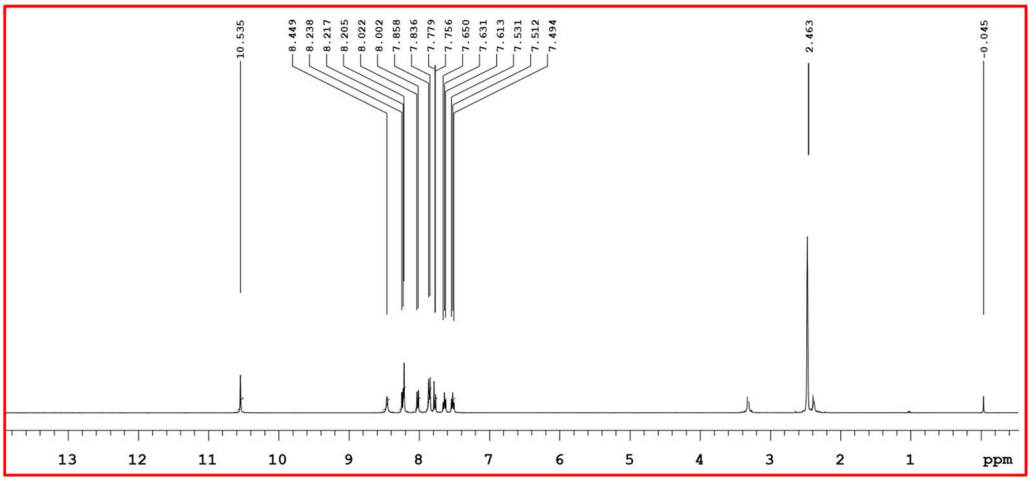


Fig.-2: NMR Spectra of Compound 3

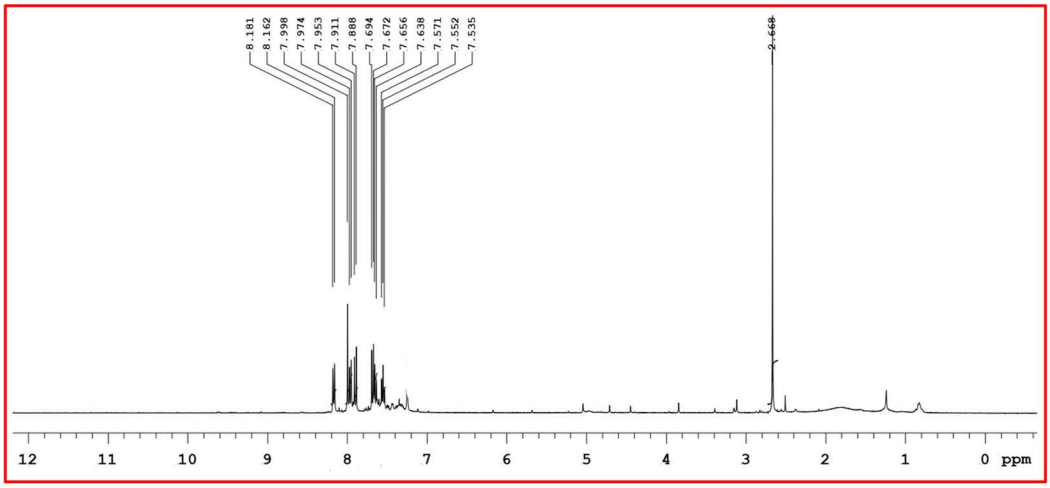


Fig.-3: NMR Spectra of Compound 4

The presence of functional groups was confirmed by the FTIR spectra Fig-(1).The compound-3 showed the band at 3360 cm^{-1} attributed to -OH stretching frequency. The compound-3 showed the band at 1755 cm^{-1} due to the carbonyl group of coumarin moiety whereas the absence of a band at 1685 cm^{-1} due to carbonyl of the acetyl group indicates the formation of Schiff base. Further, 1603 cm^{-1} attributed to C=N which confirms the formation of Schiff base. The ^{1}H-NMR spectrum of compound 3 (Fig-2) exhibited a singlet at δ 2.46 ppm due to the azomethine protons. The –OH protons are observed as a broad singlet at δ 10.53 ppm. The absence of amine protons in the ^{1}H-NMR spectrum confirms the formation of Schiff

base. Since there was a carbonyl group nearby, the aromatic protons next to it were moved downfield, and the remaining aromatic protons echoed in the expected region. The mass spectral analyses of compound 3 by MS have provided the m/z values that corresponded to its molecular mass. Metal complexes of the above-confirmed ligand were synthesized by condensation of ligand 3, with metal chloride (Co and Cu) in the presence of sodium acetate in an alcoholic medium.

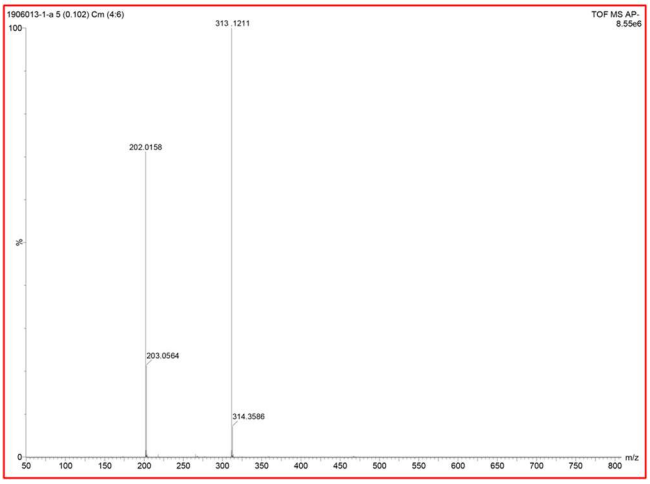


Fig.-4: Mass Spectra of Compound 3

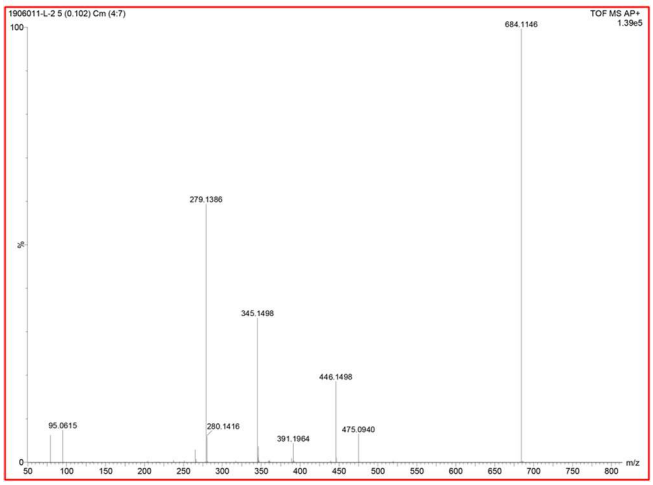


Fig.-5: Mass Spectra of Compound 5

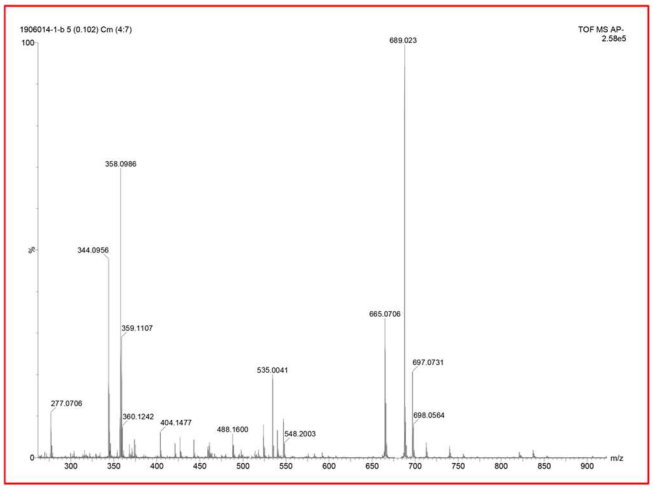


Fig.-6: Mass Spectra of Compound 5

The structure of the synthesized complexes werehypothised by FTIR, ^{1}H-NMR, and electronic spectral methods. In FTIR spectra of both complexes, the absence of the -OH band indicates the formation of complexes. In ^{1}H-NMR spectra of both the complexes the absence of -OH peak designates the involvement of -OH group in complex formation [Fig-3]. m/z peaks in mass spectra confirm the molecular ion peak of both the complexes Fig-4, Fig-5 and Fig-6.

Antibacterial Activity of (4 and 5)

The antibacterial efficacy of compounds (4 and 5) was evaluated through the utilization of the agar well diffusion assay. The presence of a well-defined circular zone on the culture plates was recorded as an indication of inhibition zones. Bacterial strains of both Gram-positive and Gram-negative types were subjected to treatment with compounds (4 and 5). Based on the findings, Compound 5 (Cu Complex) exhibited the highest level of growth inhibition for S. aureus and *B. subtilis*, with inhibition zones measuring 16±1 mm and 18.5±1.3 mm, respectively. Meanwhile, Compound 4 (Co Complex) demonstrated the maximum growth inhibition for *S. aureus* and *B. subtilis*, with inhibition zones measuring 12.5 ±0.5 mm and 14 ±0.25 mm, respectively. However, *E. coli* and *S. flexineri* displayed the lowest level of growth inhibition, with zones measuring 14±1.25 mm and 10 ±0.5 mm, respectively, upon treatment with 100 µL concentration of synthesized complexes (4 and 5). Similarly, *E. coli* and *S. flexineri* exhibited the least growth inhibition, with zones measuring 12 ±0.25 mm and 9 ±0.5 mm, respectively. Figure-7 and Fig.-8(A-D) exhibit the zone of inhibition observed against the tested pathogens at different concentrations. Meanwhile, Fig.-7 and Fig.-8(E) present a graphical representation of the measured data.

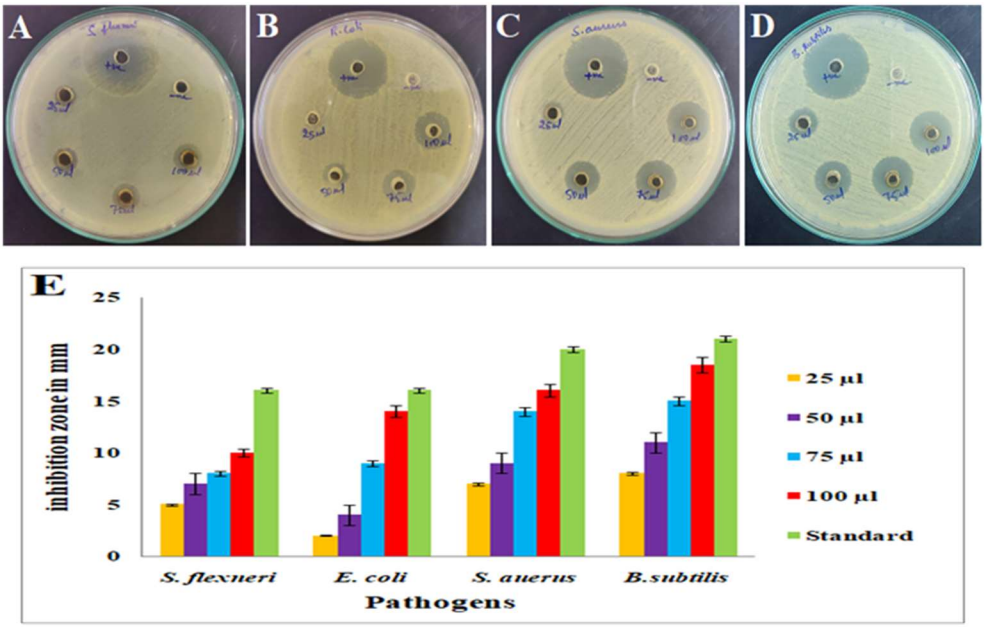


Fig.-7: Antimicrobial Activity of Different Concentrations of Cu-complex 5. A*S. flexneri*, B*E. coli*, C*S .aureus*, D*B. subtilis*, and E Graphical Representation of Antimicrobial Activity of Inhibition Zones of Cu-Complex 5 Against Tested Pathogens

In addition, the synthesized complexes 4 and 5 in the current study demonstrated a noteworthy ability to impede the proliferation of gram-positive bacteria. The presence of Compound 5 results in the inhibition of diffusible compounds, leading to the formation of a discernible zone of inhibition in the vicinity of the well. Differences in the composition and thickness of cell walls may account for the observed variability in inhibition zones, as previously noted.[24] As per the research findings, Compound 5 has the potential to interact with the bacterial membrane surface, leading to the disruption of the same. This, in turn, can result in an increase in cell permeability and alteration or inhibition of cellular respiration. Consequently, it induces enzymatic degradation, perturbs DNA proteins, and ultimately culminates in cellular demise.[25-26]

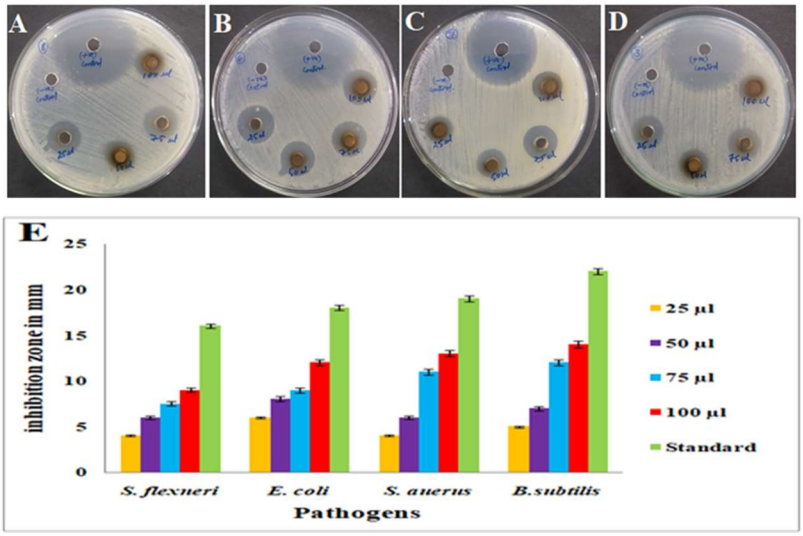


Fig.-8:Antimicrobial Activity of Different Concentrations of Co-complex 4. An*S.flexneri*, B*E.coli*, C*S.aureus*, D*B.subtilis*, and EGraphical Representation of the Antimicrobial Activity Of Inhibition Zones of Co-complex 4 Against Tested Pathogens

In-vitroanticancerActivityof Cu-complex 5 by MTT Assay

The present study employed the colorimetric MTT assay to evaluate the *cytotoxicity* of Cu-complex 5 towards human *hepatocellular adenocarcinoma cancer cells* in vitro, across a range of concentrations. The outcomes were quantified through absorbance at 570 nm, given that this particular assay is reliant on colorimetric analysis. Morphological changes were observed in Fig.-9 (A-G) in the cell that was subjected to treatment with Cu-complex 5. The Cu-complexes exhibit noteworthy *anticancer* properties, with a median lethal concentration of 143.52 µg/mL resulting in 50% cell death. A reduction in the percentage of cell viability was observed in a dose-dependent manner when exposed to Cu-complex 5 at concentrations of 12.5, 25, 50, and 100 µg/mL, resulting in values of 72.45%, 60.12%, 48.69%, and 23.25%, respectively. The percentage of cell viability further decreased to 10.23% upon treatment with 200 µg/mL of Cu-complex 5. The graphical representation of the aforementioned percentages can be observed in Fig.-9 (H). The inhibitory mechanism of Cu-complex 5 for cancer cell lines is currently lacking in comprehensive understanding. The hypothesis put forth was that Cu-complex 5 has the potential to impede the activity of signaling proteins that have undergone abnormal increases or interact with the functional groups of intracellular proteins and enzymes as well as the nitrogen bases present in DNA, thereby leading to cellular demise.[27-29]

Proton Ligand Ionization Constants

Proton or hydrogen ions are typically released when a metal interacts with an electron donor atom of a Schiff base ligand (HL). The measurement of the protons released during complexation is the basis of alkaline potentiometric titrations.[30] The primary benefit of this methodology over alternative approaches is the ability to precisely identify the pH at which complexation occurs and to observe complexation continuously as a function of pH using the titration curves. Moreover, the potentiometric titration curves can be used to compute the stability and dissociation constants. Using the Irving-Rossotti method[23], the stability constants of the binary complexes were found potentiometrically.[20] Therefore, the mixtures that contain some metal ions were titrated with standard 0.1 N sodium hydroxide solution in a potentiometer device and the titration chart was plotted Fig.-10.

The average number of protons associated with ligand (HL) at different pH values, $\overline{n_A}$, was calculated from the titration curves of the acid in the absence and presence of ligand (HL) by applying the following equation:pL values were calculated using $\bar{n}$ values to calculate the stability constants.[23]

$$\overline{n_A} = Y - \frac{(V_2 - V_1)[(N + E^o)}{(V_o + V_1)T_L^o}$$

Where Y is a number of replaceable protons, and $T°_L$ are initial concentrations of mineral acid and the reagents respectively, V_1 and V_2 are the volumes of the alkali of normality N required for the acid and reagent titrations respectively at a given pH and V_o is the initial volume of the titrating solution.Thus, the formation curves ($\overline{n_A}$ vs. pH) for the proton–ligand systems were constructed and found to extend between 0 and 1 in the $\overline{n_A}$ scale.The value formation constants corresponding to the formation of protonated ligands are obtainedin Fig.-11.[20]

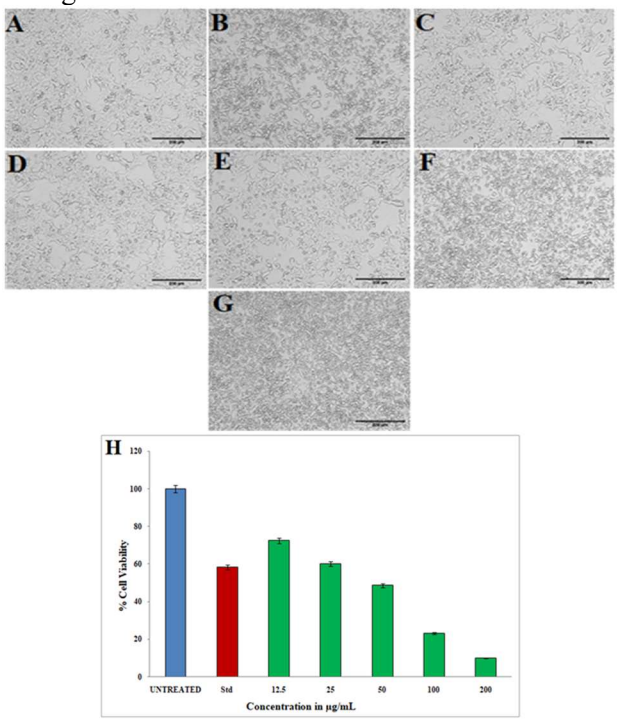


Fig.-9:Cell Viability of HepG2 Cells Treated with *Cu-complex 5:*A Negative Control, B Positive CControl, C 12.5 µg/mL,D25 µg/mL, E 50 µg/mL,F100 µg/mL,G200 µg/mL, and H Bar Graph Showing the Comparative Percentage of Cell Viability

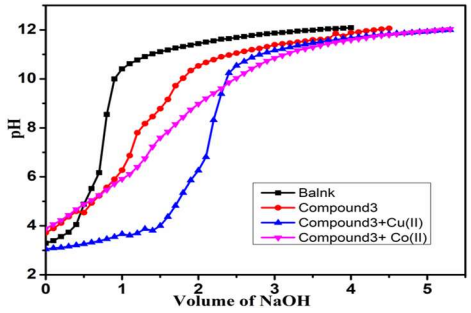


Fig.-10: Titration of Metal Ions with Sodium Hydroxide Solution

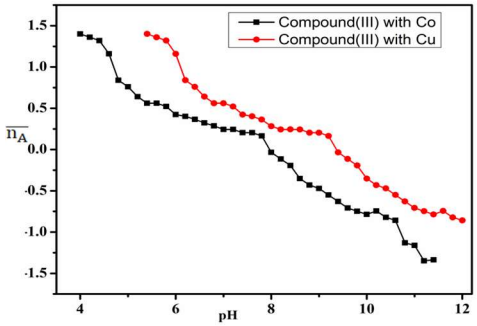


Fig.-11: Dissociation of 2-aminophenol Schiff base with Acetyl Coumarin

This indicates that there is one ionizable proton in the synthesized Schiff base. According to Irving and Rossottithe formation curves for the metal complexes were constructed by plotting the free ligand exponent (pL) against the average number of ligands attached per metal ion (nA) and the stepwise stability constants for the formation of metal complexes are obtained.[30-31] One may compute the free ligand exponent, pL, and the average number of reagent molecules bonded per metal ion $\bar{n}$, using the following equations:

$$\bar{n} = \frac{(V_3 - V_2)[(N + E^o) + T_L^o(Y - \overline{n_A})]}{(V^o + V_2)T_M^o\overline{n_A}}$$

Where, V_3 and V_2 are the volumes of alkali required for the metal complex and the reagent titrations respectively at a given pH, and T°_M is the initial concentration of metal (II) ions. The remaining quantities have the same significance as given in the equation below:

$$pL = \log\left(\frac{1 + ([H^+]/K_1) + ([H^+]^2/K_1K_2)}{T_L^o - \bar{n}T_M^o}\right)\left(\frac{V^o + 2V_2}{V_o}\right)$$

$$pL = \log\left(\frac{1 + \text{Antilog}(pK_{a1} - pH) + \text{Antilog}(pK_{a1} + pK_{a2} - 2pH))}{T_L^o - \bar{n}T_M^o}\right)\left(\frac{V^o + 2V_2}{V_o}\right)$$

There were three repeat titrations conducted, and Table-2 lists the average results that were obtained. The ligand (HL) in its fully protonated state has a single dissociable proton that dissociates within the measurable pH range. Most likely, a persistent intramolecular H-bond with the nitrogen atom of the C=N group may be formed as a result of the deprotonated hydroxy group in the Schiff base ligand. This kind of interaction decelerates the dissociation of 2-aminophenol Schiff base with acetyl coumarin, hence increasing the pK^H value 20.[32]

Table-2: Ionization Constants and Stability Constants

Ligand	p^{Ka}	Log K	
		Copper(II)	Cobalt(II)
Compound(3)	5.90	2.90	3.57

CONCLUSION

New coumarin Schiff bases (E)-3-((2-hydroxy-5-methylphenyl)imino)ethyl)-2H-chromen-2-one] and their Co and Cu metal complexes were synthesized using a facile synthetic process. Newly synthesized molecules were characterized by various spectroscopic methods viz., elemental analysis (EA), FT-IR, mass(MS), ^{1}H-NMR. The findings of antibacterial experiments revealed that compound 5 (Cu Complex of the Coumarin Schiff base), with inhibition zones of 16 mm and 18.5 mm, respectively, exhibited the greatest level of growth inhibition for S. aureus and B. subtilis., Compound 4 (Co Complex) displayedthe maximum growth inhibition for S. aureus and B. subtilis, with inhibition zones measuring 12.5 ±0.5 mm and 14 ±0.25 mm, respectively. However, both substances showed good to moderate effectiveness against other bacteria, including E. coli and S. flexineri. *In vitro,* cytotoxicity of Cu-complex 5 against human hepatocellular adenocarcinoma cancer cells at different concentrations, 12.5, 25, 50, and 100 g/mL, demonstrated a reduction in the percentage of cell viability in a dose-dependent manner, with values of 72.45%, 60.12%, 48.69%, and 23.25%, respectively.These findings demonstrated that Compound **5** is capable of interacting with the bacterial membrane surface and disrupting it.Further, thorough SAR investigations are needed to demonstrate the molecule's bioactivity. Additionally, the proton-ligand dissociation constant of HL indicated that there is one ionizable proton in the synthesized Schiff base and stability constants of Co^{2+} and Cu^{2+} were discussed based onthe potentiometric analysis method.

ACKNOWLEDGMENTS

One of the authors Vindhya R thanks to Ballari Institute of Technology and Management, Ballari for its facilities and encouragement.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

R. Vindhya https://orcid.org/0000-0003-2085-4460
T. M. Sharanakumar https://orcid.org/0000-0002-6427-9130
Veerendra Kumar A. Kalalbandi https://orcid.org/0000-0001-6577-6449
K. N. Shashiraj https://orcid.org/0000-0003-4920-6459
K. M. Nagarsha https://orcid.org/0000-0003-4743-5477
H. M. Suresh Babu https://orcid.org/0009-0007-7187-867X
Suresh https://orcid.org/0009-0004-0753-9807

REFERENCES

1. G. WHO, WHO Methods and Data Sources for Global Burden of Disease Estimates, (2000–2019), http://www.who.int/gho/mortality_burden_disease/en/index.html
2. Geneva, Department of Health Statistics and Information Systems, (2019), https://apps.who.int/iris/bitstream/handle/10665/311696/WHO-DAD-2019.1-eng.pdf
3. Meenakshi Gupta, Ruchika Sharma, Anoop Kumar, *Oriental Pharmacy and Experimental Medicine,* **19**, 259(2019), https://doi.org/10.1007/s13596-019-00359-z
4. M. Baym, L.K. Stone, R. Kishony, *Science,* **351**, 6268(2016), https://doi.org/10.1126/science.aad3292
5. Antimicrobial Resistance and Its Impact on Cancer Care, (2019), https://www.uicc.org/what-we-do/areas-focus/antimicrobial-resistance amr?gclid=CjwKCAjw__ihBhADEiwAXEazJlpy0NZcutoWledGdgTaVMN-chex2wtpat0RCLthZ4LlyeuPkWtyfRoCSPIQAvD_BwE
6. Dongxu Feng, Aihua Zhang, Yuan Yang, Peng Yang, *ArchPharm,* **353(6),** 1900380(2020), https://doi.org/10.1002/ardp.201900380
7. EsraKüpeliAkkol, Yasin Genç, BüşraKarpuz, Eduardo Sobarzo-Sánchez, Raffaele Capasso, *Cancers,***12(7)**, 1959, (2020), https://doi.org/10.3390/cancers12071959
8. I. Kostova, S. Bhatia, P. Grigorov, S.S. Balkansky, V.K. Parmar, A. Prasad, Saso L, *Current Medicinal Chemistry*, **18,** 3929(2011), https://doi.org/10.2174/092986711803414395
9. C. Marlyn, Ortiz Villamizar, E. Carlos, Puerto Galvis, Y. Leonor, Vargas Méndez, V. Vladimir, Kouznetsov, *Discovery and Development of Neuroprotective Agents from Natural Products,* **2018**, 149(2018), https://doi.org/10.1016/B978-0-12-809593-5.00005-7
10. D.J. Hadjipavlou-Litina, K.E. Litinas C. Kontogiorgis, *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry***, 6(4)**, 293(2007), https://doi.org/10.2174/187152307783219989
11. M. Iftikhar Hussain, Qamar Abbas Syed, Muhammad Nasir Khan Khattak, Baraa Hafez, Manuel, J, Reigosa, Ali El-Keblawy, *Biologia,***74,** 863(2019), https://doi.org/10.2478/s11756-019-00242-x
12. Deepjyoti Dutta, Nayan Kama Bhattacharyya, Joydeep Biswas, *Indian Journal of Chemistry,***60B**, 1478(2021), https://doi.org/10.56042/ijcb.v60i11.43174
13. P.A. Vigato, S. Tamburini, *Coordination Chemistry Reviews,* **17-20(248)**,1717(2004), https://doi.org/10.1016/j.cct.2003.09.003
14. E. Zarie, K.M. WAHDAN, A.M.H. Wahba, B.H. Heakal, A.O. Said, Z.I. Elbialy, *Egypt Journal of Chemistry,* **65(162)**, 1253(2022), https://doi.org/10.21608/EJCHEM.2022.145699.6346
15. A. M. Hassan, A. O. Said, *Advanced Journal of Chemistry, Sect. A ,* **4(2),** 87(2021), https://doi.org/10.22034/AJCA.2021.266876.1235

16. A. M. Hassan, B. H. Heakal, A. O. Said, W.M. Aboulthana, M.A. Abdelmoaz, *Egypt Journal of Chemistry,* **63(7)**, 2533(2020), https://doi.org/10.21608/ejchem.2020.21048.2255
17. S.A. Abdel-Latif, H.B. Hassib, Y.M. Issa, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* **67(3-4)**, 950(2007), https://doi.org/10.1016/j.saa.2006.09.013
18. A.A. El-Bindary, A.F. Shoair, A.Z. El-Sonbati, M.A. Diab, E.E. Abdo, *Journal of Molecular Liquids,***212**, 576(2015), https://doi.org/10.1016/j.molliq.2015.10.011
19. H.M. Irving, H.S. Rossotti, *Journal of Chemical Society,* 2904(1954), https://doi.org/10.1039/JR9540002904
20. Etelka Farkas, Hajnalka Csóka, *Journal of Inorganic Biochemistry,***89(3-4)**, 219(2002), https://doi.org/10.1016/S0162-0134(02)00379-3
21. Giorgio Valle, Jayne Besley, Alan R. Williamson, Timothy, R. Mosmann, Alan Colman, *European Journal of Biochemistry*,**132(1),** 131(1983), https://doi.org/10.1111/j.1432-1033.1983.tb07337.x
22. Kariyellappa Nagaraja Shashiraj, Sreenivasa Nayaka, Raju Suresh Kumar, Gireesh Babu Kantli, DhanyakumaraShivapoojarBasavarajappa, Pooja Vidyasagar Gunagambhire, Abdulrahman I. Almansour, Karthikeyan Peruma, *Processes*,**11(3)**, 893(2023), https://doi.org/10.3390/pr11030893
23. H. Irving, H. S. Rossotti, *Journal of Chemical Society*, 3397(1953), https://doi.org/10.1039/JR9530003397
24. D.V.M. Jun Sung Kim, M.S. Eunye Kuk, M.S. Kyeong Nam Yu, M.S. Jong-Ho Kim, B.S. Sung Jin Park, D.V.M. Hu Jang Lee, D.V.M. So Hyun Kim, D.V.M. Young Kyung Park M.S, D.V.M. Yong Ho Park, D.V.M. Cheol-Yong Hwang, Yong-Kwon Kim., Yoon-Sik Lee., Dae Hong Jeong., D.V.M. Myung-Haing Cho, *Nanomedicine: Nanotechnology, Biology and Medicine*, **3(1),** 95(2007), https://doi.org/10.1016/j.nano.2006.12.001
25. S. Rajeshkumar, *Journal of Genetic Engineering and Biotechnology,***14(1),** 195(2016), https://doi.org/10.1016/j.jgeb.2016.05.007
26. Bansuri Nandaniya, Siva Prasad Dasa, and Darshan Jani, *Current Chemistry Letters,***12**, 289(2023), https://doi.org/10.5267/j.ccl.2022.12.010
27. Mahmood Dehghani, Azadeh Delbar Saf, Asghar Vosoughi, Giti Tebbenouri, Hadi Ghazanfari Zarnagh, *Electron Physician*, **10(5)**, 6749(2018), https://doi.org/10.19082/6749
28. Sreenivasa Nayaka, Bidhayak Chakraborty, S.S. Pallavi, Meghashyama P Bhat, K.N. Shashiraj Bhagyalaxmi Ghasti, *International Journal of Pharmaceutical Research,***12(3**), 302(2020).
29. Mikołaj Sadowski, and Karolina Kula, *Current Chemistry Letters,* **13**, 15(2024), https://doi.org/10.5267/j.ccl.2023.9.003
30. D. Vilas, Athawale, S. Sareeta, Nerkar, *Monatshefteför Chemie,* **131**, 267(2000), https://doi.org/10.1007/s007060070102
31. V.D. Athawale, Vidyagauri Lele, *Journal of Chemical & Engineering Data,* **41(5)**, 1015(1996), https://doi.org/10.1021/je950306z
32. M.M. Omar, Gehad, G. Mohamed, Potentiometric, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,***61,** 929(2005), https://doi.org/10.1016/j.saa.2004.05.040

[RJC- 8886/2024]