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NOVEL SCHIFF BASE METAL (II) COMPLEXES WITH AZIDE AS COLIGANDS: A STUDY ON ANTIMICROBIAL, LARVICIDAL AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

Schiff bases are considered as promising biologically active ligand which is attained from carbonyl compounds and primary amines. It is also prepared from amino acids which are essential for living metabolism. The interactions of transition metal ions with biologically active ligands provide most fascinating area in Coordination chemistry. Schiff base metal complexes are highly significant because of their functions in various fields such as medical, industrial and agricultural. The present study deals with the synthesis of a new series of azido based transition complexes derived from o-vanillin and L-valine. The complexes were structurally characterized by spectroscopic techniques such as FTIR and UV-Vis., The FTIR spectra confirmed the tridentate coordination nature of the Schiff base *via* imine nitrogen, phenolic oxygen and one of the oxygen atom of carboxylate group. Radical scavenging activity was carried out by CUPRAC, FRAP, DPPH and H_2O_2 methods. Mosquito larvicidal and ovicidal activities against *Culex quinquefasciatus* larvae over 24 h exposure time period were performed for the synthesized compounds. Further, LC_{50} and LC_{90} values were calculated. In order to explore the bioactivity profile, the synthesized compounds were screened against four different species of bacteria and fungi. The synthesized copper complexes exhibit significant results in all these studies.

Keywords: Biologically active Schiff base, Metal complexes, Antimicrobial, Antioxidant, Larvicidal activities.

1. INTRODUCTION

At current scenario, antimicrobial resistance has become a universal concern eventually affecting human's ability to prevent and treat a growing infections caused by microbial pathogens. The management of transmissible diseases still remains an important and taxing problem because of emerging infectious diseases and the mounting number of multidrug resistant pathogens. As a result, existing drugs are inadequate. Hence there is a need to find some new drug for treatment [1-8].

Many of the transition metal complexes possess crucial role in pharmaceutical industries in several aspects because of their anticancer, antimicrobial, antioxidant and larvicidal properties [9-12]. Today, medicinal chemist has begun to develop transition metal based antimicrobial agents with great promise and remarkable success [13]. The Schiff bases are versatile organic blockers which can readily coordinate with metal ions of diverse oxidation states to form promising metal complexes [14-16]. Schiff bases are also called as imines, azomethines and these are condensed product of carbonyl compounds and amines. Schiff base transition metal complexes are one of the most adaptable and are of great interest among the researchers [17-19]. The chelating attraction towards the Schiff base and transition metal ions are highly recommended to prepare their solid complexes. These compounds reveal prolonged applications in various domains such as therapeutic, agricultural and industrial fields [20-21]. The existence of imine moiety into the structure of the coordinated complexes often resulted in essential changes in their behavior towards metal ions [22, 23]. In this regard, we made an attempt to synthesize new azido based Schiff base metal (II) complexes and to explore their biological activities.

2. MATERIAL AND METHODS

Analytical grades of chemicals were used for the synthesis of the compounds. Metal azido complexes are potentially explosive however only a small amount of materials were prepared and handled with safety measures. The electronic absorption spectra of the complexes were

recorded on a SYSTRONICS 2201 spectrophotometer at room temperature with the sample concentration of 10^{-5} M in DMSO. The FTIR spectra were recorded on SHIMADZU spectrophotometer between 4000-400 cm^{-1} range, using KBr pellet. Eggs and egg rafts of *Culex quinquefasciatus* were procured from Zonal Entomological Unit, Vellore which was free of exposure to pathogens, insecticides or repellents.

2.1. Synthesis of Schiff base ligand (L^1)

L-valine (0. 351g) and KOH (0.336 g) were dissolved in 25 mL of ethanol then o-vanillin (0.456 g) was added. The reaction mixture was continuously stirred for 2 h at 60°C in a magnetic stirrer. The solution turned pale yellow to orange yellow. The volume of the solution was reduced to half. Finally, the precipitate was filtered, washed with ethanol and dried.

2.2. Synthesis of Schiff base metal (II) complexes

To the hot ethanolic solution of Schiff base ligand (L^1), copper (II) acetate monohydrate (0.597 g) was added. The mixture was allowed to stir for 3 h at 60°C. Further, to this solution, sodium azide (0.195 g) in methanol: water (1:9) mixture was added as drops and stirred for another 2 h at the same temperature. The resultant solution was filtered and the resultant product was formed on slow evaporation of the mother liquor at room temperature.

The above mentioned procedure was carried out for the preparation of other complexes using nickel (II) acetate tetrahydrate (0.744 g), cobalt (II) acetate tetrahydrate (0.747 g) and zinc (II) acetate dehydrate (0.657g). The final product was filtered and dried.

2.3. Antimicrobial studies

The antimicrobial efficiency of the synthesized compounds was performed according to National Committee for Clinical Laboratory Standards ((1993a) [24, 25]. The bacterial strains such as *S. aureus*, *P. aeruginosa, E. coli*, *B. subtilis* and fungal strains such as *A. flavus, C. albicans, R. stolonifer, A. niger* were taken for the investigation. The stock solution was prepared with 10^{-6} M concentration of synthesized compounds in DMSO. 50 µL test solution was taken for the study. Ampicillin and Polymyxin B sulphate were taken as reference for antibacterial and antifungal activities respectively.

2.3.1. Minimum inhibitory concentration (MIC)

Inhibition of microorganism using the lowest concentration of synthesized compounds was calculated by test tube dilution method [26-29]. To examine MIC for bacterial strains, 1 mL of sterile Muller-Hinton broth was taken in a set of sterile test tubes. Stock solutions were prepared by dissolving the synthesized compounds in DMSO (1 mg/1 mL). To the first test tube, 1 mL of stock solution was added then it was diluted in sequence in six test tubes using sterile pipette so that concentrations varied. Finally, 2 drops of the test organism were added in all the test tubes. To analyze MIC for fungal strains, SDS nutrient broth was used and similar procedure was followed. Finally, the bacterial strains in the test tubes were incubated at 37°C for 24 h, whereas the fungal strains were incubated at room temperature for 48 h.

2.4. Antioxidant activity

The substance which inhibits the production of free radical or the process of oxidation are called as antioxidants. It is also described as an excessive production of reactive oxygen/nitrogen species (ROS/ RNS), known as prooxidants, which are involved in the detoxification of radicals [30-32].

Free radicals are responsible for deadly diseases like cancer. In order to avoid free radical production, drug with high antioxidant activity can be used. Hence, Scavenging activity of the complexes was analyzed by adopting standard procedure [33-35].

2.4.1. Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by adsorption at 230 nm using a UV-Vis., spectrophotometer. Compounds with the concentration of 2 mg/2 mL in DMSO were added to hydrogen peroxide and the absorption was observed at 230 nm after 10 min incubation against blank solution containing phosphate buffer without hydrogen peroxide [36]. The radical scavenging activity was calculated using following equation.

% of radical scavenging activity={(Absorbance of control -Absorbance of sample)/Absorbance of control} X 100

2.4.2. DPPH scavenging activity

DPPH radical scavenging activity was evaluated using 2 mg/2 mL of the compounds in DMSO with 2 mL of 0.05 M methanol solution of DPPH. After 30 minutes incubation period at room temperature, the antiradical scavenging ability of synthesized compounds was determined by measuring the decrease in the absorbance of DPPH at 517 nm. DPPH solution without sample was

taken as control. The percent of inhibition (I %) of free radical production from DPPH was calculated [37- 40].

2.4.3. CUPRAC Method

In this method, metal reducing antioxidant capacity was calculated by taking advantage of the reduction power of the Cu(II) to Cu(I) in neocuprine complex in ethanolic medium [41,42]. 2 mg of the synthesized compounds were dissolved in ethanol. From this, 30 µl of solution was added to 40 µl $CuCl_2$ (10 mM), 40 µl neocuproine in ethanol(7.5 mM) and 50 µl ammonium acetate buffer (1M, pH 7) solution. The mixture was left for 45 minutes after that maximum absorption was measured at 450 nm.

2.4.4. FRAP Method

Standard procedure was carried out to investigate the scavenging activity by FRAP method [43-45]. 2 mg of the synthesized compounds in 2 mL ethanol were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1 % potassium ferricyanide. Afterwards mixture was incubated at 50°C for 30 minutes, 1 mL of 10 % solution of trichloroacetic acid was added to each mixture and centrifuged. The centrifuged solutions were first mixed with distilled water and 0.1 % ferric chloride by 1:1:1 ratio. Finally scavenging activity of the compounds was measured by absorbance at 700 nm.

2.5. Larvicidal bioassay (preliminary screening)

Larvicidal activity was evaluated by following the methods of WHO with slight modifications [46-48]. The eggs and egg rafts of *Culex quinquefasciatus* were dipped into a dish containing 250 mL of dechlorinated water for 40-60 minutes to hatch out larvae. The reared larvae were maintained for 5 days in standard environment temperature (37°C) of the laboratory as per the literature [49]. Mosquito larvae were fed with powered nutrients broth once a day. After 4 days, the hatched larvae turned into larvae in early forth stage and were subjected to be employed for further experiment.

A total of 20 reared *Culex quinquefasciatus* larvae were placed in 100 mL of double distilled sterilized water containing 2 mg of synthesized complexes as well as ligand. Sterile distilled water without ligand and the complexes served as a control. Triplicates were maintained for each assay. Total number of dead larvae in each batch was counted and mortality rate were registered after 24 h exposure period. Mortality was converted into percent mortality as follows:

% of mortality = {(No. of dead larvae)/(No. of larvae used)} X 100

2.5.1. Dose dependent larvicidal activity

Dose dependent larvicidal activity was evaluated according to the standard procedure [50]. Based on the preliminary larvicidal screening results, different concentrations of Schiff base ligand and the complexes (4 mg, 2 mg, 1 mg, 0.5mg/100 mL in sterilized double distilled water) were prepared for dose dependent larvicidal assay. Briefly, 20 reared *Culex quinquefasciatus* larvae were placed in the test solution. Sterile double distilled water without Schiff base ligand and complexes was taken as control. Triplicates were maintained for each assay. Percentage of mortality was assessed after 24 h incubation at room temperature. The percentage of mortality is reported as the average of triplicates.

2.5.2. Ovicidal activity

Ovicidal activity was evaluated by the standard procedure [51-53]. Freshly laid 20 eggs of *C. quinquefasciatus* were taken in beakers containing various concentrations of synthesized Schiff base ligand and the metal complexes (4 mg, 2 mg, 1 mg, 0.5mg/100mL in sterilized double distilled water). Sterile distilled water without ligand and the complexes were served as control. The no hatched eggs with unopened opercula were counted in each treatment and the percent mortality was calculated using the following formula and analyzed.

% of egg mortality = {(No. of unhatched eggs)/(Total No. of eggs)} X 100

3. RESULTS AND DISCUSSION

3.1. Analytical data of the synthesized compounds

Schiff base ligand (L^1): Dark brown precipitate; Molecular Formula: $C_{13}H_{17}NO_4$; MW: 251.8; M.p. (decomp. T): 260°C. Complex **1**: brown solid; Molecular Formula: $C_{13}H_{15}N_4NaCoO_4$; MW: 373.20; M.p. (decomp. T): 280°C; Λ_M (DMF)=53.23 $cm^2\Omega^{-1}M^{-1}$. Complex **2**: green solid; Molecular Formula: $C_{13}H_{15}N_4NaNiO_4$; MW: 372.97; melting point=M.p. (decomp. T): 290°C; Λ_M (DMF) = 55.27 $cm^2\Omega^{-1}M^{-1}$.Complex **3**: dark green crystalline solid; Molecular Formula: $C_{13}H_{15}N_4NaCuO_4$; MW: 377.82; M.p. (decomp. T): >300°C; Λ_M (DMF)=56.82 $cm^2\Omega^{-1}M^{-1}$. Complex **4**: pale yellow solid; Molecular Formula: $C_{13}H_{15}N_4NaZnO_4$; MW: 379.66; M.p. (decomp.T): >300°C; Λ_M (DMF) = 59.22 $cm^2\Omega^{-1}M^{-1}$.

[where, L^1 = Schiff base ligand derived from L-valine and o-vanillin, Complex **1** $Na[CoL^1N_3]$, Complex **2** = $Na[NiL^1N_3]$, Complex **3** = $Na[CuL^1N_3]$, Complex **4** = $Na[ZnL^1N_3]$.

All the synthesized compounds are stable at room temperature and are freely soluble in DMF, DMSO, CH_3CN, C_2H_5OH and CH_3OH. The synthesized complexes (10^{-3} M) are found to be 1:1 electrolytes since the molar conductance value of the complexes fall in the range 58-80 unit in DMF [54 -56].

3.2. UV-Visible spectra

The electronic spectrum of Schiff base ligand showed λ_{max} at 281 nm and 380 nm corresponds to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions respectively. This shows the formation of azomethine (>C=N) linkage present in the ligand. The synthesized complexes showed band around 263 to 282 nm correspond to the intraligand transition ($\pi\rightarrow\pi^*$). All the complexes showed bands in the high energy 350-390 nm region which consistent with the LMCT bands comprising of transitions from the coordinating atoms of respective Schiff base to the metal centre apart from O→Metal(II) and N→Metal(II) transitions [57]. Except complex **4**, all other synthesized complexes exhibits broad band in the region of 635-680 nm which confirms that these complexes possess d→d transitions due to presence of unpaired electrons [58, 60]. Due to lack of unpaired electrons, d→d transition was not observed for the zinc complex.

3.3. FTIR spectra

FTIR absorption band appeared around 1625 cm^{-1} confirms the formation of imine linkage in the ligand. This imine absorption band is shifted to higher frequency in the complexes due to the coordination to the metal ions. The difference between asymmetric and symmetric stretching frequencies ($\Delta\upsilon = [\upsilon_{as}\ COO^- - \upsilon_s\ COO^-]$) of the carboxylate group in the Schiff base transition metal complexes were found to be higher than the corresponding free carboxylate anion. This confirmed the monodentate coordination of the carboxylate anion present in the Schiff base ligand with the metal ions [59]. The bands appeared around 470 cm^{-1} and 560 cm^{-1} are assigned to the formation of M-O and M-N coordination respectively [59, 60]. The FTIR spectra confirmed the tridentate coordination nature of the Schiff base *via* imine nitrogen, phenolic oxygen atom and one of the oxygen atom of carboxylate group. Hence, the Schiff base ligand is tridentate in nature. Further, the IR spectra of the complexes also exhibit peaks in the region of 630-650 cm^{-1} corresponding to bending mode $\delta(N_3)$ of the pseudohalide present respectively. One sharp and strong peak appeared at 2042-2407 cm^{-1} in the complexes indicates the presence of nitrogen atom of an azide bonded to the metal ion [61, 62].

3.4. Larvicidal and ovicidal activities

From the study of larvicidal and ovicidal activity against *C. quinquefasciatus,* it was observed that all the larvae present in control were very active and exhibited normal movement in a beaker. But in the complex solution, restless movement of the treated larvae was observed.

This movement of the larvae decreased gradually with respect to time. The movement of the larvae was noted for 24 h with respect to time intervals. After some period of time, tremor was observed in all the treated larvae and dead larvae settled down in the bottom of the beaker. Number of dead larvae was counted and the percentage of mortality was calculated. Synthesized Schiff base ligands exhibits low mortality when compared to the complexes, hence metal ions in a complex plays a major role for the killing of the larvae. LC_{50} and LC_{90} and significant chi-square values are also observed. The data are given in Tables. 1 and 2. Among the synthesized complexes, the copper (II) complexes exhibits better activity against *C. quinquefasciatus*.

Table 1: Larvicidal activity: Statistical analysis of synthesized compounds against fourth instars *Culex quinquefasciatus*

Compound	Concentration / % Mortality ± SD				LC_{50} mg/ 100 mL	LC_{90} mg/ 100 mL	$\chi2$	df
	4 mg/100 mL	2 mg/100 mL	1 mg/100 mL	0.5mg/100 mL				
L^1	10±9.56	5±10.58	NiL	NiL	-	-	-	3
Complex **1**	65±6.30	55±0.48	40±6.92	30±5.10	2.13	6.31	3.54	
Complex **2**	70±0.26	55±0.48	45±0.34	45±0.34	1.38	6.66	3.40	
Complex **3**	95±0.16	65±6.46	50±0.06	50±0.06	0.76	3.71	10.5	
Complex **4**	80±0.67	60±0.16	45±0.34	45±0.34	1.16	4.94	8.16	

Values are represented in Mean ±SD values, Control-Nil mortality, df- significant at p<7.81, LC_{50}-50 % killing effect of the synthesized compounds exposed larvae, LC_{90} - 90 % killing effect of the synthesized compounds exposed larvae, $\chi2$- Chi square.

Table 2: Ovicidal activity data of the synthesized compounds

Compound	Concentration / % Mortality ± SD				LC_{50} mg/ 100 mL	LC_{90} mg/ 100 mL	$\chi 2$	df
	4 mg/100 mL	2 mg/100 mL	1 mg/100 mL	0.5 mg/100 mL				
L^1	NiL	NiL	NiL	NiL	-	-	-	
Complex **1**	45±5.60	35±6.31	25±6.69	10±6.15	4.24	8.71	7.23	
Complex **2**	50±6.52	45±7.38	35±7.82	20±5.65	3.54	8.90	6.82	3
Complex **3**	65±5.64	55±6.48	40±8.28	30±6.66	2.13	6.32	8.29	
Complex **4**	70±4.77	40±6.48	35±3.54	35±3.09	2.35	6.19	9.28	

Values are represented in Mean ±SD values, Control-Nil mortality, df- significant at p<7.81, LC_{50}-50 % killing effect of the synthesized compounds exposed larvae, LC_{90}-90 % killing effect of the synthesized compounds exposed larvae, χ^2- Chi square.

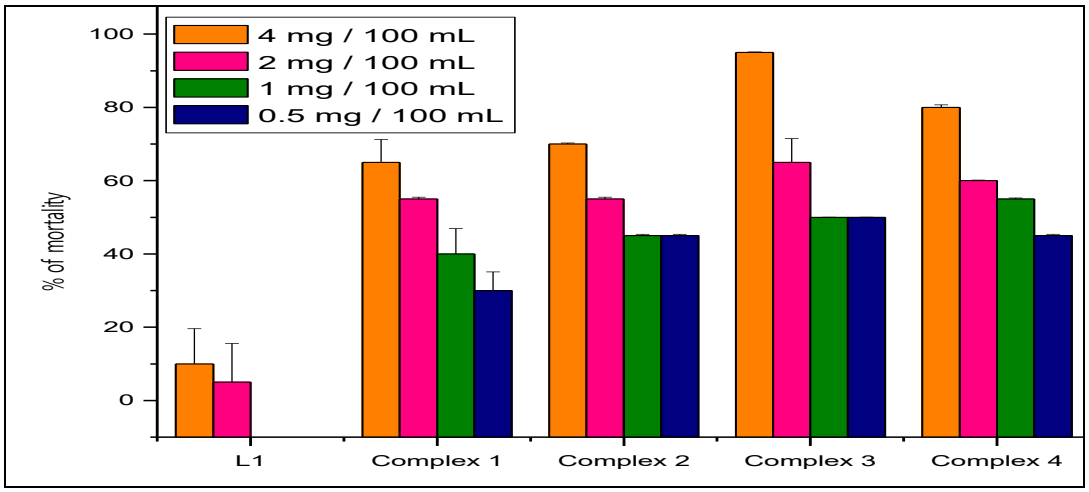

Fig. 1: Graphical representation of the larvicidal activity

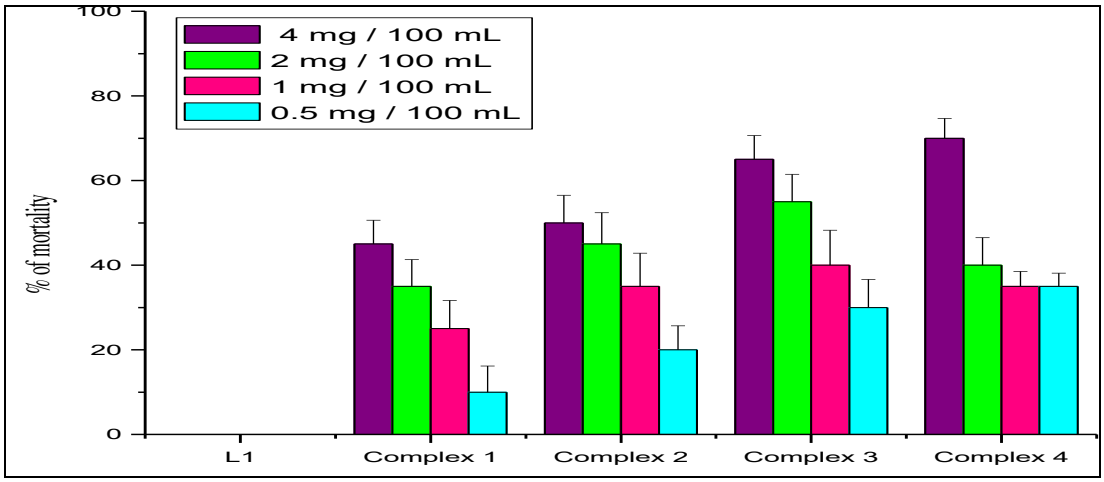

Fig. 2: Graphical representation of the ovicidal activity

Due to 1:1 electrolyte nature, the complexes get ionized in water. These ions gets into the larvae and are responsible for denaturation of the sulphur or phosphorous containing compounds like DNA that leads to the denaturation of organelles and enzymes and thus reduces the cellular membrane permeability and finally causes the lost of the cellular function and cell death. So, the nature of the metal in a complex plays an effective role in killing the larvae. The percentage of mortality is directly proportional to the concentration of the synthesized compounds and the data are represented graphically in figures.1 and 2.

In the case of ovicidal activity, eggs of *C. quinquefasciatus* exhibits more tolerance to ligand than in complexes. In

complexes, treated *C. quinquefasciatus* did not hatch and some were hatched in an abnormal way at higher concentration and the larvae died before completion of eclosion. But it was quite opposite in control where hatchability of eggs was normal and percentage of mortality decreases in control than complexes.

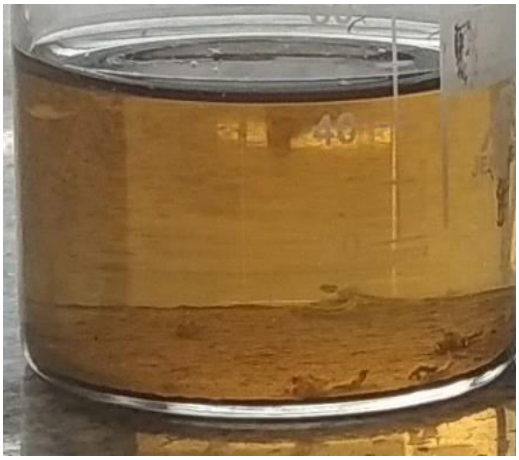

Fig. 3: Image of the larvicidal activity

3.5. Antimicrobial activity

The obtained area of inhibition zones in diameter around the well are summarized in figures 4 and 5. It was reported that the antibacterial activity of the ligand could be improved when it is chemically or physically modified by metal ions [63-65]. In this work, the results also showed the moderate to stronger antibacterial behavior against gram positive and gram negative bacteria. It was noted that zone of inhibition values enhanced in complexes compare to ligand. Complexes **1** and **2** showed moderate activity against selected bacterial as well as fungal stains. Complexes **3** and **4** exhibited marginally higher activity against *S. aureus* and *B. subtilis*. These complexes also exhibit better antifungal activity against *R. stolonifer and A. niger*. This superior antibacterial property of the complex is due to the release of complex ions into the bacterial cell membrane this upshot in bacteria cell wall leakage. Once the complex ions get into the bacteria cell, the interaction with the enzyme prosthetic group arises that can inhibit the replication of DNA. It is interesting to note from that the Complex **3** showed relatively better antibacterial activity as compared to other complexes. This is due to effective chelating of the Cu(II) ion to the ligand and also the change in the structure of the compounds, based on the polarity of the metal contents in the complex may be in part the reason behind such difference in the activity of the complexes.

It is also evident that our synthesized compound was electrolytes so it is readily dissociate in to constituent ions when it is poured into well that get into cell wall of bacteria and inhibit the replication of DNA. Hence, zone appeared due to cell death. The antibacterial activity depends on the sharing nature of the positive charge of the metal with donor groups of the ligands. The proposed mechanism for antibacterial property of synthesized compound is shown in the figure. 7.

MIC for antibacterial and antifungal activities of the synthesized compounds are depicted in Tables. 3 and 4. Except L^1 all other complexes show minimum to maximum MIC values. Overall, Complex **3** exhibits satisfactory results in antimicrobial and MIC analysis. So, this follows the order as follows: Complex **3**> Complex **4**>Complex **2**>Complex **1**>L^1

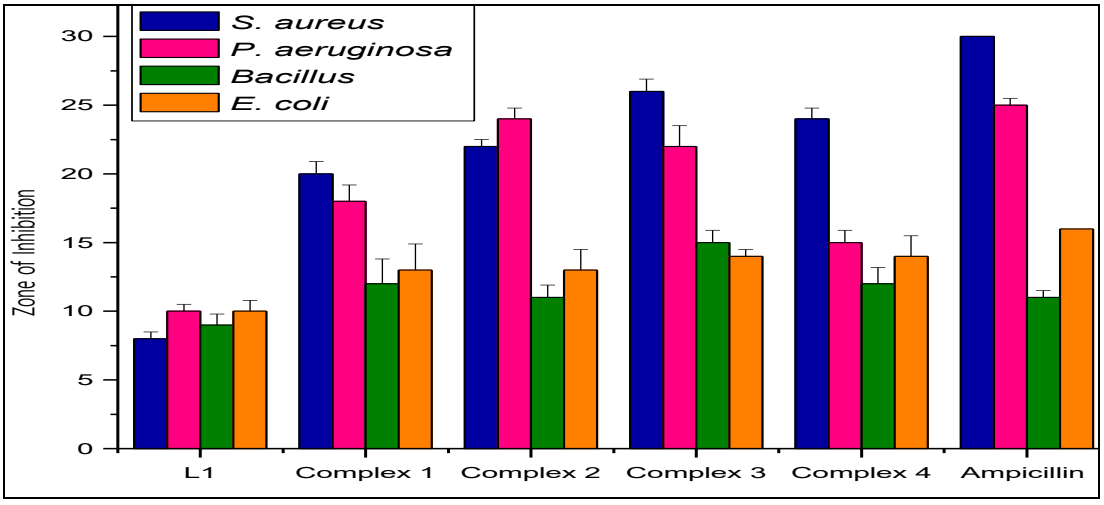

Fig. 4: Antibacterial activity of the synthesized compounds

Table 3: Antibacterial MIC values of the Schiff base ligand and its complexes in µg /mL

Compound	*S. aureus*	*P. aeruginosa*	*B.subtilis*	*E. coli*
L^1	5	5	5	5
Complex **1**	5	2.5	2.5	2.5
Complex **2**	2.5	1.25	5	2.5
Complex **3**	1.25	2.5	5	1.25
Complex **4**	1.25	1.25	5	2.5

Table 4: Antifungal MIC values of the Schiff base ligand and its complexes in µg /mL

Compound	*A. niger*	*A. flavus*	*C.albicans*	*R. stolonifer*
L^1	5	5	5	5
Complex **1**	2.25	5	2.5	1.25
Complex **2**	1.25	1.25	5	2.25
Complex **3**	1.25	2.5	2.5	2.5
Complex **4**	2.5	5	5	2.5

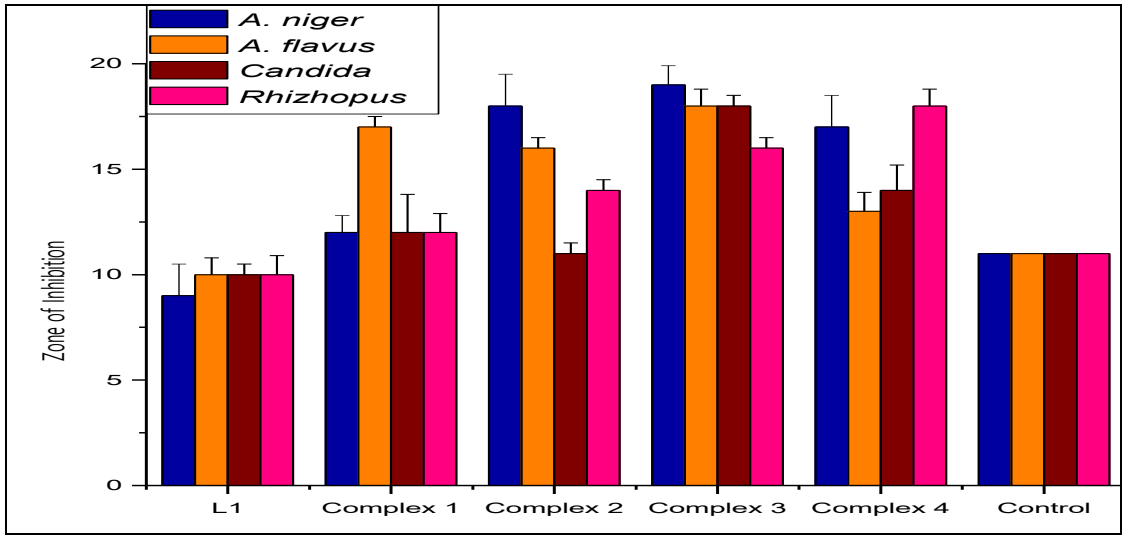

Fig. 5: Antifungal activity of the synthesized compounds

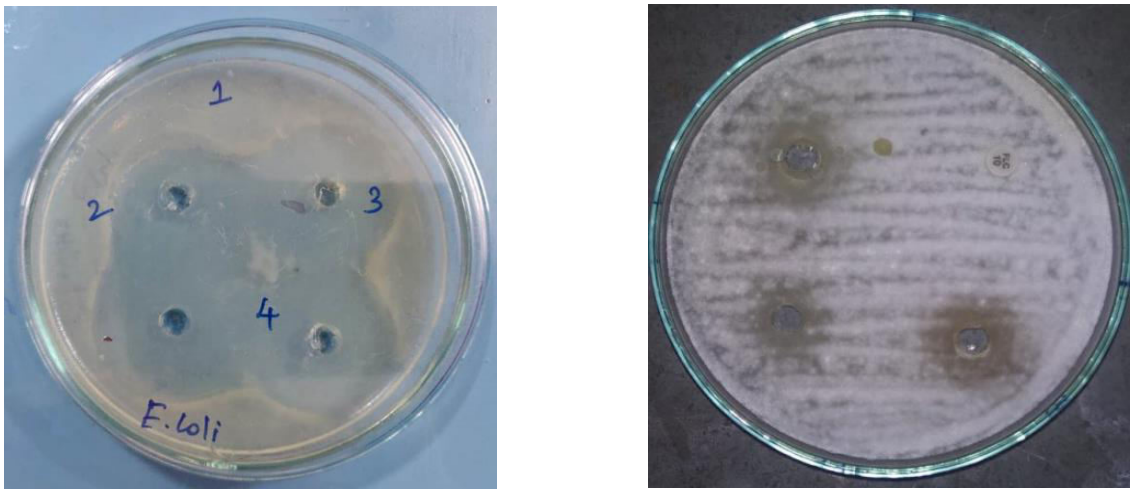

Fig. 6: Zone of inhibition of the synthesized compounds

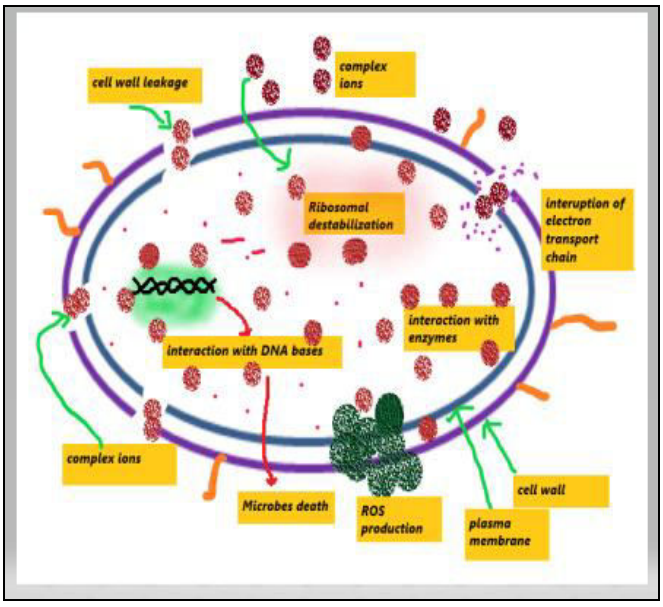

Fig. 7: Proposed mechanism of the antibacterial activity

3.6. Antioxidant activity

The percentage of free radical scavenging activity of the synthesized compounds (figure. 8) is mentioned in Table 5. DPPH method exhibits better results compare to all other methods. It has been proved that Schiff base metal complexes showed significant free radical scavenging action. This may be due to the presence of nitrogen atom in imine moiety which may donate an electron to form a stable free radical. The results from this study revealed that synthesized compounds are capable of donating electron or hydrogen atom and subsequently react with free radicals or terminate chain reactions in a dose dependent pattern. The complexes show stronger scavenging effects than the ligand. Hence, a powerful synergic antioxidant effect of the azide coordinated complexes was proved [66].

Table 5: Scavenging activity of the synthesized compounds

Compound	Scavenging activity			
	DPPH	H_2O_2	CUPRAC	FRAP
L^1	10	7	10	5
Complex **1**	72	58	70	55
Complex **2**	63	60	65	65
Complex **3**	80	78	78	72
Complex **4**	75	70	75	78

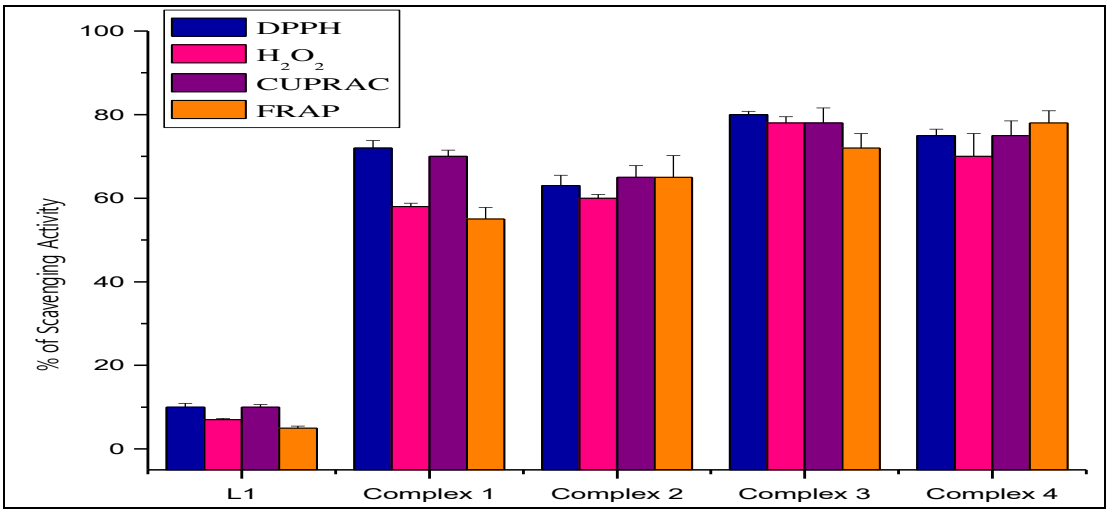

Fig. 8: Scavenging activity of the synthesized compounds

4. CONCLUSION

In the present study, azido based Schiff base Co(II), Ni(II), Cu(II) and Zn(II) complexes were synthesized and characterized. According to different physical and spectral analysis it was proved that synthesized Schiff base ligand act as tridendate. All the complexes are 1:1 electrolyte. The results of larvicidal and ovicidal activity against *C. quinquefasciatus* revealed that the complexes possess effective activities than Schiff base ligand. Antimicrobial activity of the complexes shows better zone of inhibition values against selected pathogenic microorganism and obtained MIC values of the complexes are also good. Radical scavenging activity of all the complexes exhibited better results. In particular, the Complex **3** showed 80 % of scavenging activity in DPPH method. Therefore, this study provides useful insight into the development of new route in medical field.

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