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Electrochemical study and microbial evaluation of bioactive schiff base and its metal complex

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Abstract

The novel Schiff base ligand has been produced in methanol medium by combining 2-hydroxy-2-methylpropiophenone and sulphapyridine. Complex of this Schiff base ligand with Cu (II) ion has been prepared with ligand metal salt ratio of 2:1. Spectroscopic techniques and elemental analysis have been used to clarify the structures of the produced compounds. The electrochemical behavior of the synthesized ligand, designated HMPSP, was investigated using cyclic voltammetry (CV) in Britton-Robinson (BR) buffer and aqueous phosphate buffer at GCE. CV investigations reveal that HMPSP ligand exhibits dielectric, diffusion control, irreversible reduction peak and the product of this reduction are secondary amines. Whereas the Cu (II)/Cu (I) redox systems are quasi-reversible necessitate a monoelectron transfer. The various kinetic parameters were also computed, including the diffusion coefficient ($D_0^{1/2}$), charge-transfer coefficient (α_n), and rate constant ($K^0_{f,h}$). The synthesized Schiff base ligand and its metal complex have been shown to be biologically effective against the fungi (*Aspergillus niger* & *Penicillium chrysogenum*) as well as the bacteria (*Bacillus subtilis* & *Escherichia coli*). In comparison to the standard, the microbiological result shows moderate activity.

Keywords: Schiff base, cyclic voltammetry, irreversible, quasi-reversible, anti-microbial activity

Introduction

Schiff's bases are an ensemble of physiologically active pharmaceutical compounds exhibiting diverse pharmacological properties that have garnered the attention of pharmaceutical researchers [1, 4]. Transition metal complexes of Schiff bases, derived from amines and aldehydes, have been extensively studied. The Schiff base comprises nitrogen and oxygen donor atoms which provide it easiest to coordinate with transition metal ions to produce more thermally resistant and vibrant metal complexes [5, 6]. Incorporating heteroatom-containing organic ligands as coordination sites has been a key strategy in coordination chemistry, yielding a variety of intriguing and beneficial compounds. Because it enables the formation of mono-, di-, or multinuclear complexes, the highly efficient chelating ability of ligands containing multiple donor heteroatom's have garnered attention globally. The majority of pharmacological medicines comprise metal compounds that are now being used in clinical contexts, and new application areas are opening up gradually. The coordination chemistry of the metallic complexes of Schiff bases produced from sulfa medicines has attracted a lot of attention due to their significance biological action [7, 9]. In addition to being physiologically active, the condensation products of sulfa drugs with aldehydes and ketones also combine with metal ions to form complexes. Numerous chemotherapeutically significant sulfa drugs, such as sulfadiazine, sulfa pyridine, and sulfa merazine, have the crucial toxophoric functional group $-SO_2NH-$. Azomethine have been used as catalysts, analytical reagents, and azo compounds for use as pigments and dyes. In experiments using H_2SO_4 at different temperatures and concentrations, they also showed that mild steel corrosion may be inhibited [10].

Using the cyclic voltammetry approach, the electrochemical behavior of bioactive synthetic substances was investigated. This method is highly sensitive and effective in providing important information such as the stability of the compound under study, the kinetics of electrochemical processes occurring at electrode surfaces, and redox characteristics.

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The resulting graph, known as a cyclic voltammogram, is obtained by plotting the current density against the applied potential. Any electrode response that is occurring will show a peak in the measured current at that voltage. The electrode material, electrolyte content, and sweep rate can all affect a process's peak breadth and height. The function of adsorption, diffusion, and linked homogeneous chemical reaction may be investigated with the use of a cyclic voltammogram ^[11, 12].

In this study, an effort has been made to produce Schiff base by combining sulphapyridine and 2-hydroxy-2-methylpropiophenone, as well as its complex with the Cu (II) ion. Characterization was carried out using an elemental analysis and spectral techniques. Additionally, the antibacterial and electrochemical properties of the ligand and its Cu (II) complex were investigated.

2. Experimental

2.1. Materials and Apparatus

All chemicals were analytical grade and of the highest available purity, and used without further purification. For the preparation of Schiff base ligand and their complexes, 2-hydroxy-2-methylpropiophenone, sulfa pyridine and Cu (II) salts were obtained from Sigma-Aldrich. For the preparation of phosphate buffer and BR buffer,

disodiumhydrogenphosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), acetic acid, phosphoric acid and boric acid were obtained from Sigma- Aldrich. Double distilled water (DDW) and purified solvent were used for cyclic voltammetric studies. Cyclic voltammetric measurements were performed using computerized constant current source provided by Retrace Electronics which is fully digitally controlled. The digital pH meter (Elico) was used to measure the pH of solutions. The infra-red spectra of synthesized compounds were recorded on KBr pellets in the range $4000\text{--}400\text{ cm}^{-1}$ on an FT-IR spectrometer (Schimadzu).

2.2. Preparation of the Schiff base and its Copper (II) complex

2.2.1. Preparation of the Schiff base (HMPSP)

Chemicals of the AR grade were used in this method. In an equimolar ratio, heated methanolic solutions of 2-hydroxy-2-methylpropiophenone and sulphapyridine were combined while being constantly stirred. The resultant mixture was refluxed at 60°C for five to six hours. The reaction mixture's volume was cut in half, or to one-fourth of the original quantity by refluxing. After cooling, the solid bulk separated was filtered off, cleaned with ethanol, and vacuum-dried over anhydrous CaCl_2 ^[13].

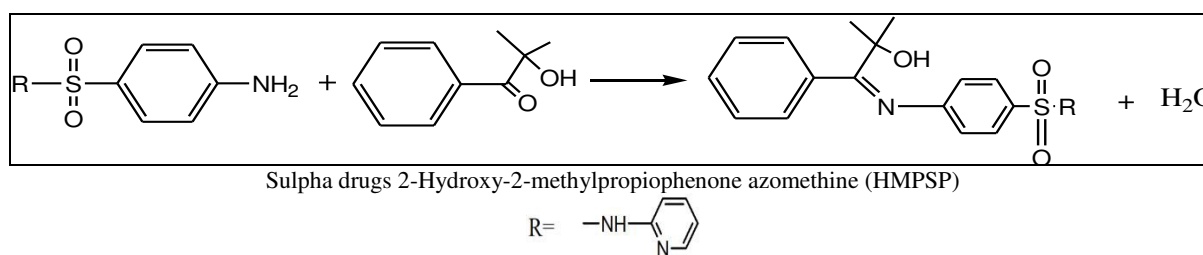


Fig 1: Synthesis reaction of Schiff base ligand

2.2.2. Preparation of the Copper (II) complex

A heated solution of the produced ligand in the methanol was combined with a Methanolic solution of $(\text{CH}_3\text{COO})_2\text{Cu} \cdot \text{H}_2\text{O}$ in a 1:2 molar ratio to produce the copper complex. The resulting reaction mixture was stirred before being heated at 50°C under reflux for three to four

hours. Dark color complex then began to form and were filtered, methanol cleaned, and vacuum-dried over anhydrous calcium chloride. The formation reaction and structure of prepared coordination compounds complex are shown in fig.2.

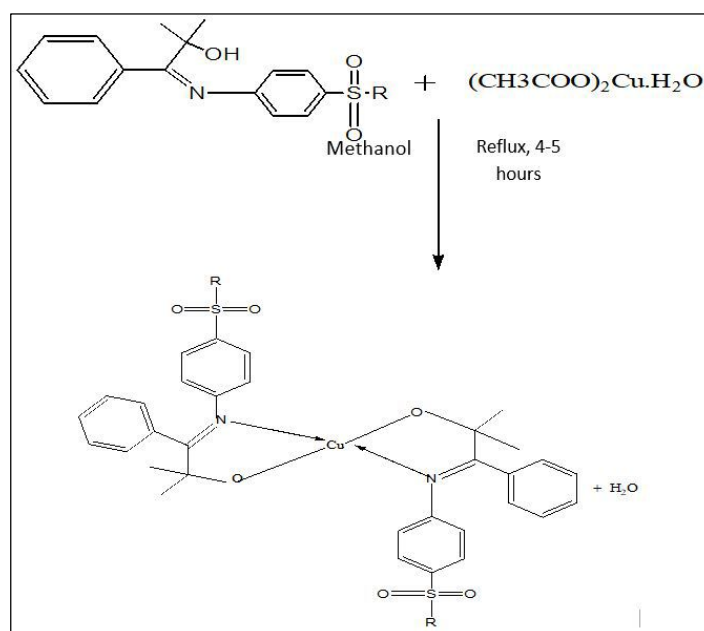


Fig 2: Synthesis reaction of Cu (II) complex of Schiff base ligand

2.3. Cyclic voltammetric Experiments

Cyclic voltammetric measurements were performed using computerized constant current source which is fully digitally controlled. A three electrode system consisting of glassy carbon electrode (GCE) as the working electrode, Ag/AgCl as the reference electrode and platinum wire as the counter electrode was used [14]. In the typical cyclic voltammetric experiment, before recording the current voltage curve, the experimental solution was deoxygenated by purging a stream of nitrogen gas for 10-15 minutes prior to the experiments in order to remove dissolved oxygen from the media. The pH of experimental solutions varied from 5.0 to 8.2 and the scan rate was varied from 50 mVs⁻¹ to 250 mVs⁻¹.

All experiments were investigated at room temperature.

2.4. Antibacterial experiments

The synthesized compounds were tested against two bacterial strains (*Bacillus subtilis*, *Escherichia coli*) and two fungi (*Aspergillus niger*, *Penicillium chrysogenum*) while Ciprofloxacin and Ketoconazole were used as the standard for bacterial and fungal studies, respectively. The agar well diffusion method [15], a common microbiological methodology, was performed for the in-vitro antimicrobial screening.

Calculation the activity index: It was calculated as follows for the plant compounds

$$\text{Activity index (A.I.)} = \frac{\text{Mean of inhibition zone of the compound}}{\text{Inhibition zone obtained for standard antibiotic}}$$

Minimum Inhibitory Concentration (MIC)

This concentration is the lowest amount of an antibiotic that will prevent a microbe from growing visibly following incubation. By diluting a certain amount of the substance to different concentrations, the Macro-broth dilution approach was employed to calculate the value of MIC of the samples (Baron and Finegold, 1990).

3. Results and Discussion

The structure of novel Schiff base was elucidated by

several analytical and physical techniques. The metal (II) complex was formed using the synthesized Schiff base in 1:2 ratios. The obtained analytical and physical results were in good agreement with the suggested composition of Schiff base and its metal (II) complex. The synthesized metal (II) complex is colored, stable in air and insoluble in water, but completely soluble in DMSO and DMF solvents. The physico-chemical data of the complexes are summarized in (Table-1).

Table 1: Micro analytical data of Schiff base and its Cu (II) complex

S. No.	Composition of ligand/complex	Colour & state	Yield (%)	M. Wt. Found (Calcd.)	Elemental Analysis (%): found (Cal)			
					C Found (Calcd.)	H Found (Calcd.)	N Found (Calcd.)	Cu Found (Calcd.)
1	C ₂₁ H ₂₁ N ₃ SO ₃ (HMPSP)	Pale yellow solid	80	395.47 (395.40)	63.72 (63.78)	5.40 (5.35)	10.60 (10.63)	-
2	C ₄₂ H ₄₀ CuN ₆ S ₂ O ₆ (HMPSP-Cu)	Like pulm solid	79	852.40 (852.48)	59.14 (59.18)	4.69 (4.73)	9.82 (9.86)	7.43 (7.45)

3.1 FTIR spectral studies: The infra-red spectra of HMPSP ligand and its Cu (II) complex were recorded on KBr pellets in the range 4000-400 cm⁻¹ on an FT-IR spectrometer (Schimadzu). The binding site of the ligand in metal (II) complexes can be determined a lot by comparing the infrared spectra of the free ligand and its metal (II) complexes (Table-2) lists the selected IR vibrations and approximate assignments for the ligand HMPSP and its metal (II) complex. The infrared spectra of Schiff base showed a prominent absorption band at 3420 cm⁻¹ and a band at 1633 cm⁻¹, which were attributed to the stretching vibrations of ν(O-H) and azomethine (-CR=N-) group respectively [16, 18]. The frequency for (-CR=N) bond decreased by 13-20 cm⁻¹ for all metal complexes, indicating coordination of the nitrogen atom of ligand to M

(II) ion. The broad and strong band due to ν(OH) 3420 cm⁻¹ in ligand spectra, disappear in the complex formation, showing oxygen coordination to metal ion by deprotonation. The appearance of new bands in regions 460-465 cm⁻¹ and 560-570 cm⁻¹ assignable to νM-O and νM-N respectively attributed to the bonding of metal ions to oxygen and nitrogen atoms in Cu(II) [16, 18]. The frequency for the Schiff base because of ν_{asy}(SO₂) and ν_{sy}(SO₂) was observed at 1325-1340 cm⁻¹ and 1145-1128 cm⁻¹, respectively. No change occurs in these bands in the complexes which show no involvement of-SO₂ group for coordination. Water vibration bands were absent in Cu (II) complex spectra indicating that it was not present in the Cu (II) complex [16, 18]. This conclusion was further reinforced by the results of the CHN analysis.

Table 2: IR data of Schiff base and its Cu (II) complex

S.no.	Syn. compound	ν (C=N) (cm ⁻¹)	ν _{asy} (SO ₂) (cm ⁻¹)	ν _{sy} (SO ₂) (cm ⁻¹)	ν(OH)(cm ⁻¹) phenolic	ν (C-O) (cm ⁻¹) phenolic	ν (NH) (cm ⁻¹)	ν (M-O) (cm ⁻¹)	ν (M-N) (cm ⁻¹)
1.	C ₂₁ H ₂₁ N ₃ SO ₃ (HMPSP)	1633	1330	1145	3420	1470	3370	-	-
2.	C ₄₂ H ₄₀ CuN ₆ S ₂ O ₆ (HMPSP-Cu)	1605	1330	1145	-	1445	3360	560	460

3.2 NMR studies

The ^1H NMR spectra were recorded in DMSO using TMS as an internal standard. The ^1H NMR spectra of the HMPSP have expected characteristic signals. The methyl protons show singlet at δ 2.48 (6H, s), hydroxyl group proton at δ 4.78, pyridine ring protons at δ 8.22- 6.62 (4H, m), benzene ring protons at δ 7.60 (5H, m), sulphapyridine benzene ring proton δ 6.46 (2H, m), 7.50 (2H, m) and ($\text{SO}_2\text{-NH-}$) proton at δ 11.10 (1H, s) ^[16, 18]. The hydroxyl proton resonates as a singlet at δ 4.78 ppm in case of ligand and disappears in the Cu (II) complexes which indicate the involvement of hydroxyl group oxygen in the coordination via deprotonation, which has already been shown by IR spectral data. These observations support the following assigned structure of HMPSP as well as Cu (II) complex.

3.3. Electrochemical studies

3.3.1 Electrochemical studies of HPSP ligand

The cyclic voltammetric data were recorded with fully computer controlled electro analytical system, using three electrode cell assemblies with glassy carbon as working electrode. The cyclic voltammograms of above introduced ligand were recorded between +800mV to -1600mV in the scan rate interval of 50- 250 mV/sec, at constant pH and concentration of experimental solution. The electrochemical behavior of HMPSP was recorded in acetones, methanol solvents, using phosphate buffer and BR buffer of different pH values. The cyclic voltammetric data for ligand are summarized in table 3-6 and fig. 3-6 showed the cyclic voltammograms of HMPSP in different conditions.

Table 3: Sweep rate vs voltammetric measurements for 1mM HMPSP in propanone-phosphate buffer at three distinct pH levels (5.0, 7.0, and 8.2).

pH	ν (mV/s)	E_{pc} (mV)	I_{pc} (μA)	$E_p/2$ (mV)	$I_{pc}/\nu^{1/2}$	α_n	$D_{O1}/2$ (cm^2/s)	$k^{\circ}f, h$ (cm/s)
5	50	-878	25.9	-780	3.6628	0.4867	33.048045	4.15×10^{-9}
	100	-905	30.5	-795	3.0500	0.4336	29.155074	1.91×10^{-9}
	150	-918	33.7	-810	2.7515	0.4416	26.062383	1.27×10^{-8}
	200	-927	38.2	-820	2.7011	0.4457	25.465853	1.06×10^{-8}
	250	-943	40.8	-840	2.5804	0.4631	23.868589	4.55×10^{-9}
7	50	-910	28.9	-810	4.0870	0.4770	37.250389	3.57×10^{-9}
	100	-933	31.2	-823	3.1200	0.4336	29.824207	1.21×10^{-8}
	150	-952	39.7	-843	3.2414	0.4376	30.844384	9.67×10^{-9}
	200	-972	44.6	-862	3.1536	0.4336	30.146311	8.99×10^{-9}
	250	-979	46.4	-876	2.9345	0.4631	27.144670	2.70×10^{-9}
8.2	50	-917	33.8	-814	4.7800	0.4631	44.214863	6.02×10^{-9}
	100	-946	42.6	-825	4.2600	0.3942	42.709084	5.69×10^{-8}
	150	-967	48.3	-858	3.9436	0.4376	37.526039	9.12×10^{-9}
	200	-982	50.5	-867	3.5708	0.4147	34.901432	1.77×10^{-8}
	250	-995	53.4	-875	3.3773	0.3975	33.719401	2.96×10^{-8}

Table 4: Sweep rate vs voltammetric measurements for 1m HMPSP in $\text{CH}_3\text{OH-}$ phosphate buffer at three distinct pH levels (5.0, 7.0, and 8.2).

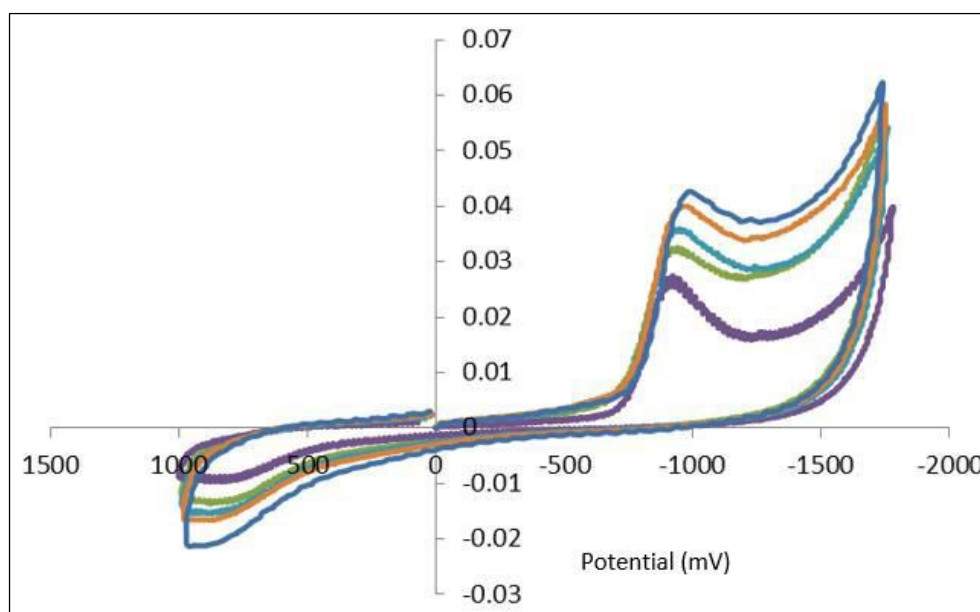
pH	ν (mV/s)	E_{pc} (mV)	I_{pc} (μA)	$E_p/2$ (mV)	$I_{pc}/\nu^{1/2}$	α_n	$D_{O1}/2$ (cm^2/s)	$k^{\circ}f, h$ (cm/s)
5	50	-920	30.1	-790	4.2567	0.3669	44.235521	1.59×10^{-8}
	100	-935	28.29	-812	2.8290	0.3878	28.595881	5.65×10^{-8}
	150	-947	31.6	-828	2.5801	0.4008	25.652689	3.25×10^{-8}
	200	-954	39.9	-845	2.8213	0.4376	26.846589	9.40×10^{-9}
	250	-969	41.9	-834	2.6499	0.3533	28.062663	1.84×10^{-7}
7	50	-937	32.4	-832	4.5820	0.4542	42.792990	5.55×10^{-9}
	100	-953	41.1	-820	4.1100	0.3586	43.200186	1.85×10^{-7}
	150	-970	44.1	-853	3.6007	0.4076	35.497998	2.45×10^{-8}
	200	-983	50.6	-858	3.5779	0.3816	36.459314	6.21×10^{-8}
	250	-1000	45.3	-870	2.8650	0.3669	29.772674	7.64×10^{-8}
8.2	50	-950	42.05	-832	5.9467	0.4042	58.876228	3.65×10^{-8}
	100	-970	44.6	-845	4.4600	0.3816	45.44727	6.64×10^{-8}
	150	-992	40.9	-859	3.3394	0.3586	35.101160	1.07×10^{-7}
	200	-1002	46.9	-850	3.3163	0.3138	37.264698	6.11×10^{-7}
	250	-1020	51.8	-872	3.2761	0.3222	36.325248	3.87×10^{-7}

Table 5: Sweep rate vs voltammetric measurements for 1mM HMPSP in CH₃OH-BR buffer at three distinct pH levels (5.0, 7.0, and 8.2).

pH	N (mV/s)	E _{pc} (mV)	I _{pc} (μA)	E _p /2 (mV)	I _{pc} /v ^{1/2}	a _n	D ₀₁ /2 (cm ² /s)	k ⁰ f,h (cm/s)
5	50	-930	31.6	-830	4.4689	0.4770	40.730529	2.69×10 ⁻⁹
	100	-945	33.2	-835	3.3200	0.4336	31.736015	1.06×10 ⁻⁸
	150	-961	40.6	-840	3.3149	0.3942	33.234646	4.31×10 ⁻⁸
	200	-979	44.5	-845	3.1466	0.3559	33.198265	1.54×10 ⁻⁷
	250	-990	46.6	-850	2.9472	0.3407	31.783221	2.49×10 ⁻⁷
7	50	-947	41.1	-800	5.8124	0.3244	64.229340	7.07×10 ⁻⁷
	100	-956	44.1	-820	4.4100	0.3507	46.873353	2.55×10 ⁻⁷
	150	-983	49.6	-853	4.0498	0.3669	42.084842	1.07×10 ⁻⁷
	200	-1002	55.2	-858	3.9032	0.3312	42.689719	3.64×10 ⁻⁷
	250	-1015	58.8	-867	3.7188	0.3222	41.234065	4.67×10 ⁻⁷
8.2	50	-970	44.5	-848	6.2932	0.3909	63.353834	4.65×10 ⁻⁸
	100	-992	50.6	-840	5.0600	0.3138	56.857831	7.45×10 ⁻⁷
	150	-1015	58.2	-859	4.7520	0.3057	54.095063	8.89×10 ⁻⁷
	200	-1030	62.4	-870	4.4123	0.2981	50.868339	1.08×10 ⁻⁶
	250	-1050	67.5	-860	4.2690	0.2510	53.632562	6.37×10 ⁻⁶

Table 6: Sweep rate vs. voltammetric measurements for 1mMHMPSP in propanone-BR buffer at three distinct pH levels (5, 7, and 8.2)

pH	v(mV/s)	E _{pc} (mV)	I _{pc} (μA)	E _p /2(mV)	I _{pc} /v ^{1/2}	a _n	D ₀₁ /2(cm ² /s)	k ⁰ f,h(cm/s)
5	50	-912	31.6	-815	4.4689	0.4917	40.114919	2.23×10 ⁻⁹
	100	-920	38.1	-822	3.8100	0.4867	34.376051	2.76×10 ⁻⁹
	150	-934	40.2	-850	3.2823	0.5678	27.418143	1.17×10 ⁻⁹
	200	-940	47.5	-840	3.3587	0.4770	30.612344	3.36×10 ⁻¹⁰
	250	-962	49.2	-855	3.1116	0.4457	29.336274	7.44×10 ⁻⁹
7	50	-908	33.7	-820	4.7659	0.5420	40.747804	4.33×10 ⁻¹⁰
	100	-930	37.9	-850	3.7900	0.5962	30.895994	4.30×10 ⁻¹¹
	150	-941	42.6	-823	3.4782	0.4042	34.436812	4.25×10 ⁻⁸
	200	-946	45.3	-855	3.2031	0.5241	27.849787	5.04×10 ⁻¹⁰
	250	-970	48.3	-857	3.0547	0.4221	29.596089	1.55×10 ⁻⁸
8.2	50	-923	34.02	-806	4.8111	0.4076	47.430774	3.98×10 ⁻⁸
	100	-939	41.1	-813	4.1100	0.3785	42.047973	1.08×10 ⁻⁷
	150	-948	40.8	-859	3.3313	0.5359	28.643594	2.82×10 ⁻¹⁰
	200	-956	44.0	-855	3.1112	0.4722	28.498128	2.76×10 ⁻⁹
	250	-973	51.5	-850	3.2571	0.3878	32.923633	5.79×10 ⁻⁸



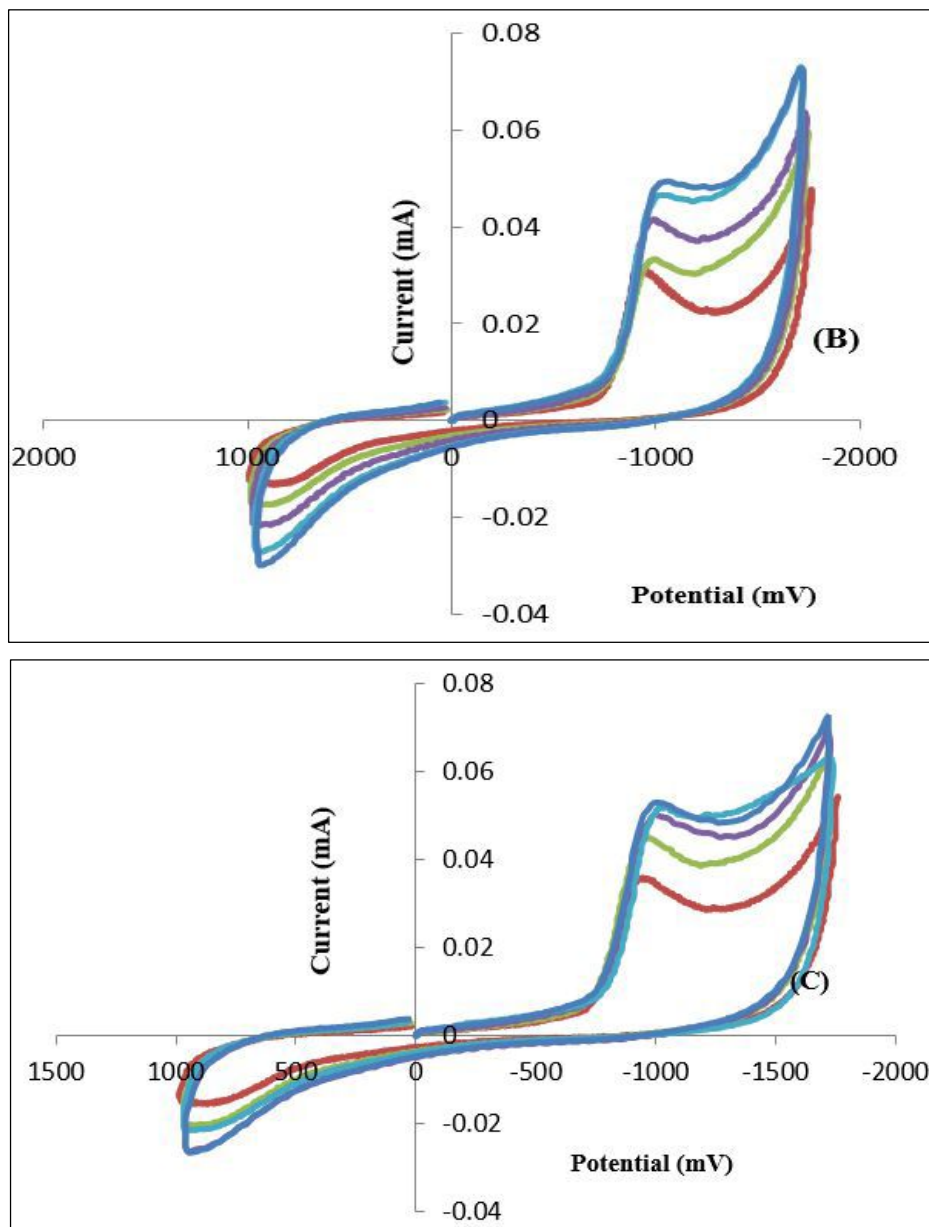
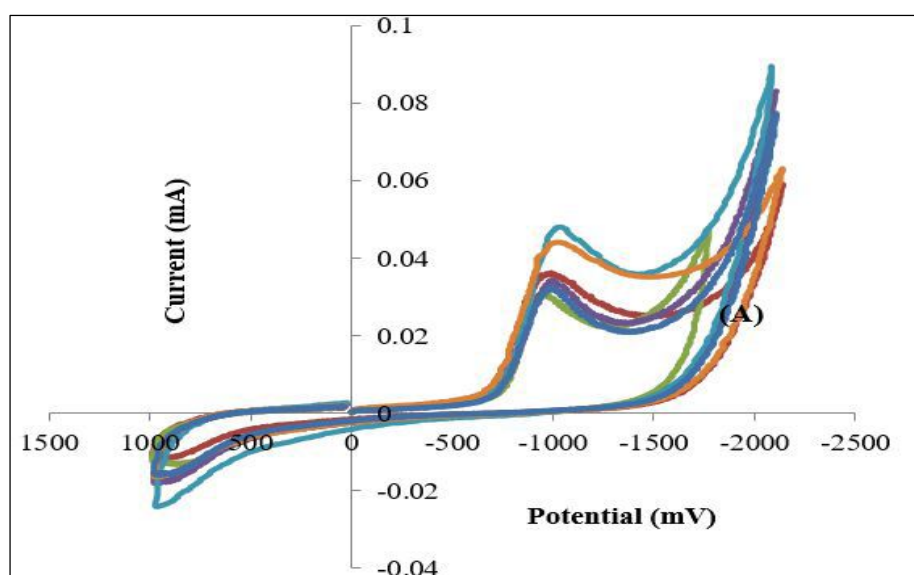


Fig 3: Cyclic voltammograms of HMPSP propanone - phosphate buffer at three various pH values 5.0 (A), 7.0 (B) and 8.2(C).



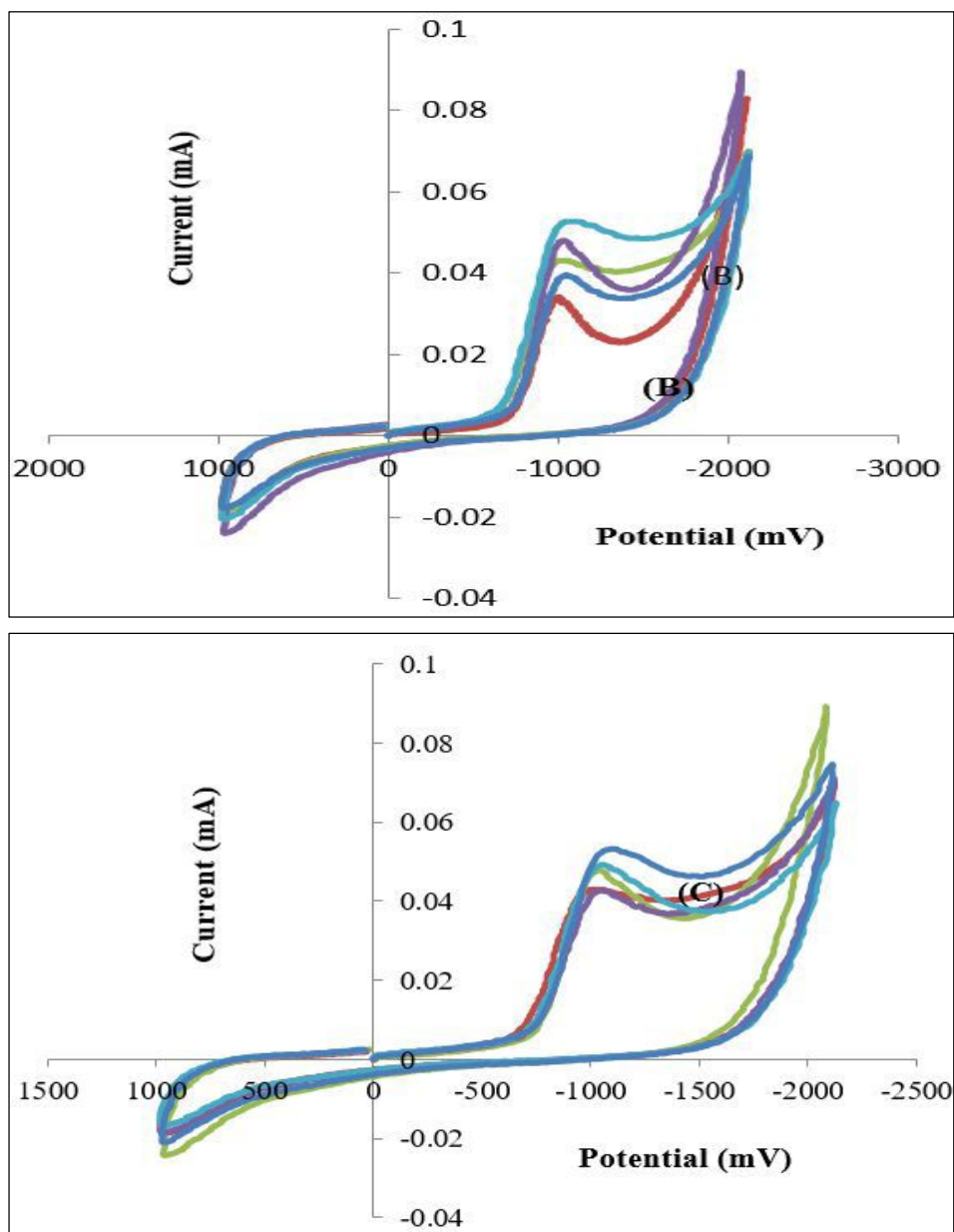
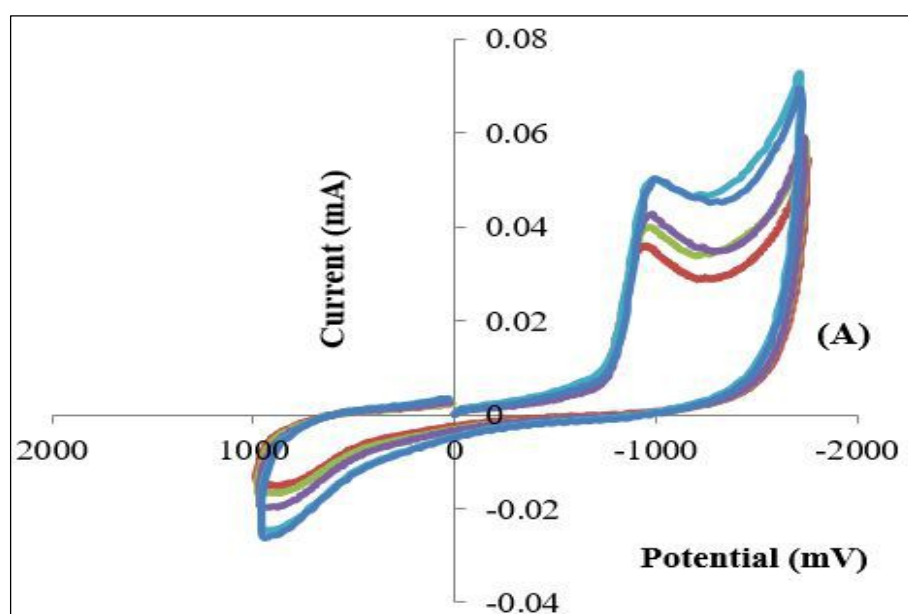


Figure 4: Cyclic voltammograms of HMPSP in CH_3OH - phosphate buffer at three various pH values 5.0 (A), 7.0 (B) and 8.2(C).



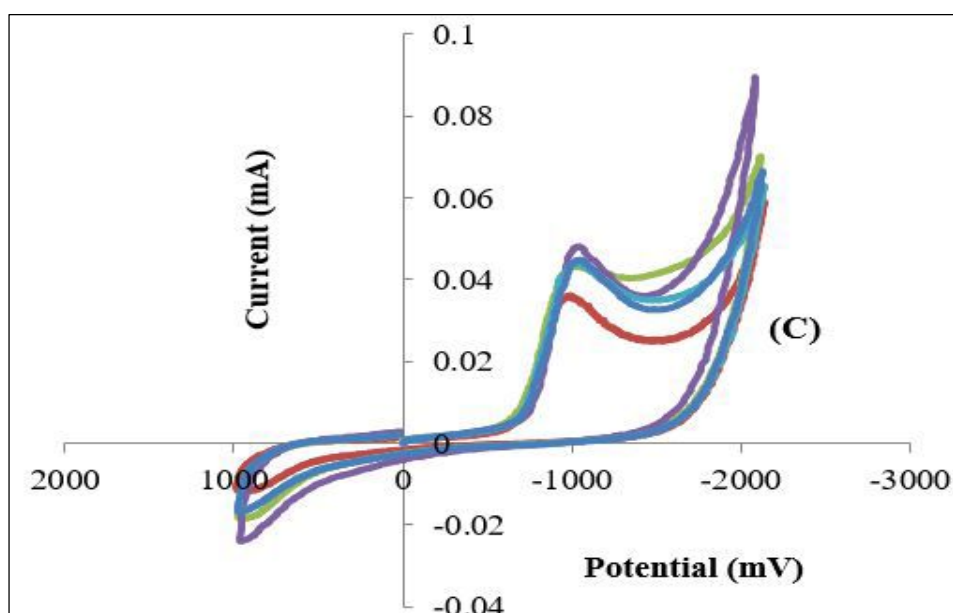
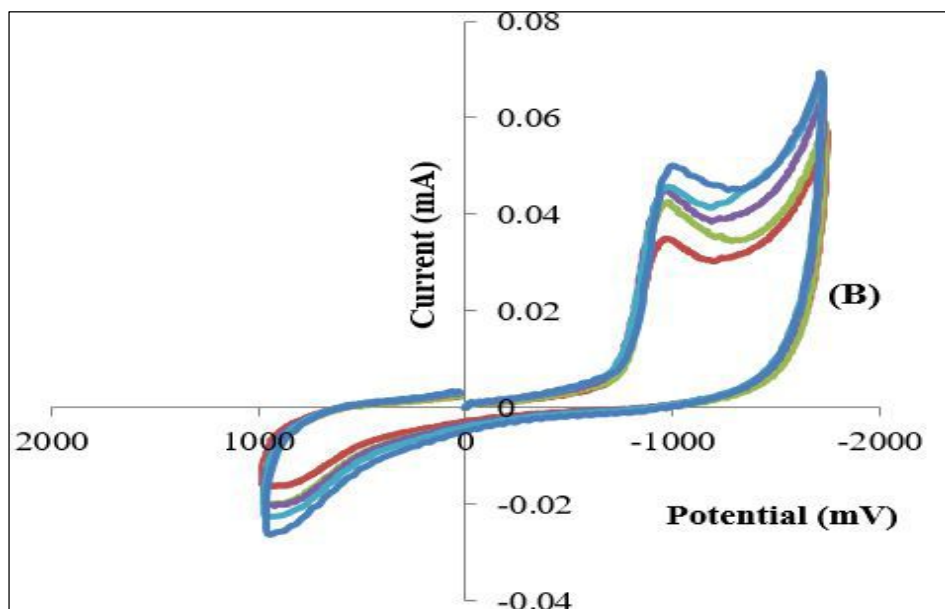
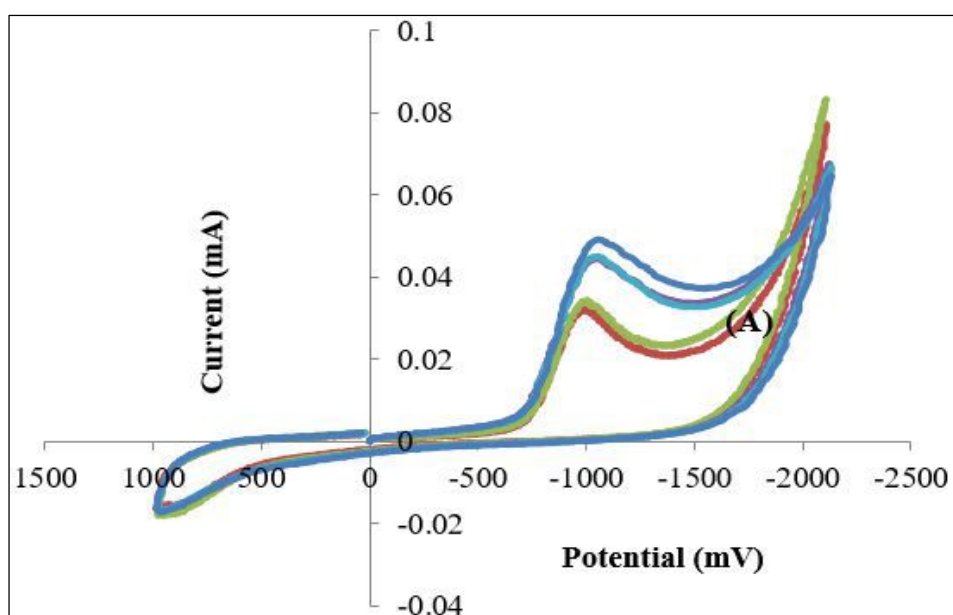


Fig 5: Cyclic voltammograms of HMPSP in propanone - BR buffer at three various pH values 5.0 (A), 7.0 (B) and 8.2(C).



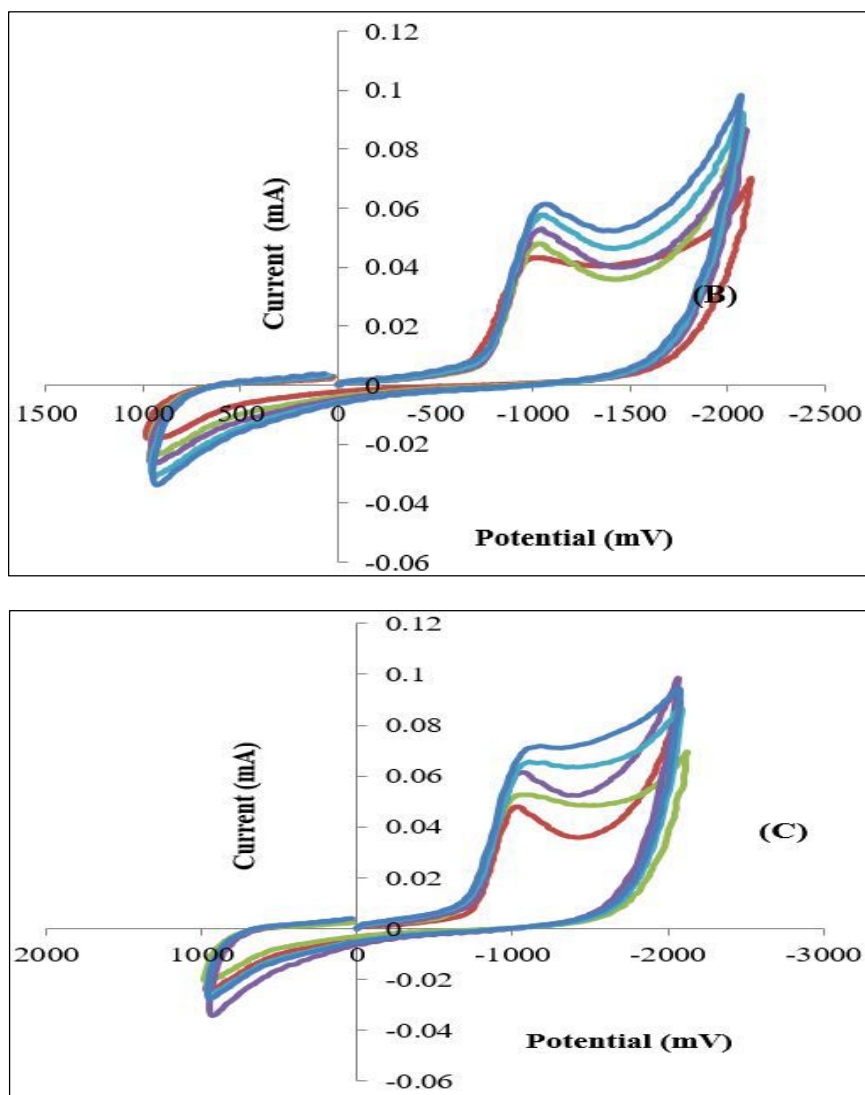


Fig 6: Cyclic voltammograms of HMPSP in CH₃OH - BR buffer at three various pH values 5.0 (A), 7.0 (B) and 8.2(C)

In all the cyclic voltammograms HMPSP displays only one irreversible cathodic wave. In the anodic side of the reverse scan, no peak could be seen, suggesting irreversible nature of the electrodes process¹⁹. In all the cyclic voltammograms the current function ($I_{pc}/v^{1/2}$) has

been found to be fairly constant with respect to scan rate showing that the electrode process is diffusion controlled. The various kinetic parameters assessed with the help of CV graphs, are reported in the tables

Table 7: Effect of variables on cyclic voltammograms parameters of HMPSP ligand

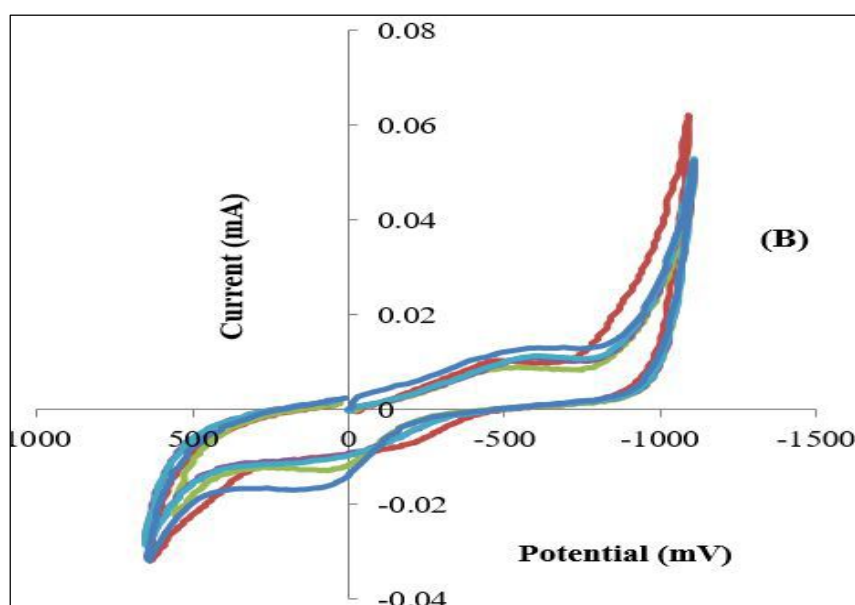
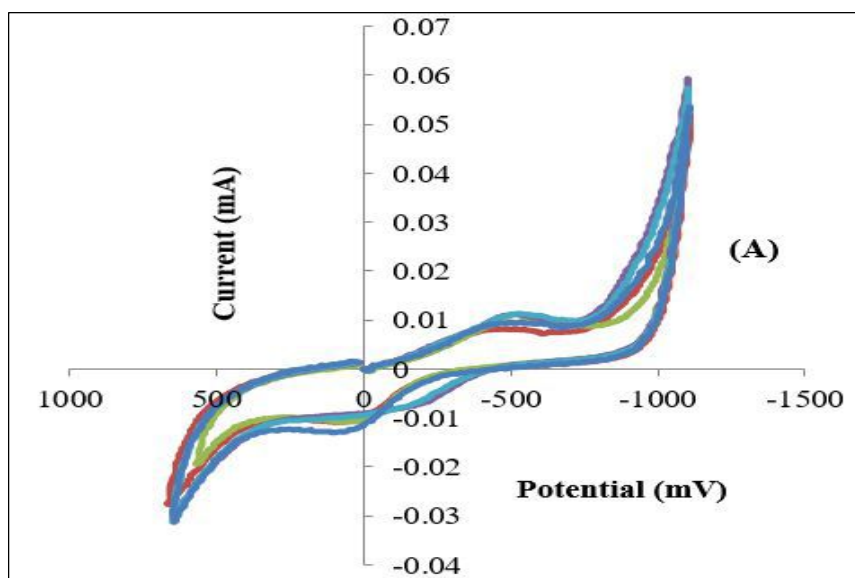
S. no.	Effect of variables	Experiment	Observation	Results
1	Effect of Scan rate	A series of cyclic voltammograms of HMPSP were recorded by varying the scan rate from 50,100,150,200,250(mV/s)	It is found that the cathodic peak potential (E_{pc}) and peak current (I_{pc}) increases linearly and swept to more negative value with the increasing scan rate. (shown in tables 3 to 6)	It is showing that the electrode process is diffusion controlled and irreversible [20, 22]
2	Effect of Solvent	Cyclic voltammograms of HMPSP were recorded in acetone and methanol solvents with phosphate buffer and BR buffer at pH 5.0, 7.0 and 8.2.	It showed that the negative peak potential value is maximum in methanol solvent and minimum in acetone solvent (shown in tables 3 to 6)	The electrochemical reduction is more facial in more polar solvent [20, 22].
3	Effect of pH	The pH effect on electrochemical behavior of HMPSP was also investigated at pH 5, 7 and 8.2.	The CV graphs shows that the cathodic peak shifts towards more negative potential direction with increasing pH value (shown in tables 3 to 6)	This indicates participation of proton during electrode process [20, 22].
4	Effect of buffer medium,	Cyclic voltammograms of HMPSP were recorded in phosphate buffer and Britton-Robinson buffer (BR) with acetone solvent at various sweep rates	These CV graphs show that cathodic peak potential swept towards high negative value in BR buffer solution compare to phosphate buffer (shown in tables 3 to 6)	This indicates that the polarity of buffer solution affected electrochemical behavior of redox species [20, 22]

3.3.2 Electrochemical studies of Cu(II) complex of HMPSP ligand: The electrochemical behavior of Cu (II) complex has been investigated by cyclic voltammetric technique in DMF solvent containing NaClO₄ as supporting electrolyte with different scan rates varying

from 100, 150, 200, 250, 300 mVs⁻¹. The cyclic voltammetric data for ligand are summarized in table 8 and fig.7 showed the cyclic voltammograms of Cu (II) complex in different concentrations.

Table 8: Influence of concentrations and sweep rate on cyclic voltammogram parameters of Cu (II) complex of 2-hydroxy-2-methylpropionophenone sulphapyridine (HMPSP) in DMF solvent contains NaClO₄ as supporting electrolyte.

Complex con.	ν	E_{pc}	$E_{p,a}$	δE_p	$E_{1/2}$	I_{pc}	I_{pa}	I_{pa}/I_{pc}	$I_{p,c}/\nu^{1/2}$
1mM	100	-380	-90	290	-210	0.0073	0.0064	0.876712	0.000730
	150	-420	-80	340	-230	0.0087	0.0070	0.804598	0.000710
	200	-440	-90	350	-245	0.0110	0.0082	0.745455	0.000707
	250	-445	-80	365	-250	0.0099	0.0080	0.808081	0.000626
	300	-450	-70	380	-260	0.012	0.0080	0.666667	0.000693
2mM	100	-420	-110	310	-220	0.0095	0.0065	0.684211	0.000950
	150	-450	-90	360	-230	0.011	0.0078	0.709091	0.000898
	200	-500	-80	420	-275	0.0098	0.0082	0.836735	0.000693
	250	-520	-60	460	-290	0.0108	0.0072	0.666667	0.000683
	300	-530	-50	480	-310	0.0131	0.0092	0.70229	0.000756
3mM	100	-440	-100	340	-346	0.0069	0.0052	0.764706	0.000680
	150	-460	-80	380	-230	0.0101	0.0085	0.841584	0.000825
	200	-520	-90	430	-245	0.0125	0.0112	0.751678	0.001054
	250	-540	-80	460	-260	0.0129	0.0090	0.708661	0.000803
	300	-560	-70	490	-280	0.0144	0.0114	0.791667	0.000831



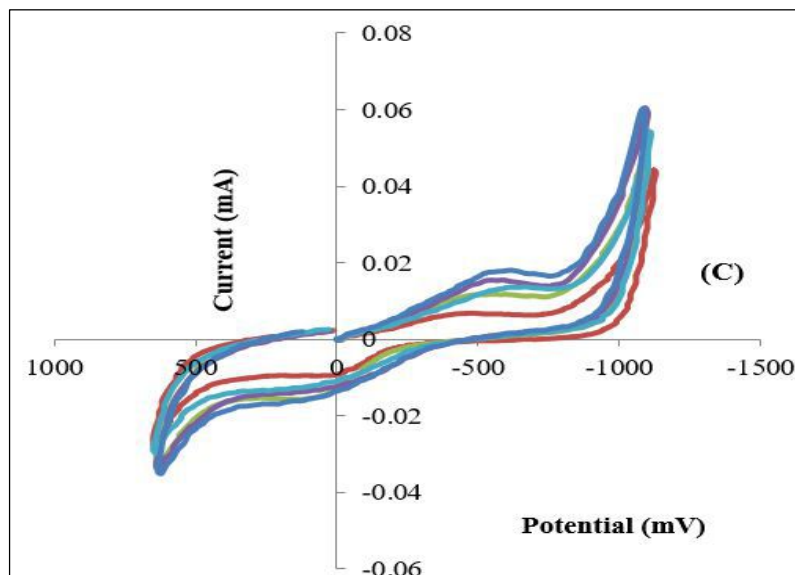


Fig 7: Cyclic voltammograms of Cu (II) complex of HMPSP in DMF -NaClO₄ at three various concentrations values 1mM (A), 2mM(B) and 3mM(C)

The CV of complex shows a quasi-reversible peak corresponding to the formation of Cu (II)/Cu (I) couple at given potential. The ratio between the anodic and cathodic peak current is less than unity ($I_{pa}/I_{pc} < 1$) corresponding to a simple one electron transfer process.²³ A linear

dependence between cathodic peak current (I_{pc}) and the square of the scan rates ($v^{1/2}$) have been also observed. This fact implies that these electrochemical process are mainly diffusion controlled.

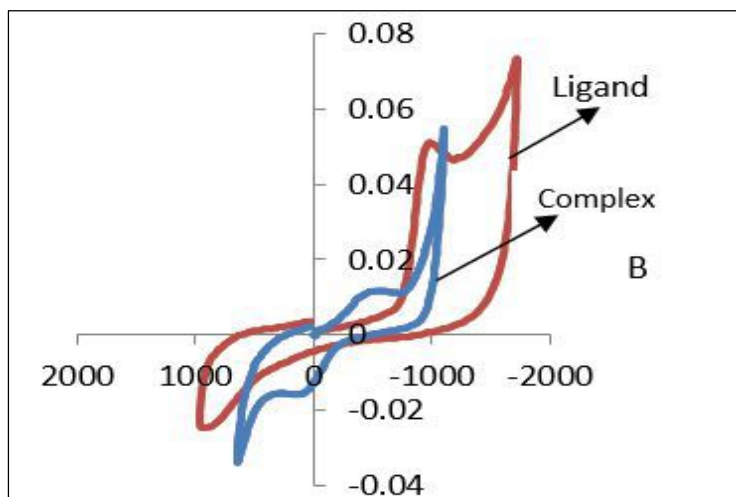
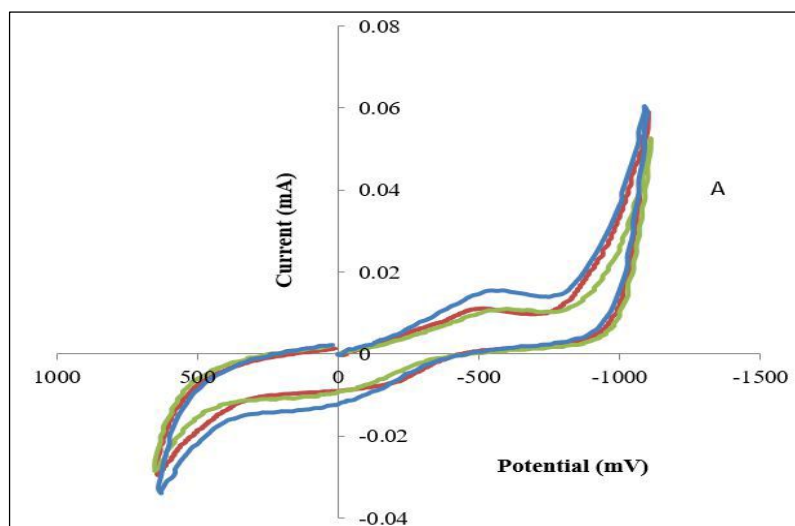


Fig 8: [A] Cyclic voltammograms of Cu (II) Complex of HMPSP 1mM-3mM conc. in DMF-NaClO₄ at 200 scan rate.[B] Cyclic voltammograms of Cu (II) complex and its ligand indicating that the redox process is assigned to the copper centre only.

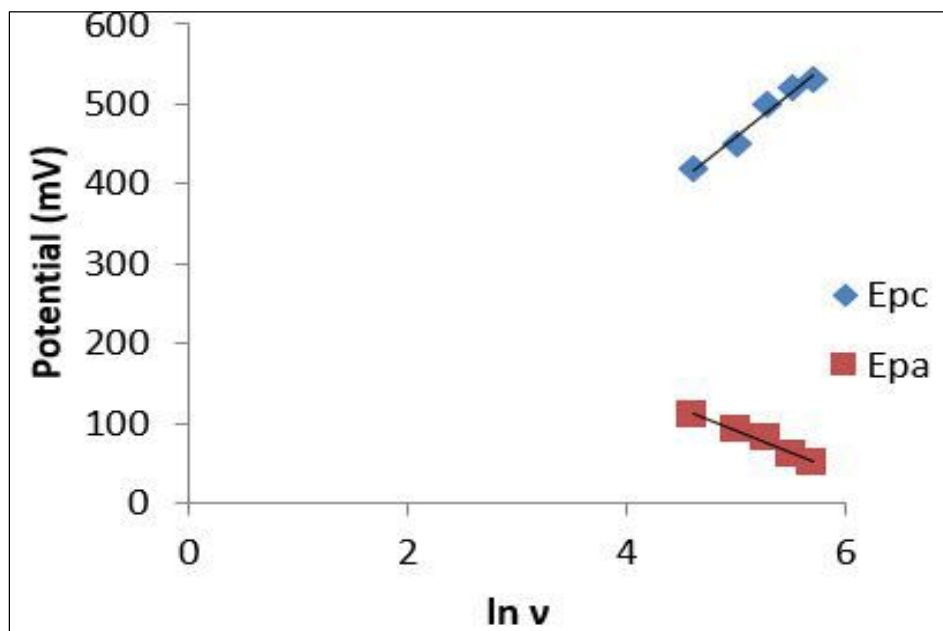


Fig 9: Representation of linear relationship of the E_{pc} & E_{pa} with $\ln v$.

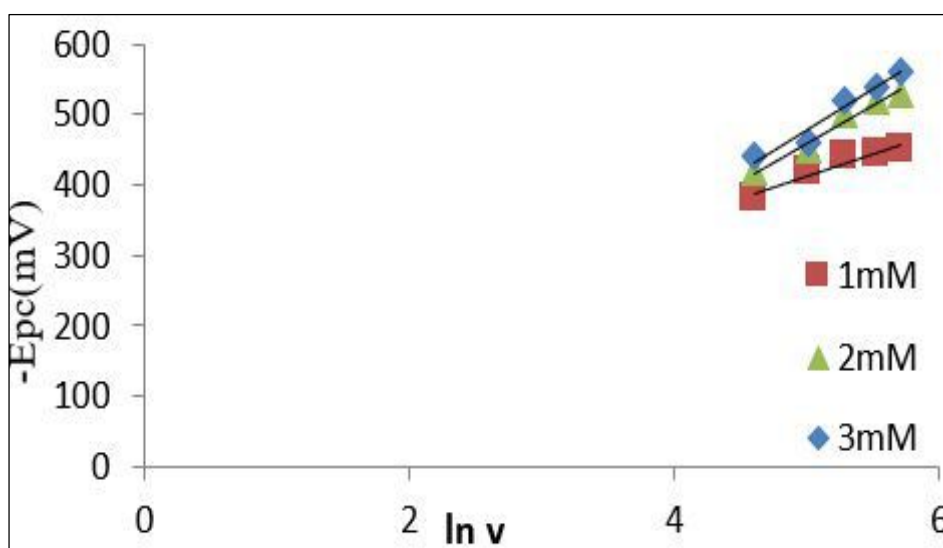


Fig 10: A graphical representation E_{pc} vs $\ln v$ for Cu (II) complex

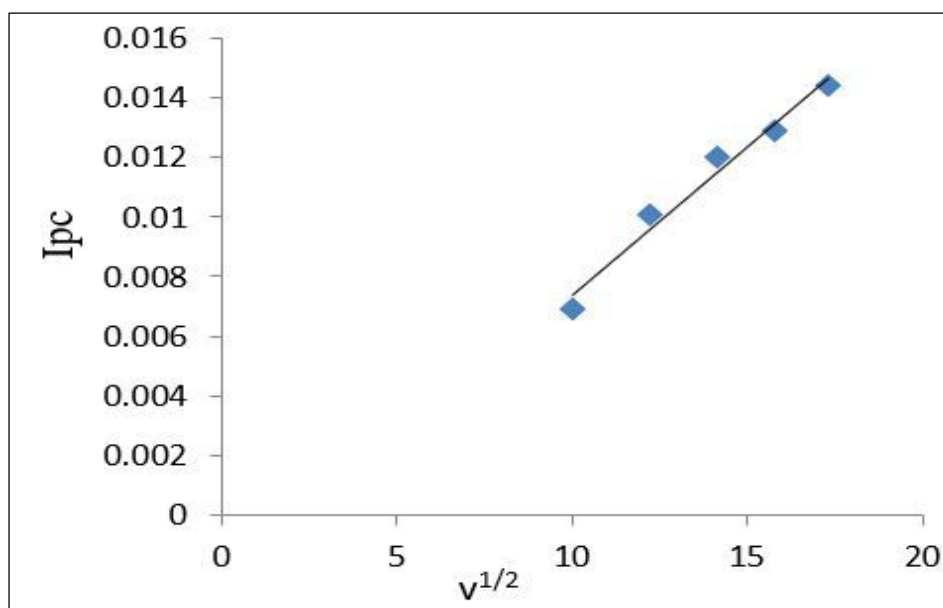


Fig. 11: A graphical representation I_{pc} vs $v^{1/2}$ for Cu (II) complex at 3mM concentration

3.4 Microbial studies

3.4.1 Antimicrobial activity of the HMPSP ligand and its Cu (II) complex

The Schiff bases ligand and its Cu complex were tested against two bacterial strains (*Bacillus subtilis*, *Escherichia coli*) and two fungi (*Aspergillus niger*, *Penicillium chrysogenum*) while Ciprofloxacin and Ketoconazole were

used as the standard for bacterial and fungal studies, respectively. The Agar Well Diffusion method, a common microbiological methodology, was performed for the in-vitro antimicrobial screening. In order to create solutions with varying concentrations of synthesized compounds as shown in tables.

Table 9: Antibacterial screening data for the HMPSP and its Cu (II) complex with respect to antibacterial standard drug ciprofloxacin (1mg/ml concentration)

Conc. of ligand Or complex $\mu\text{g/ml}$	<i>Bacillus subtilis</i>								<i>Escherichia coli</i>							
	HMPSP				Cu (II) complex				HMPSP				Cu (II) complex			
	IZ of S	IZ (MM)	AI	MIC	IZ Of S	IZ	AI	MIC	IZ Of S	IZ (mm)	AI	MIC	IZ Of S	IZ (mm)	AI	MIC
30	22	6	.27	30	21	8	.38	20	30	NA	NA	40	30	NA	NA	40
60		9	.40			10	.47			6	.20			7	.23	
90		13	.59			19	.90			14	.46			15	.43	

Table 10: Antifungal screening data for the HMPSP and its Cu (II) complex with respect to antifungal standard drug ketokenazole (1mg/ml concentration)

Conc. of ligand or complex $\mu\text{g/ml}$	<i>Aspergillus Niger</i>								<i>Penicillium Chrysogenum</i>							
	HMPSP				Cu (II) complex				HMPSP				Cu (II) complex			
	IZ Of S	IZ (MM)	AI	MIC	IZ Of S	IZ	AI	MIC	IZ Of S	IZ (mm)	AI	MIC	IZ Of S	IZ (mm)	AI	MIC
30	30	NA	NA	40	31	8	.25	30	13	NA	NA	90	11	NA	NA	60

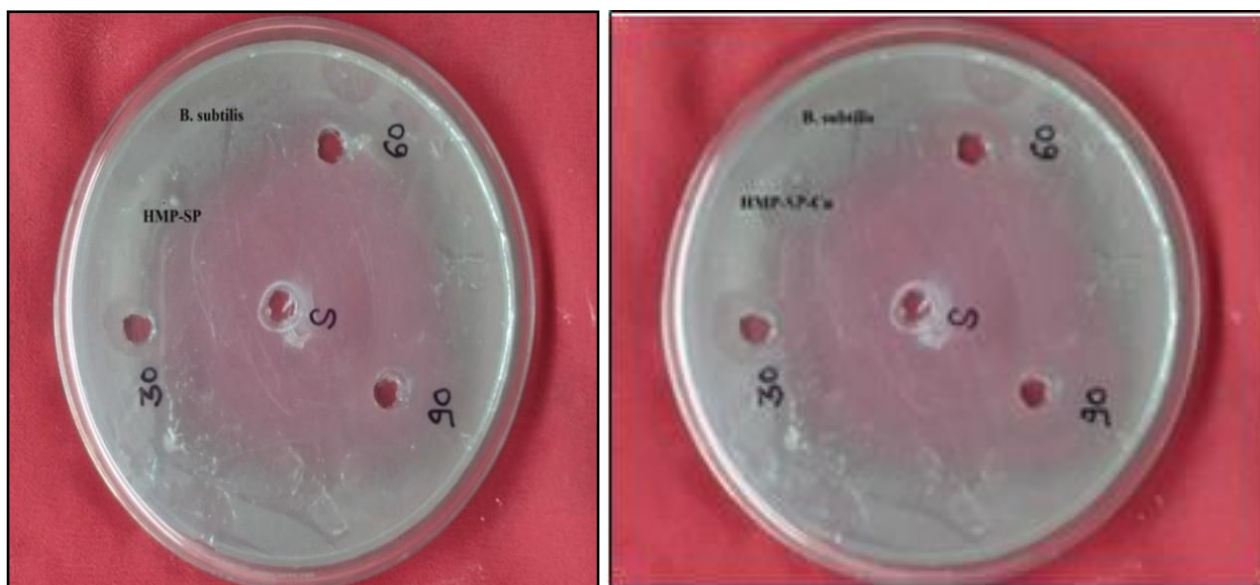


Fig 10: Antibacterial screening effects of the HMPSP and its Cu (II) complex against *Bacillus subtilis*

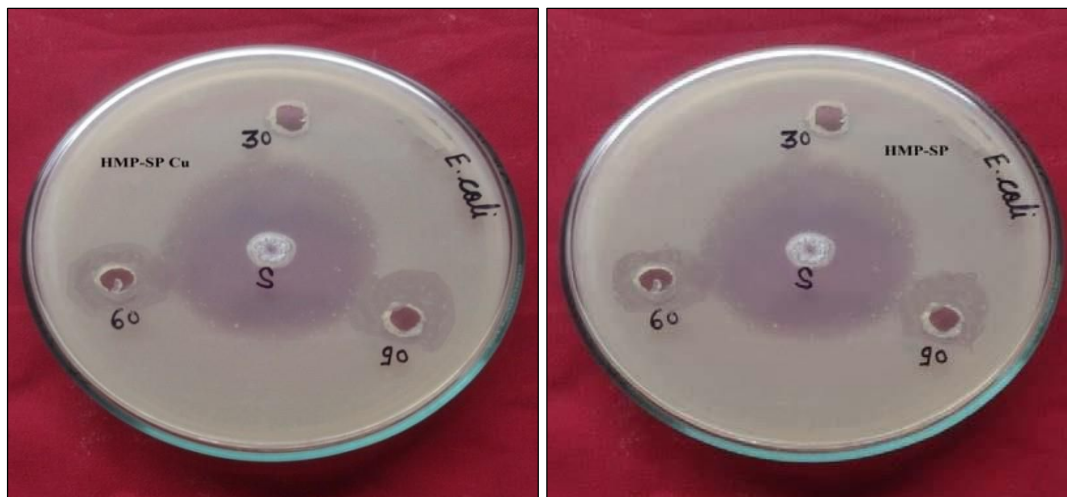


Fig. 11: Antibacterial screening effects of HMPSP and its Cu (II) complex against *E. coli*

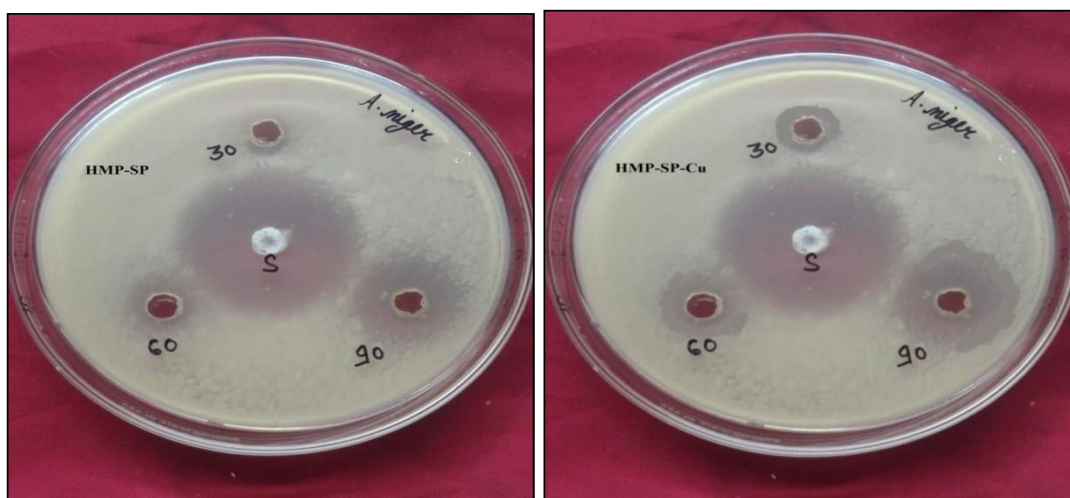


Fig.12: Antifungal screening effects of the HMPSP and its Cu (II) complex against *A. niger*

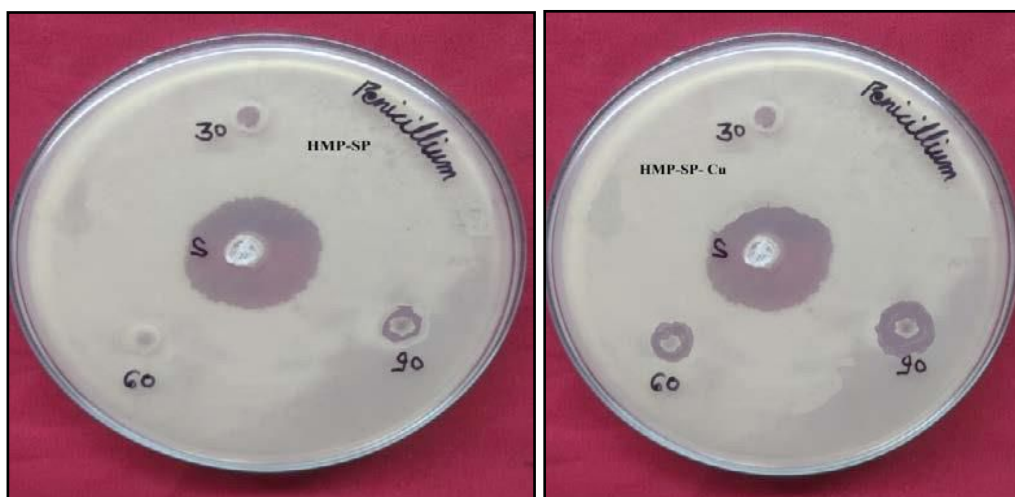


Fig 13: Antifungal screening effects of the HMPSP and its Cu (II) complex against *P. chrysogenum*

The results revealed that the synthesized compounds exhibited various levels of inhibition. It was found that the Cu (II) complex shows less MIC value compared to HMPSP ligand reveals that Cu (II) complex have greater activity against tested organisms compared to HMPSP ligands as shown in histogram. This enhancement in the activity of the metal complexes can be explained on the basis of the

chelation theory [24, 25]. Chelation reduces the polarity of metal ions to a greater extent so the complex becomes more lipophilic, which is an essential element in governing antibacterial action. High lipophilicity increases the ability of complexes to penetrate lipid membranes, which in turn prevents protein synthesis and restricts organism development

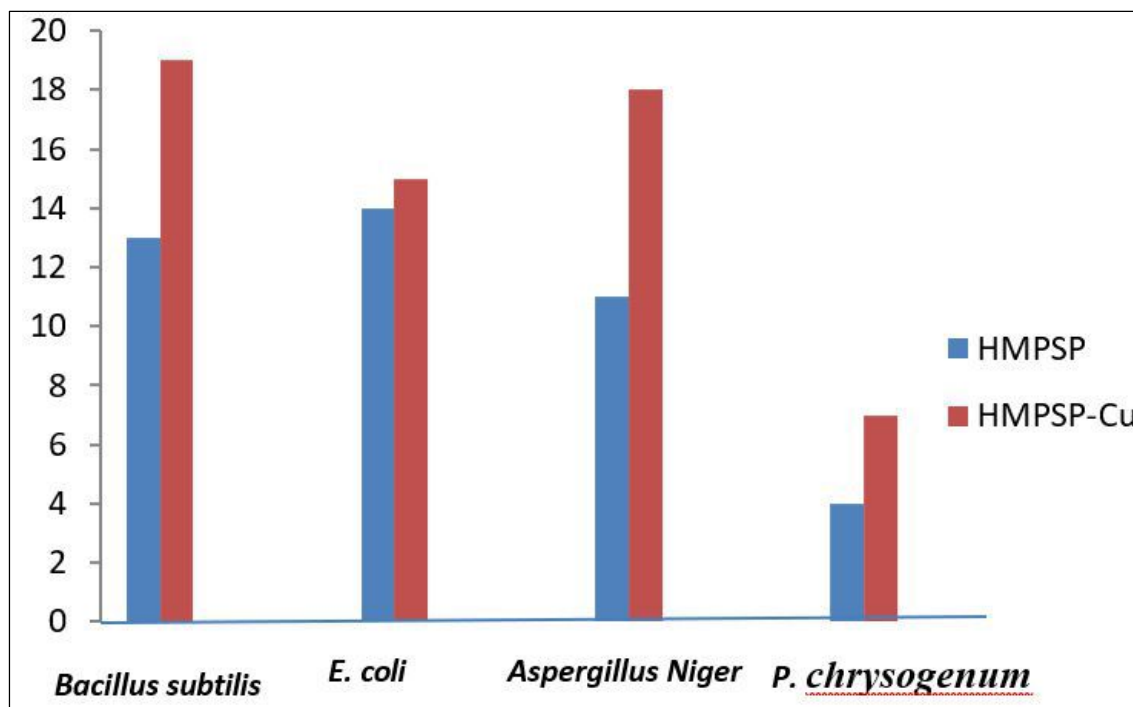


Fig 14: Comparison of the diameters of Zone of the Inhibition (ZI) between the ligand and its Cu complex at 90 µg/ml concentration

Conclusion

We have prepared and characterized a novel bidentate Schiff base ligand HMPSP (L) which coordinates easily to Cu (II) ion to form square planar CuL_2 complex. The electrochemical data reveal that the electrochemical reduction of HMPSP ligand is irreversible and diffusion controlled in nature under the pH 5 to 8.2 and scan rate range from 50 mV/s to 250 mV/s. The effect of solvents, buffers, sweep rates and pH were also studied on peak potential and peak current. It was also found that redox behavior of Cu (II)/Cu (I) is quasireversible in DMF solvent containing NaClO_4 as supporting electrolyte with different scan rates varying from 100, 150, 200, 250, 300 mVs⁻¹. Antimicrobial studies show that the complexes were more active than the free ligand

Conflict of interest

The authors declare no conflict of interest for this manuscript.

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