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RESEARCH ARTICLE

SYNTHESIS, PHYSICOCHEMICAL STUDIES AND BIOLOGICAL APPLICATIONSOF OXOVANADIUM(IV) COMPLEXES WITH SCHIFF BASES DERIVED FROM 3-PHENYL-4-AMINO-5-MERCAPTO-1, 2, 4-TRIAZOLES AND BENZIL

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Abstract

A series of some mononuclear oxovanadium(IV) complexes have been synthesized by the reactions of oxovanadium(IV) sulfate with Schiff base ligands derived from 3-(phenyl / substituted phenyl)-4-amino-5-mercapto-1, 2, 4-triazoles of aromatic carboxylic acids and benzyl. The complexes were analyzed by different spectroscopic methods (FTIR, UV-Vis., EPR), X-ray diffraction (XRD) analysis, elemental analysis, conductivity measurement, and cyclic voltammetry (CV) analysis. The non-electrolytic nature of the complexes was determined on the basis of the molar conductance values. The powder X-ray diffraction pattern has also been used to determine crystal size and type. On the basis of the physicochemical data, the tentative square- pyramidal geometry of the complexes has been proposed. In vitro antibacterial andantidiabeticproperties by alpha amylase inhibition activities of the selected complexes have also been determined.

Introduction:-

Schiff bases are versatile ligands and played influencing role in the development of coordination chemistry and were involved at key points in the development of the relationship between organic synthesis, inorganic chemistry and biochemistry[1,2].Schiff base derivatives in various processes promoted the researchers for designing the novel heterocyclic Schiff bases for the development of new environmental-friendly technology[3]. Nowadays, the bioinorganic chemists target the nitrogen-containing organic compounds as heterocyclic ligands and their metal complexes to study their pharmacology as the focus of research due to their wide range of biological activities such as antibacterial, antifungal, antidiabetic[4,5,6], anticancer[7]and antiviral activities[8]. The chemistry of oxovanadium(IV) has received significant attention, as the VO^{2+} unit can coordinate a different type of donor atoms to form different types of complexes.[9,10,11]. Nowadays, most of the research directed on coordination complexes of VO^{2+} because it is probably the most relevant species present in biological systems since a number of systematic model studies have carried out on the interaction of this oxo-cation with different biomolecules and other ligands of biological and pharmacological interest in order to contribute to a better understanding of its possible roles and functions in living organisms[12,13].Keeping in view the medicinally important properties of Schiff bases[14,15], and potential bioactivity of vanadium metal, researcher find it valuable to combine both the chemistry of these two moieties and evaluate their coordination behaviour and bioactive nature[16] which results into the study of coordination behaviour and biological activity of different acyclic and macrocyclic Schiff base ligands towards

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oxovanadium(IV)[17]. The present work aims the synthesis and characterization of oxovanadium(IV) derivatives with triazole based multidentate Schiff base ligandsand toscreen for their antibacterialactivity and antidiabetic properties by alpha amylase inhibition activities of the complexes. Alpha-amylase (1, 4 -α- D-glucan- glucanohydrolase, EC 3.2.1.1) is an endoglucanase that hydrolyses internal α(1- 4) glycosidic linkages of starch and other related polysaccharides[18]. Human alpha amylase is secreted by the salivary gland and pancreas that plays an important role in the digestion of starch. Finding of inhibitor of alpha-amylase may have benefits for the prevention of obesity and diabetes [19].

Experimental

Materials And Measurements:-

The solvents and chemicals used during the study were commercially available and used as received without further purification.Hydrazine hydrate was procured from SD Fine Chemicals from Maharashtra, India and oxovanadium(IV) sulphate was procured from Aldrich Chemical Co., England.

The UV-Vis. spectra of all the samples prepared were analyzed using UV-Vis. electronic spectra on a double beam spectrophotometer of Labtronics Model LT-2802. The FTIR spectra wereanalyzed by using Fourier Transform Infrared Spectroscopy (IR Tracer 100, Shimadzu, Japan). The differentoxovanadium(IV) complexes wereanalyzed for the phase detection using an X-ray Diffractometer with monochromatic Cu-Kα radiation (D_2 phase Diffractometer, Bruker, Germany). The samples were scanned at a 2θ angle of 2 to 80 degrees.

The cyclic voltammetry was conducted on HOKUTODENKO (model no. 151, made in Japan) Potentiostat/Galvanostat by using a graphite electrode as working electrode, another graphite electrode as supporting/counter electrode and SCE as a reference electrode. The solution of the sample of complex[$VO(L_1)$], was prepared in synthetic grade DMSO solution (manufactured in Mumbai, India) in 10^{-3} M concentration. The molar conductance of three sample solutions was measured by using DELUX CONDUCTIVITY METER of model-601 and serial no. of 17051032 made in India. 0.1M solution of tetrabutylammonium bromide (TBAB) is prepared in acetonitrile by taking 2.344 g of it and used as supporting electrolyte. 20ml of the sample solution and 5 ml of supporting electrolyte were mixed together in a beaker and was subjected for current measurement at the fixed potential range of -0.2V to 1.0V.The solid-state X-band EPR spectrum for oxovanadium(IV) complex has been recorded at room temperature at field set 3200 G (= 0.32 T), microwave power 5 mW and microwave frequency 9.1 GHz.

Synthesis of Schiff bases

Aromatic carboxylic acids (benzoic acid, 4-nitrobenzoic acid, 2-chlorobenzoic acid, 4-chlorobenzoic acid and 4-methylbenzoic acid), when allowed to reflux with methanol in the presence of few drops of sulfuric acid at 50℃, results into the formation of ester. The ester so formed was extracted with ether and dried. Hydrazides were synthesized by the reaction of hydrazine hydrate and methyl ester of carboxylic acids inequimolar ratio (1:1) refluxing for ca. 4 h in ethanol and then cooled. The crystals separated were filtered off, washed with aqueous ethanol and then crystallized from hot water.To prepare mercaptotriazolesmethanolic solution (20 cm^3) of substituted acid hydrazide (0.01 mol), potassium hydroxide (0.01 mol) and carbon disulfide(0.01 mol) were stirred for ca. 2 h. A solid mass was obtained which was refluxed with hydrazine hydrate (0.012 mol) for ca. 4 h. The reaction mixture was cooled, poured in cold water and neutralized with dilute hydrochloric acid. The product, thus obtained, was filtered off, washed with ether and crystallized from methanol [20, 21].

In order to prepare Schiff base, an ethanolic solution (20 cm^3) of mercaptotriazole(0.2 mol) derived from different carboxylic acids (benzoic acid / 4-nitrobenzoic acid / 2-chlorobenzoic acid / 4-chlorobenzoic acid / 4-methylbenzoic acid) andbenzil (0.1 mol), added few drops of glacial acetic acid and refluxed for ca. 6-7 h. The precipitate, thus obtained, was filtered off and washed with ethanol and ether [22].

The reaction pathway is shown below (Fig. I):

Figure 1:- Synthesis of Schiff bases.

Synthesis of oxovanadium(IV) complexes

A methanolic solution (15 cm^3) of the ligand (0.005 mol) was added to a methanolic solution (15 cm^3) of oxovanadium(IV) sulfate(0.005 mol), and the mixture was allowed to reflux for ca. 12 h. The volume was reduced to 20 cm^3 and allowed to settle overnight. Green or yellowish greencolored precipitate obtained was filtered, washed thoroughly with cold methanol and ether (1:1) and dried under vacuo. In this way oxovanadium(IV) complexes with Schiff bases were derived from 3-(phenyl/substituted phenyl)-4-amino-5-mercapto-1, 2, 4-triazole and benzil.

Biological activity

Antibacterial activity (in vitro)

Two synthesized triazole Schiff bases (L_1H_2 and L_2H_2) and their oxovanadium(IV) complexes [$VO(L_1)$] and [$VO(L_2)$] were screened in vitro for their antibacterial activity against three Gram-negative (E. coli, K. pneumonia and P. aeruginosa) and two Gram-positivebacterial strains Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Susceptible Staphylococcus aureus (MSSA) by the agar-well diffusion method. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer. Bacterial inocula (2-8 hrs old) containing approximately 10^4-10^6 colony-forming units (CFU/ml) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (4mg/ml, 6mg/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug gentamycin, served as negative and positive controls, respectively. The plates were incubated at 37^{o}C for 24 hrs. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to evaluate the interfering effect of DMSO on the biological screening, alternate studies on DMSO solution showed no activity against any bacterial strains. The percentage inhibition of the growth of the test organism was calculated by the following formula[23];

Inhibition (%) = Cd/Td x 100,

where Cd is colony diameter of control and Td is colony diameter of treated set.

Minimum inhibitory concentration (MIC)

Compounds containing promising antibacterial (above 80%) activity were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion techniqueby preparing discs containing 25, 50, 100 and 200 µg/ml concentrations of the compounds along with standards at the same concentrations [24].

Protocol for Alpha-Amylase Inhibition Assay

The alpha-amylase inhibition activity was evaluated in 50mM phosphate buffer (pH=6.8). 80 µL of Porcine pancreatic alpha- amylase (PPA) at the final concentration of 1.5 units/mL (was prepared in Buffer) was added into the various concentrations of 20 µL test compounds on 96 well plate and incubated at 37º C for 15min. The reaction was started by the addition of substrate , CNPG3, at 375 µM final concentrations prepared in the buffer. The change in absorbance by released p-nitroaniline was continuously monitored at 405 nm [25]. All the experiments were performed in triplicate in a final volume of 200 µL, by using a micro-plate reader (Epoch2, BioTek, Instruments, Inc., USA)

$$\%\text{inhibition} = \frac{\text{Ao} - \text{At}}{\text{Ao}} * 100$$

$$\text{Ao} = \text{Absorbance of Control}$$

$$\text{At} = \text{Absorbance of Sample}$$

Results And Discussion:-

The reactions of oxovanadium(IV) sulfate with Schiff base ligands (LH_2) derived from 3-(phenyl / substitutedphenyl)-4-amino-5-mercapto-1,2,4-triazoles and benzil have been carried out in ethanol in 1:1 molar ratio. The complexes of the type [$VO(L_n)$] are obtained according to the following reaction:

$VOSO_4 + LH_2 \longrightarrow$

(LH_2 = L_1H_2, L_2H_2, L_3H_2, L_4H_2,L_5H_2)

The complexes are green in color, soluble in DMF, DMSO, nitrobenzene and are stable at room temperature.The analytical data(Table 1) are compatible with a 1:1 metal to ligand stoichiometry. Electrical conductance measurements in dimethylformamidereveal that they are essentially non-electrolytes.

Magnetic Moment and Electronic Spectra

The magnetic moments of the oxovanadium(IV) complexes lie in the range 1.70–1.78 B.M at room temperature. These values are well within the range reported [27] for monomeric oxovanadium(IV) complexes. In the electronic spectra, three bands are observed in the regions 10750–12200, 15500–16000 and 21800–22870 cm^{-1}**(Table 2)**. These bands are nearly the same as reported [28] for other five-coordinated oxovanadium(IV) complexes. Several schemes have been advanced to interpret the electronic spectra of oxovanadium(IV) complexes[29]. Three-band spectrum has been predicted for five–coordinate complexes possessing the effective C_{4v} symmetry of a square pyramid. Ballhausen and Gray[30] proposed, the energy level scheme for $[VO(H_2O)_5]^{++}$. In this model, the unpaired electron resides in a largely non bondingd_{xy} orbital (z–axis is taken as the vanadyl bond) with lobes pointing between the equatorial ligands. The interaction of d_{xz}, d_{yz} with the ligand may be π–acceptor in character and the relative order of the B_2 and E orbitals could be inverted. Usually, three optical bands are observed.

1. $^2B_2(d_{xy}) \longrightarrow {}^2E(d_{xz}, d_{yz})$
2. $^2B_2(d_{xy}) \longrightarrow {}^2B_1 (d_{x^2-y^2})$
3. $^2B_2(d_{xy}) \longrightarrow {}^2A_1 (d_{z^2})$

For some complexes, four bands are observed, possibly arising from the splitting of d_{xz}, d_{yz} levels in low symmetry.

Accordingly, the observed bands can be assigned to $b_2 \rightarrow e_\pi^*(^2B_2 \rightarrow {}^2E)$, $b_2 \rightarrow b_1^*(^2B_2 \rightarrow {}^2B_1)$and $b_2 \rightarrow a_1^*(^2B_2 \rightarrow {}^2A_1)$ transitions in increasing order of energy. The second transition corresponds to 10 Dq. One more band is observed at ca. 32000–35000 cm^{-1}, which may be due to intra-ligand transition. The electronic spectral bands of two representative complexes $[VO(L_1)]$ and $[VO(L_2)]$ are represented in the **Figure 2.**

Infrared (IR) Spectra

IR spectra provide valuable information regarding the nature of the functional group attached to the metal atom. The bonding sites of Schiff base ligands involved in the oxovanadium(IV) complexes have been determined by careful comparison of the IR spectra of the complexes with the spectra of ligands[31].

Schiff bases appear to exist in both thiol and thionetautomeric forms[32].Investigations showed that thione structure dominate in the solid state and thiol in solution. Thiols and thio-substituted compounds can be considered to be the direct analogs of the equivalent oxygenated compounds such as alcohols and ethers. Unlike the oxygen containing analogs, the equivalent C-S and C-S-H stretching vibrations tend to give rise to very weak absorptions in the IR spectrum. The higher mass of sulphur, compared with oxygen, results in the characteristic groupfrequencies occurring at noticeably lower frequencies than the oxygen containing analogues. The IR spectra of Schiff bases in solution show a broad band at ca. 2600-2480 cm^{-1}assignable to ν(S-H)[33]. In complexes ν(S-H) band disappears indicating the deprotonation of thiol group and formation of bond between VO^{2+} and sulphur. This is further confirmed by appearance of new band in complexes at ca. 380 - 350 cm^{-1} assignable to ν(V-S).Schiff bases exhibit strong band at ca.1620 cm^{-1}, assigned[34] to ν(C=N). In complexes, this band shifts to lower frequency at 1605-1598 cm^{-1} indicating the participation of azomethine nitrogen in bond formation with VO^{2+} ion. Moreover, new bands appear in oxovanadium(IV) complexes at ca. 415 -395 cm^{-1} due to ν(V-N) vibrations. The band due to ν(C=N) (triazole ring) appears at ca. 1570 cm^{-1} in the ligands which remains almost at the same position in the complexes indicating non-coordination of ring azomethine nitrogen in bond formation[35].

The spectra of all oxovanadium(IV) complexes show a new characteristic band around 975 - 970 cm^{-1} due to ν(V=O) vibrations.All Schiff bases and their corresponding oxovanadium(IV) complexes show bands at ca. 3050-2880 cm^{-1} due to ν(Ar-H)[36] and alkyl groups. Thus, the IR spectral studies indicate that the Schiff base ligands, behave as dibasic, tetradentate chelating agents having coordination sites at two thiol sulphur atoms and two azomethine nitrogen atoms.

Conductance and Cyclic Voltametry

The specific conductance of the different sample solutions was measured at 27.2 ℃ and the cell constant at 1.000 (acceptance 1±10%). The molar conductance of [VO(L_1)], [VO(L_2)]and[VO(L_3)] solutions were found to be 19.4 x103 µS/M, 20.0 x103 µS/M, and 18.3 x103 µS/M respectively.

The cyclic voltammetry data**(Table 3)** obtained at a scan rate of 100 mV/s on a 10^{-3}M solution of [VO(L_1)] complex in DMSO containing 0.1M aqueous solution of tetrabutylammonium bromide as supporting electrolyte are presenting in **Figure 3**. The analysis of the cyclicvoltammogram of the complex [VO(L_1)] shows the value of peak potential (ΔEp) is equal to 70 ± 2 mV and peak current ratio (I_a/I_c) is equal to 1.00± 0.08 (approximately 1).From the study of cyclic voltammogram of the complex, in an ideal reversible process, the peak-to-peak separation, ΔE_p is ~59 mV and 30 mV for a one-electron and two-electron processes, respectively, and I_a/I_c is ~1 for a reversible process. The I_a/I_c being approximately 1, it may be assumed that the complex is electrochemically pseudo-reversible in nature indicating the good electrochemical stability[37].

Electron Paramagnetic Resonance (EPR) Spectra

^{51}V is in 99.8% natural abundance and has a nuclear spin I = 7/2. In fluid solution, eight hyperfine lines are observed exhibiting a variation in line width. Hyperfine coupling are large (~100 gauss), but a large nuclear moment and spin cause a large line-width which tends to obscure super hyperfine splitting. Almost all vanadium compounds have symmetry less than Oh and Td, and so EPR signals are readily observed because of the longer relaxation time. At room temperature, there is free rotation of the vanadyl group, as manifest by the isotropic spectrum. At lower temperature the spectrum becomes anisotropic. EPR studies on the large number of vanadyl complexes, shows that most VO^{2+} complexes have the square–pyramidal structure and may frequently add on Lewis bases to the sixth coordination position.Splitting of the vanadium perpendicular features into A_x and A_y has only been seen in a few cases and arises from the asymmetry of the chelating ligands. In all cases, $g_{||} < g_{\perp} < 2$, which are in agreement with prediction[38].

The X-band EPR spectra of oxovanadium(IV) complexes were recorded at room temperature and at liquid nitrogen temperature. The polycrystalline sample data show significant temperature dependence. At ambient temperature, intense EPR spectra are observed. These spectra display typical eight line pattern of vanadium (^{51}V; I =7/2) with isotropic g_0 values and hyperfine coupling parameters A_0.The g average values, determined from the spectra, are ca. 1.98, similar to the spin-only values (free electron value) of 2.00 suggesting little spin orbital coupling. At the liquid nitrogen temperature, the spectra display well resolved axial anisotropy with two sets of eight-line pattern[39]. The $g_{av,}$ $g_{||}$, $g_{\perp}$, A_{av}, $A_{||}$ and $A_{\perp}$ values are given in **Table 4**. The values are typical of the spectra displayed by square pyramidal oxovandium(IV) complexes with unpaired electron in an orbital of mostly d_{xy} character. Inspection of these data reveals a close agreement between the spectral parameters obtained at room temperature and at liquid nitrogen temperature; [$g_{av.} = 1/3(g_{||} + 2\ g_{\perp})$; $A_{av.} = 1/3(A_{||} + 2A_{\perp})$], thus indicating the complexes retain theirstructural identity throughout this temperature range. EPR spectra of one representative complex, [VO(L_2)], at room temperature and at liquid nitrogen temperature are shown in **Figure 4.**

X-ray Powder Diffraction Pattern

The XRD pattern clearly indicates the formation of the nanocrystal. The crystallite size has been calculated by using Debye-Scherer formula[40] given as below and presented in **Table 5:**

$$D = \frac{0.94\lambda}{\beta cos\theta}$$

where D is the crystallite size, λ is the wavelength of X-ray used; β is the full width at half maximum (FWHM) in radians and θ is Bragg's angle in radians. The observation of multiple sharp peaks in the XRD pattern tells that the sample possesses a high degree of crystallinity.

The average crystallite size of the oxovanadium(IV) complexes was found to be 17.020–22.189 nm range.This tells that the average size is in the nano-scale range[41].

Thus, on the basis of above studies, the following structure (Figure 5)is tentatively assigned for [VO(L)] complexes:

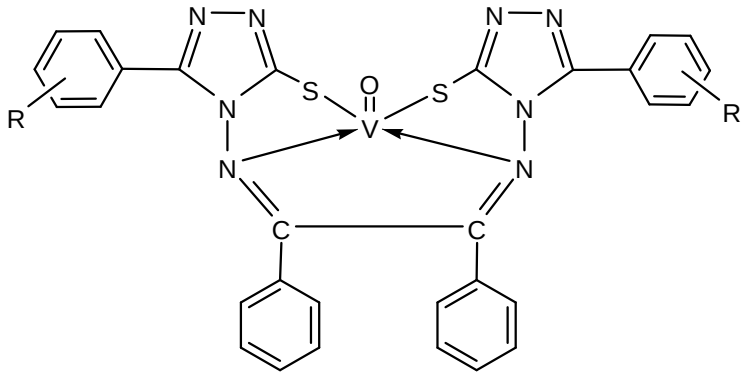


Figure 6:- Tentatively proposed structure of the complexes.

Biological activity

Antibacterial bioassay (in vitro)

The synthesized triazole Schiff's bases and their oxovanadium(IV) complexes[$VO(L_1)$]and [$VO(L_2)$]were screened for in vitro antibacterial activity against three Gram-negative (E. coli, K. pneumonia and P. aeruginosa) and two Gram-positive bacterial strains(MRSA and MSSA) by the agar-well diffusion method (concentration 4mg/ml)[42]. The results obtained were compared with that of the standard drug Gentamycin and are reported in **Table 6** and as **Figure 6**. The activity of synthesized compounds (in % age) was compared with the activity of the standard drug considering its activity as 100%. The synthesized compounds showed varying degree of inhibitory effects: low (up to 33%), moderate (up to 53%) and significant (above 53%). The ligand (L_1H_2) possessed a significant (53-84%) activity against (b), (c), and (d) bacterial strain and moderate (50%) activity against (a). Similarly, the ligand (L_2H_2) exhibited significant (59-75%) activity against (a), (b), (c), (d), and (e). Complex [$VO(L_1)$], showed significant (56-83%) activity against (a), (b), (c), (d), and (e). Similarly, complex [$VO(L_2)$] possessed significant (70-80%) activity against (a), (b), (c), (d), and (e). Similar results were obtained at concentration 6mg/ml. It is interesting to note that antibacterial activity of simple ligands is increased upon coordination that confirmed our previous studies. It is, therefore evident that coordination makes the ligands strongly antibacterial agent and inhibits the growth of bacteria more than the parent Schiff's base ligands[43]. The antibacterial results of the oxovanadium(IV) complexes [$VO(L_1)$] and [$VO(L_2)$] were considered the most active compounds due to the presence of more number of nitrogen atoms, hence carried out their MIC screening.

Minimum inhibitory concentration

The data of preliminary antibacterial screening showed that the oxovanadium(IV) complexes [$VO(L_1)$]and [$VO(L_2)$]against MRSA, P. aeruginosa, E. coli and K. pneumonia were the most active compounds. Therefore, these compounds were selected for the MIC studies[44]. The results obtained were compared with that of the standard drug Gentamycin and are reported in **Table 7** and as **Figure 7**.The activity of synthesized compounds (in % age) was compared with the activity of the standard drug considering its activity as 100%. The synthesized complex[$VO(L_1)$]showed varying degree of inhibitory effects: low (up to 33%), moderate (up to 53%) and significant (above 53%). The complex [$VO(L_1)$]possessed a significant (53-61%) activity against (a), (b), (c), and (d) bacterial strain at 200 µg/ml; moderate (46%) against (a) and significant (61-66%) activity against (b), (c), and (d) at 100 µg/ml; moderate (47%) against (a) and significant (56-75%) activity against (b), (c), and (d) at 50 µg/ml; moderate (45%) against (a) and significant (57-70%) activity against (b), (c), and (d) at 25 µg/ml. Similar results were obtained for complex [$VO(L_2)$] at four different concentrations.

All complexes show higher activity against bacteria as compared to Schiff's base. Such increased activity of the metal complexes can be explained on the basis of oxidation state of the metal ion, Overtone's concept and Tweedy's chelation theory [45].It has been suggested that the ligands with N and S donor system might have inhibited enzyme production, since enzyme which requires a free hydroxyl group for their activity appears to be especially susceptible to deactivation by the ions of the complexes.

Chelation reduces the polarity of central ion mainly because of partial sharing of its positive charge with donor groups and possible p-electron delocalization within the whole chelating ring. This chelation increases the lipophylic nature of the central atom which favors its permeation through lipid layer of cell membrane. Furthermore, the mode

of action of compounds may involves the formation of hydrogen bonds through the azomethine (C=N) group of complexes with the active centers of cell constituents resulting in the interference with normal cell process[46]. Though the complexes possess activity, it could hardly reach the effectiveness of the standard drug such as Gentamycin. Some compounds are less effective, the variation in effectiveness depends on either on the impermeability of the cells of the microbes or on differences in the ribosome of microbial cells. It is also well known fact that heterocyclic ring has cell wall damaged capacity. The newly synthesized complexes have triazole ring which on contact with the microbes, damages cell wall and also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of microorganism[48]. The toxicity of compounds is directly proportional to the concentration.

Alpha-Amylase Inhibition Assay (in vitro)

Vanadium, complexed with other molecules, has been reported to be inhibitors of alpha-amylase and as anti-diabetics[47].Ligand without vanadium complex would be a weak inhibitor of alpha-amylase [48], strongly supporting vanadium may interact with the alpha-amylase enzyme. However, vanadium complexed with other ligand may help to fit in the pocket of enzyme as vanadium alone has not been reported to inhibit the activity of enzyme. In our study, ligand with electron withdrawing moiety (-NO_2) is reducing the inhibitory potential (IC_{50} = 385.8 µg/mL) compared to without such moiety (IC_{50} = 201.3 µg/mL). Electron withdrawing moiety might have interfered vanadium to interact with the amylase enzyme(Table 8).

Conclusion:-

A series of oxovanadium(IV) complexes have been synthesized from Schiff bases derived from 4-amino-3-phenyl-5-mercapto-1, 2, 4–triazole with and benzil. These Schiff's bases form stable complexes with VO^{+2} ion. Schiff bases act as dibasic, tetradentate chelating agents and coordination takes place through azomethine nitrogen and thiol sulphur via deprotonation. XRD spectra indicate that complexes are in nano range. A square-pyramidal geometry of complexes has been established by spectral studies. Oxovanadium(IV) complexes [$VO(L_1)$] and [$VO(L_2)$] synthesized have been studied against three Gram-negative (E. coli, K. pneumonia and P. aeruginosa) and two Gram-positive (MRSA and MSSA) bacteria. The complexes [$VO(L_1)$] and [$VO(L_2)$]show significant activity with the change in the nature of the ligand attached to VO^{+2} ion. The complexes show higher antimicrobial activity as compared to free ligands.

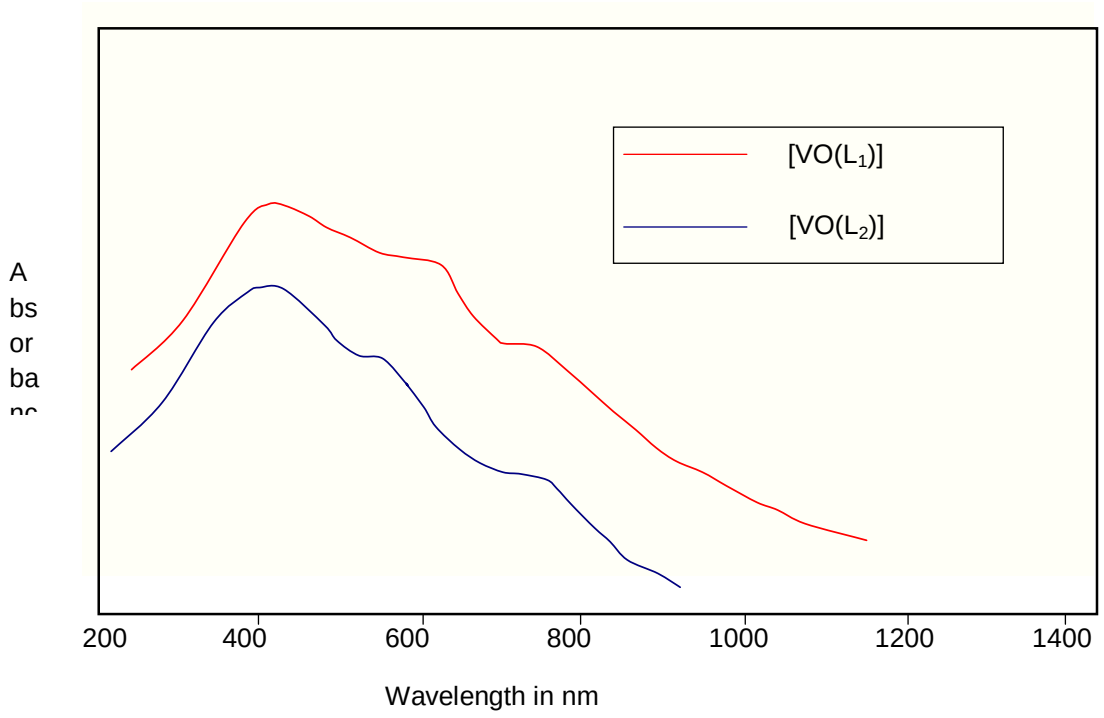


Figure 2:- Electronic spectra of [$VO(L_1)$] and [$VO(L_2)$].

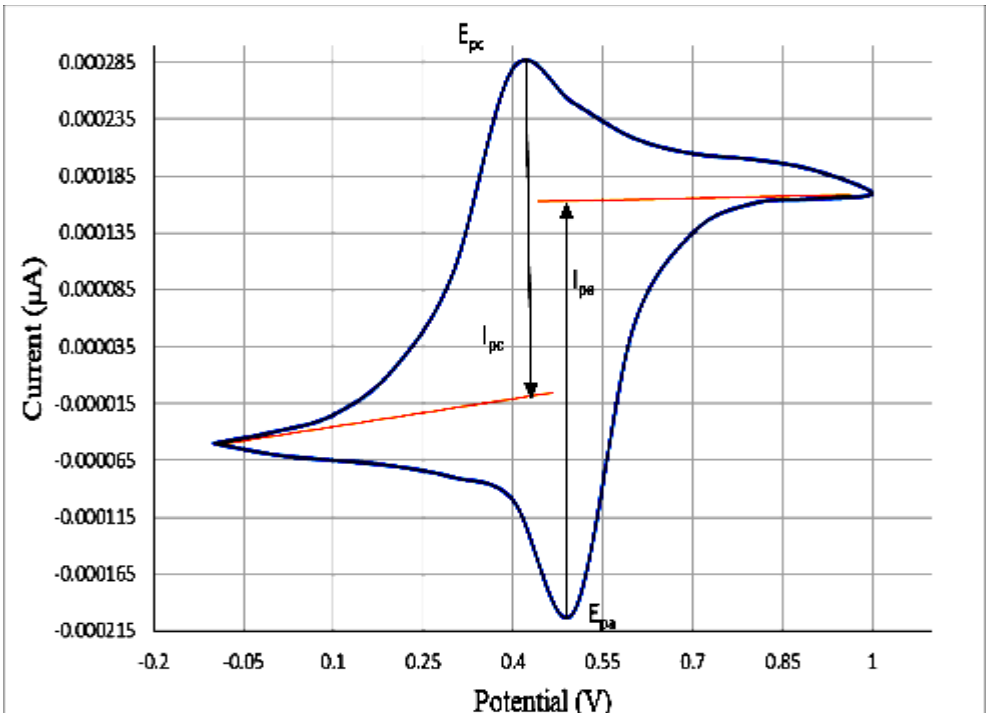
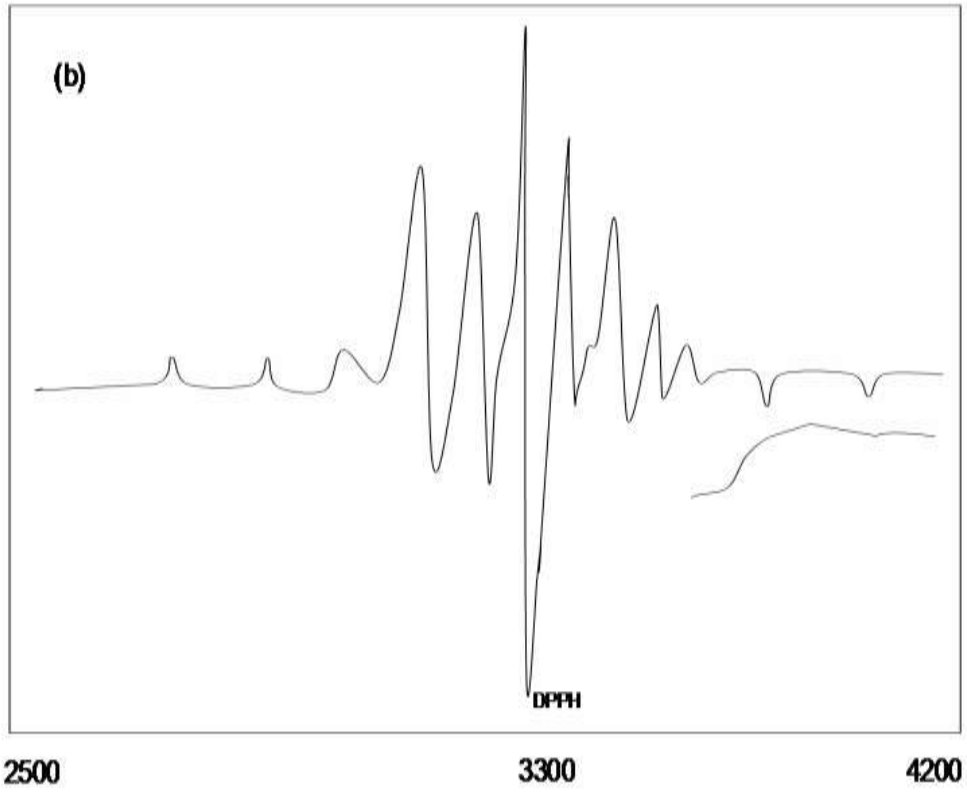


Figure 3:- Cyclovoltametric diagram of complex [$VO(L_1)$].



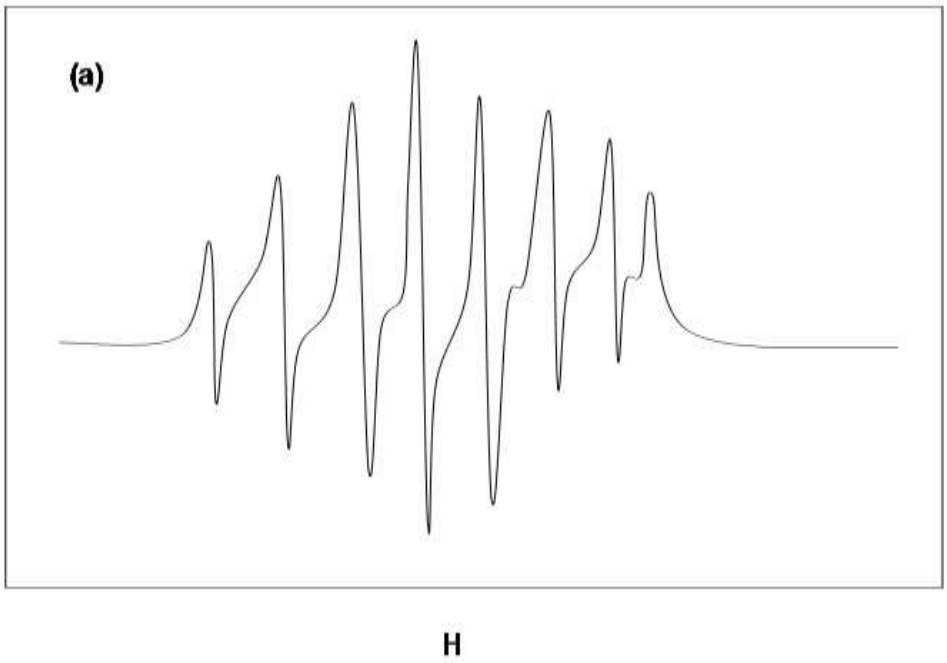


Figure 4:- EPR spectra of [VO($C_{30}H_{18}N_8S_2Cl_2$)] in DMSO at (a) room temperature and (b) liquid nitrogen temperature.

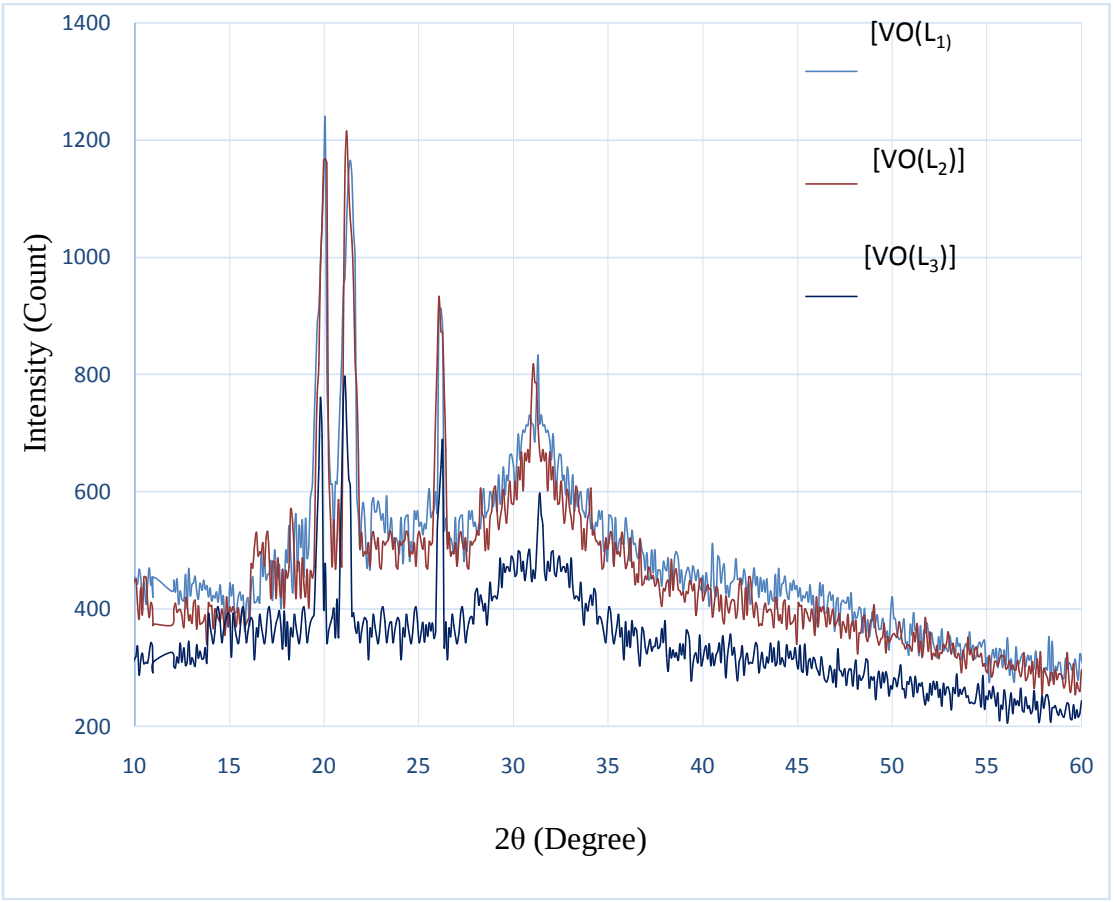


Figure 5:- Powder X-ray diffraction pattern ofoxovanadium(IV) complexes.

Table 1:- Elemental analyses of oxovanadium(IV) complexes with Schiff bases derived from 3-phenyl-4-amino-5-mercapto-1, 2, 4-triazoles and benzyl.

Complex	**Molecular formula**	**Analyses (%) Found (Calcd.)**			
		C	**H**	**N**	**V**
$[VO(L_1)]$	$[VO(C_{30}H_{20}N_8S_2)]$	57.72 (57.78)	3.12 (3.23)	17.88 (17.97)	8.10 (8.17)
$[VO(L_2)]$	$[VO(C_{30}H_{18}N_{10}O_4S_2)]$	50.42 (50.49)	2.46 (2.54)	19.54 (19.63)	7.02 (7.14)
$[VO(L_3)]$	$[VO(C_{30}H_{18}N_8S_2Cl_2)]$	51.98 (52.03)	2.54 (2.62)	16.10 (16.18)	7.15 (7.36)
$[VO(L_4)]$	$[[VO(C_{30}H_{18}N_8S_2Cl_2)]$	52.00 (52.03)	2.55 (2.62)	16.02 (16.18)	7.28 (7.36)
$[VO(L_5)]$	$[VO(C_{32}H_{24}N_8S_2)]$	58.93 (58.98)	3.63 (3.71)	17.04 (17.20)	7.65 (7.82)

Table 2:- Magnetic moments and electronic spectral bands ofoxovanadium(IV) complexes.

Complexes	μ_{eff}	**Electronic spectral bands (cm^{-1})**		
	(B.M) at 300K	$^2B_2 \rightarrow {}^2E_1$	$^2B_2 \rightarrow {}^2B_1$	$^2B_2 \rightarrow {}^2A_1$
$[VO(L_1)]$	1.75	10750	15800	21800
$[VO(L_2)]$	1.78	11800	15500	22500
$[VO(L_3)]$	1.7	12200	16000	22000
$[VO(L_4)]$	1.72	11500	15600	22200
$[VO(L_5)]$	1.74	11550	15650	22150

Table 3:- EPR parameters of oxovanadium(IV) complexes.

1) Complexes	g_{av}	g_{II}	$g_{\perp}$ $x10^4$	A_{av} cm^{-1}	A_{II}	$A_{\perp}$
$[VO(L_1)]$	1.979	1.952	1.991	87	161	50
$[VO(L_2)]$	1.974	1.950	1.986	88	161	52
$[VO(L_3)]$	1.969	1.948	1.979	87	160	50
$[VO(L_4)]$	1.972	1.950	1.980	90	165	52
$[VO(L_5)]$	1.971	1.949	1.982	89	165	51

Table 4:- XRD data of three oxovanadium(IV) complexes.

Samples	Peak Position (2Theta)	FWHM	Crystalline Size, D (nm)	Average D (nm)
$[VO(L_1)]$	20.09	1.345	21.134	20.112
	21.26	0.822	19.089	
$[VO(L_2)]$	19.97	0.482	17.495	17.020
	21.32	0.511	16.545	
$[VO(L_3)]$	19.85	0.392	21.465	22.189
	21.09	0.368	22.913	

Table 5:- Antibacterial activity of compounds at concentration (4 mg/ml in DMSO).

ZONE OF INHIBITION (mm) **Concentration (4 mg/ml in DMSO) /(6mg/ml in DMSO)**										
	BACTERIA									
Compounds	**MRSA**		**MSSA**		**P. aeruginosa**		**K. pneumoniae**		**E. coli**	
L_1H_2	16	15	17	16	21	22	20	22	19	21
L_2H_2	18	20	19	20	17	18	19	20	21	23

[VO(L_1)]	18	17	20	18	18	19	21	22	23	25
[VO(L_2)]	19	20	19	21	18	20	20	21	22	24
STANDARD (Gentamycin)	30	30	30	30	26	26	30	30	30	30

Table 7:- α-Amylase inhibitory of activity of oxovanadium(IV) complexes.

Compound	Inhibition (%)	IC_{50} (µg/mL)
[VO(L_1)]	68.3	201.3± 0.3
[VO(L_2)]	62.1	385.8 ± 0.5
Acarbose*	85.2	6.1±0.1

*Acarbosewas used as the standard for α-amylase inhibition.

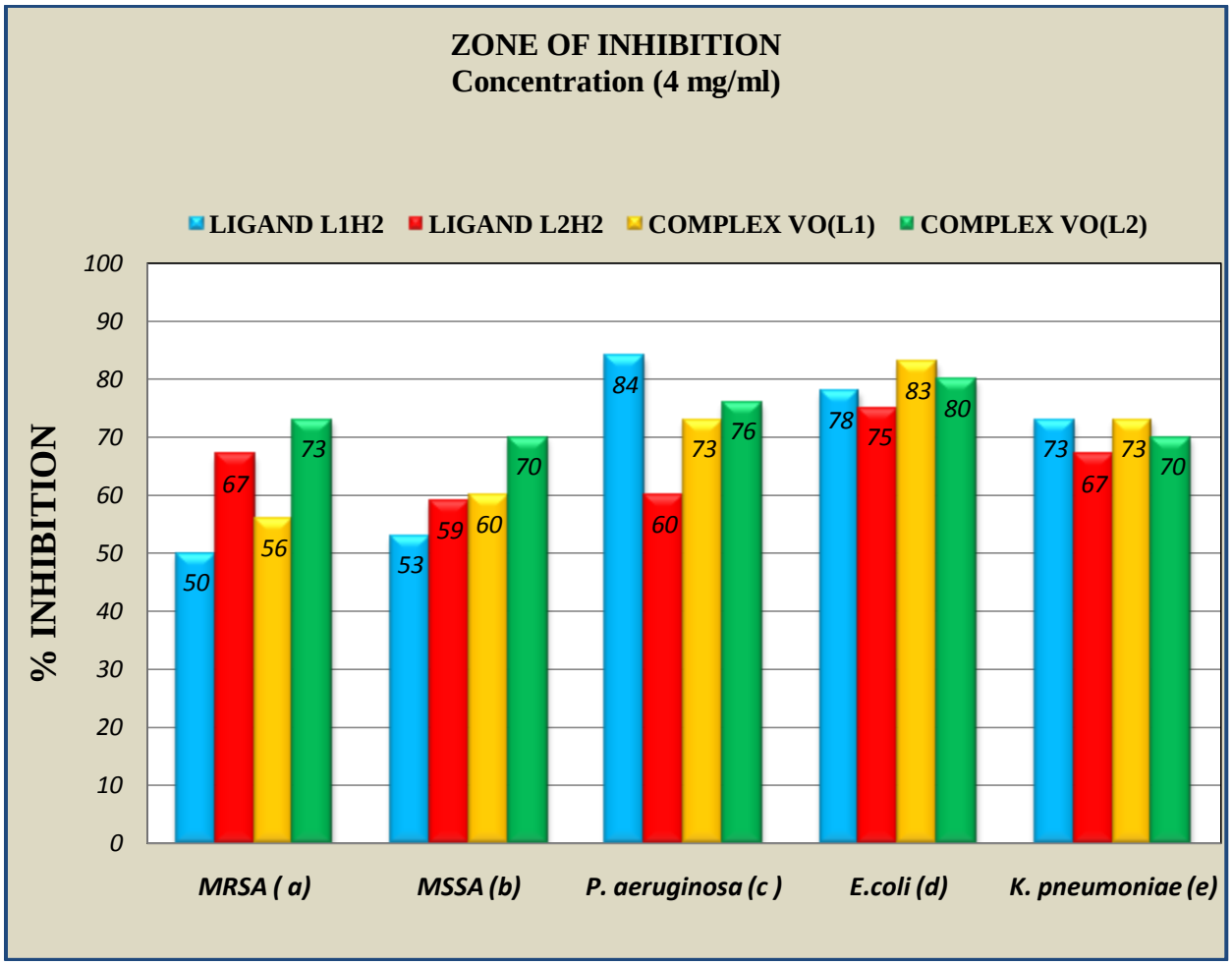


Figure 7:- Comparison of antibacterial activity of free Schiff's base ligands (L_1H_2 and L_2H_2) and complexes [VO(L_1)]and [VO(L_2)]at concentration (4 mg/ml in DMSO).

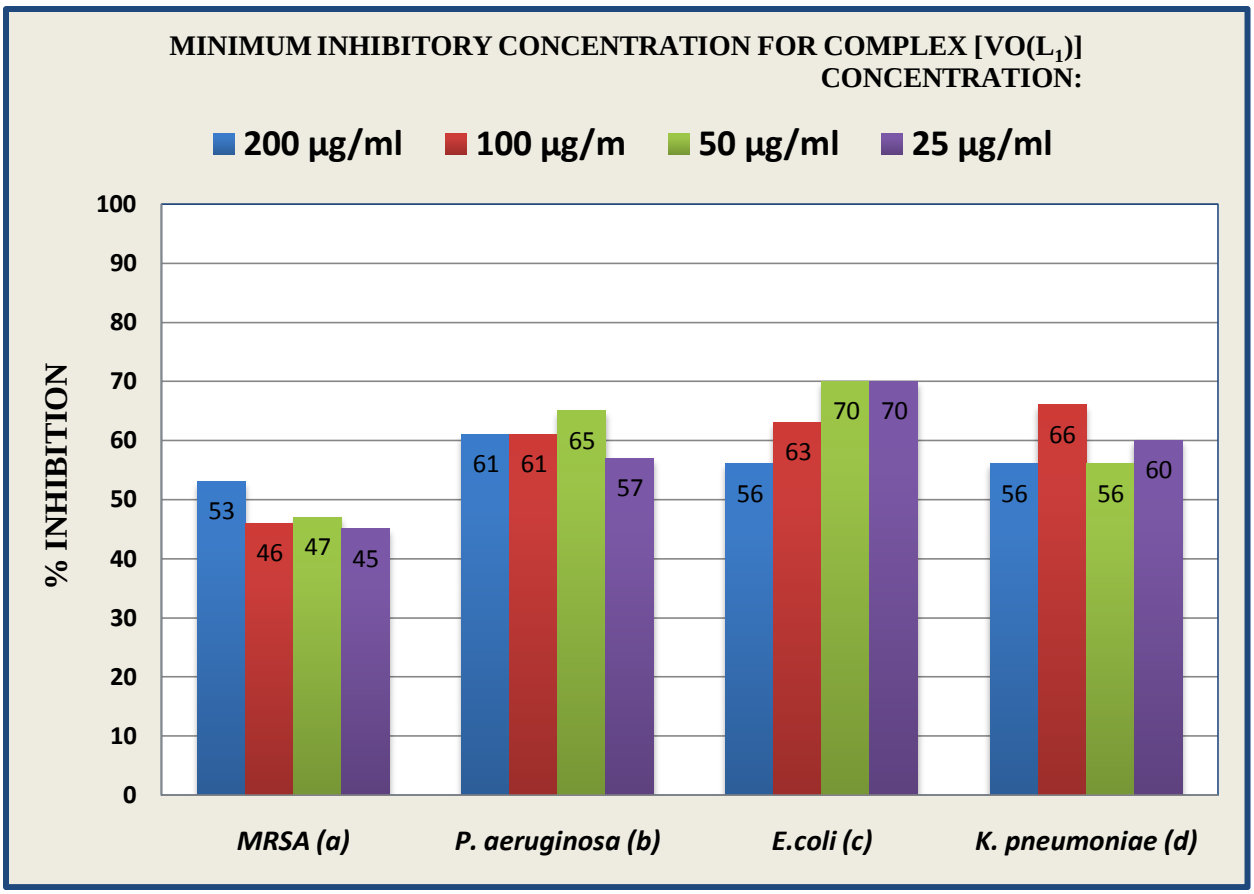


Figure 8:- Minimum Inhibitory Concentration (MIC) of complex [$VO(L_1)$] against selected bacteria.

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