EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 2
198-204
Year: 2021

SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL ACTIVITY OF 4-(1-HYDROXY-2-METHYLPROPAN-2-YLIMINO)METHYL)-2,6-DIMETHXYPHENOL

J. Sreeramulu[1], Nadipineni Nagaraju[1*], P. Malleswarareddy[2] G. Ramamohan[2] and M. Rafiyuddin[3]

Department of Chemistry, S.K. University College, S.K.University, Ananthapuramu, Pincode 515003.

***Corresponding Author: Nadipineni Nagaraju**
Department of Chemistry, S.K. University College, S.K.University, Ananthapuramu, Pincode 515003.
DOI: https://doi.org/10.17605/OSF.IO/SG94X

Article Received on 09/12/2020 **Article Revised on 29/12/2020** **Article Accepted on 19/01/2021**

ABSTRACT

A new organic Schiff base compound [4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6, Dimethxy Phenol] was synthesized by condensation of 2-amino-2-methyl-1-propanol with 4-hydroxy-2.6- di methoxy benzaldehyde and characterised by elemental analysis, electronic, Infra-red spectra, polarography and coulometry. The electrochemical studies of Schiff base in presence of cadmium as reference standard were carried out in Briton-Robinson buffer solutions of pH 2.1 – 10.1. Effect of Mercury column height was carried out at pH 4.10. Kinetic parameters of redox behavior were carried out at pH 2.10-10.10. Millicoulometric analysis was carried out to know the number of electrons involved in the redox behavior of different functional groups from this study the author has calculated an important parameter $K^0_{f,h}$ (in cm sec^{-1}) at P^H 2.1 4.1 6.1 8.1 10.1 as $5.18x10^{-4}$, $2.76x10^{-4}$, $9.97x10^{-5}$ $10.4x10^{-5}$,$10.4x10^{-5}$ respectively. Further the free energy change at different P^H values was calculated. The details of results are well presented in the present research investigation. The test Schiff base 4-((1-hydroxy-2-methylpropan-2-ylimino)methyl)-2,6-dimethxyphenol shows significant antibacterial activity against pathogenic bacterial strains (*Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus*) causes infectious diseases in humans.

KEYWORDS: 2-Amino-2-Methyl-1-Propanol, antibacterial activity, Kinetic parameters and coulometry.

1.0. INTRODUCTION

Organic compounds of varied nature are widely employed as analytical reagents. Schiff bases of aldehydes and ketones occupy an important place amongst other organic compounds like oximes, semicarbazones and thiosemicarbazones as complexing agents. The complex forming ability of schiff bases is attributed to the presence of basic site i.e., >C=N-. The more ease, with which it donates lone pair of electrons, more will be the stability of the complex. This depends on the presence of substituent groups in the compound. Schiff bases are not only used in inorganic analysis but they also find importance in many fields. Schiff bases and their metal complexes have been used as anti-inflammatory, analgesic, anticancer, tuberculostatic, antiviral, anticataract, fungicidal, pesticidal, bactericidal, insecticidal, herebicidal and growth regulating agents. Schiff bases derived from 2,4- dinitro phenyl ether of vanillin exhibited significant fungicidal activity against all the test fungi. Besides Schiff bases of vanillin, the fungicidal activity was also exhibited by chloronitro benzenes,[1] halogeno nitro derivatives of naphthalene.[2] Literature survey revealed that Schiff bases are found to posses pronounced biological activities their ease of synthesis and myriad properties have contributed greatly to their popularity and to the study of many biological systems. They also presented the Polarographic evidence for Cadmium Schiff base complexes with ST, BT and also with Vanillin Tris (VT)[3] The same authors while studying the complexation of cadmium ion with ST established a new method for the determination of ligand-proton stability constant by Polarography method.[4] The stability constants and molar absorptivities of complexes of copper (II) with 2-Amino-2-Mehty1-Propanol (AMP) in aqueous medium were determined employing spectrophotometric method.[5] even the available few references on metal complexes of AMP not emphasized the applications of those complexes in biological fields. Synthesis, Structural, Magnetic and Spectral properties of alkanolamine complexes of Pt,W ,Ni,Ag,Au and Zn have been reported.[,6,7,8,9,10,11,12] In addition to Tris, AMP is one of the important compounds of unique series of alkanolamines. Literature survey reveals that significant Alkanolamine such as Tris has also been employed extensively as a complexing agent in polarographic analysis.[13]

2.0. EXPERIMENTAL

2.1. Synthesis of 4-((1-hydroxy-2- methyl propan-2-ylimino) methyl)-2,6-dimethoxyphenol

0.05moles of 4-hydroxy-2.6- di methoxy benzaldehyde was added to a mixture of 50ml of methanol 45 ml and 2-Amino-2-methyl-1-propanol 5ml; (0.05mole) and 50ml of distilled water. The reaction mixture was taken in a clean 250ml round bottom flask and stirred well with a magnetic stirrer. Then it was refluxed for 8 hours. A Buff colored product was formed. It was separated by filtration and washed several times with hot water and methanol and dried in vacuum. This compound was recrystalized from methanol. The percentage of yield was 65% and melting point of the compound was 80-84^{0}C. The structure of ligand was established by IR, UV and elemental analysis.

2.2.0. Electrochemical analysis

2.2.1. Cyclic voltametric analysis

The cyclic voltammetric CH experiments Schiff base was conducted at the Silver electrode in Britton - Robinson buffer solutions of P^H values 4.1,and, 8.1 at different scan rates viz. 10mvs^{-1}, 20 mvs^{-1}, 50 mv S^{-1}, 100 mv S^{-1}, 200 mVS^{-1}, and 500 m VS^{-1}. It was noticed from the Table cathodic peak was noticed in all sweep rates in the solutions of pH 4.1 – 8.1. Peak potentials and peak currents change with change in the scan rates. The voltommograms of Schiff base typical pH values 4.1 and 8.1 at 100 mVS^{-1} scan rate.

2.2.2. Polarographic analysis

Recrystallised Schiff base is not freely soluble in water, 40% V/V DMF-water medium was maintained uniformly throughout the experiment. The electrochemical studies of Schiff base were carried out ,In Briton-Robinson buffer solutions of pH 2.1 – 10.1.Effect of Mercury column height was carried out at pH 4.10. Kinetic parameters of redox behavior were carried out at pH 2.10-10.10. Millicoulometric analysis was carried out for I to know the number of electrons involved in the redox behaviour of different functional groups present in Schiff base. The ionic strength was kept constant at 0.1M by using potassium nitrate.

0.002% Triton-X-100 was used as maximum suppressor. Double distilled mercury was employed for dropping mercury electrode. Purified mercury was employed for deoxygenation of all test solutions prior to recording. All the solutions were prepared in double distilled water. An ELICO DC recording polarograph model CL-357 (Digital) was used for recording the polarograms. Saturated calomel electrode (SCE) was used as reference electrode.

An ELICO glass capillary having the following characteristics m = 1.7434 mg sec^{-1}, t = 4.00 sec at constant height of mercury head 67.0 cm (in 0.1M KNO_3 in open circuit) was used. The pH measurements of the solutions were recorded with an ELICO digital pH meter model (LI 120) and pH adjustments were made using 0.1 M HNO_3 and 0.1 M NaOH. Solutions were taken in the H-type cell of Lingane and Laitimer for recording polarograms. Toshniwal Thermostat type GL-15 was used to keep the temperature at 30$\pm$0.1^{0}C.[14]

2.3.0. Antimicrobial Activity By Kirby - Bauer Disc Diffusion Method

The Kirby-Bauer disk diffusion susceptibility test is widely used method to determine the sensitivity or resistance of pathogenic bacteria to various antimicrobial compounds. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

2.3.1. Method

2.3.1.a. Preparation of filter paper discs

Whatman's no.1 filter paper discs of 6 mm diameter were prepared and autoclaved by keeping in a clean and dry petriplate. The filter paper discs were saturated with 20-25 µl of different concentrations of stock solution prepared in DMSO solution. Later, the filter paper discs were carefully dried on the laminar airflow chamber and used for antibacterial studies.

Microorganisms used

The following microorganisms (human pathogenic) were used as test organisms for antimicrobial activity of the test sample. The organisms listed were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Gram – Positive strains

1. Bacillus cereus (MTCC – 1272) Gram + ve bacterium
2. Staphylococcus aureus (MTCC – 7443) Gram + ve bacterium

Gram – negative strains

1. Escherichia coli (MTCC – 1668) Gram – ve bacterium
2. Klebsiella pneumoniae (MTCC – 7028) Gram – ve bacterium
3. Salmonella typhi (MTCC – 3224) Gram – ve bacterium

2.3.1. b. Culture media

Mueller-Hinton agar is considered the best medium used for bacterial growth, was used for the incubation and standardization of the microorganisms. The composition as follows,

1.	Beef extract	-	2 g/l
2.	Casein hydrolysate	-	17.5 g/l
3.	Agar-Agar	-	17 g/l
4.	Starch	-	1.5g/l
5.	Distilled water	-	1 Lt.
6.	pH	-	7.0 – 7.2.

2.3.1. c. Preparation of growth media

Media was sterilized in an autoclave at 15 psi pressure and 121°C for 15 min. After sterilization, nutrient agar media was poured aseptically into sterilized petri plates in a laminar flow chamber. All the steps were performed in sterile environment in order to prevent contamination. The media was allowed to be solidified in petri plates for about an hour and then placed in an incubator at 37°C for 24 h. The next day, uncontaminated plates were used for culturing of the microorganisms.

2.3.1. d. Disc diffusion susceptibility method

Petri plates containing 10ml of respective medium were seeded with a selected bacterial strain. The sterile Whatman filter paper discs of 6 mm diameter containing solution of new schiffs base different concentrations (1ml each) were aseptically placed onto the surface of agar plates and were incubated at 37°C for 18 to 24 h in an incubator. The chemicals diffused from the discs into the agar media thus preventing the growth of microorganisms (if susceptible) in the area around the disc known as zone of inhibition. The diameter of zones of inhibition around each disc measured and recorded at the end of the incubation period. The filter paper discs immersed in absolute ethanol and distilled water were prepared and used as control to understand the inhibitory effect of solution on microbial populations.

3. 0. Statistical analysis

Cycli voltametry was recorded e- chem software. The data obtained was analyzed by using devries and Kroonn[14] method is used to arrive at the number of electrons involved in reduction.The heterogeneous rate constant (kf,h) calculated from the Meites–Israel equation.[15]

4.0. RESULTS AND DISCUSSION

Elemental analysis data of new Schiff base ligand was found %61.61 carbon, 7.4 percentage of hydrogen , 5.49 percent of nitrogen all this data confirms the 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol ($C_{13}H_{19}NO_4$) with molecular weight 253. Electronic spectra (Figure 1) of the 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol in UV region show an intense band at 270 nm and weaker band at 290 nm which are assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ Transitions respectively.

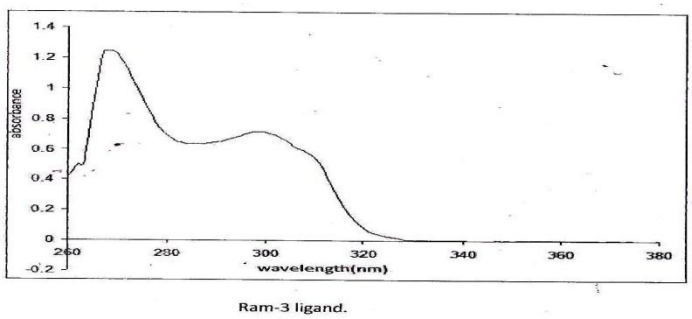


Figure 1: Electronic spectra of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)- 2,6,Dimethxy Phenol.

The IR spectra of newly synthesized Schiff base 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol was recorded in KBr Pellette in the IR range of 4000-400 cm^{-1} and IR data (Figure:2) establishes the Molecular structure of schiff base 3240 cm^{-1} (intra molecular H- bonding), 3010 cm^{-1} (Ar-H, stretching), 2980 cm^{-1} (Aliphatic C-H stretching), 1686 cm^{-1} (>C=N stretching), 1245 cm^{-1} (C-O stretching vibrations of Alcohol / Phenol).

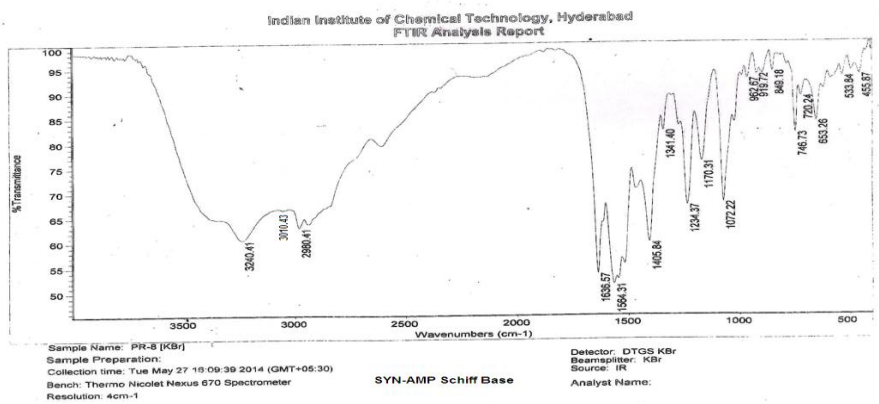


Figure 2: Vibrational spectra of 4-((1-hydroxy-2-methyl propan-2-ylimino) methyl)-2,6, dimethxy phenol.

In Briton-Robinson buffer solutins of pH 4.1 (Table:-1) and 8.1(Table:-2) Schiff base exhibit two cathodic peaks in all sweep rates. For a reversible charge transfer, O +ne → R, the peak potential is independent of scan rate and the separation of anodic peak and cathodic peak potentials (E_{pa} - E_{pc} = ΔE_p) is 60/n mV at 25°C and the ratio of anodic peak current to cathodic peak current was unity (Figure :3&4). On the other hand, a plot of $i_{pc} / \upsilon^{1/2}$ versus sweep rate is a straight line parallel to sweep rate axis and it is contrary to the behavior of a reversible system, the irreversible nature of the electrode process is characterized by dependence of peak potentialon sweep rate, the absence of anodic peak in the reverse scan, in acidic pH solutions.

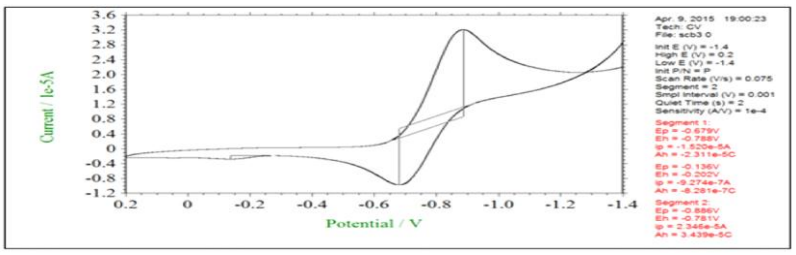
Table- 1. Cyclic voltammetric results of Schiff bases ($1x10^{-3}$M) Medium: aqueous dimethyl foramamide (40%v/v): at pH=8.1.

Schiff Base	Sweep rate (mv/sec)	Cathodic peak potentials (Epc)	Cathodic peak currents (ipc)	Anodic peak potentials (Epa)	Anodic peak currents (ipa)
		I	I	I	I
S YN-AMP	0.010	1.01	1.1	0.76	0.2
	0.020	1.07	1.6	0.82	0.3
	0.050	1.13	2.5	0.88	0.4
	0.100	1.19	3.6	0.94	0.7
	0.200	1.25	5.1	1.0	0.9
	0.500	1.34	8.2	1.09	1.5

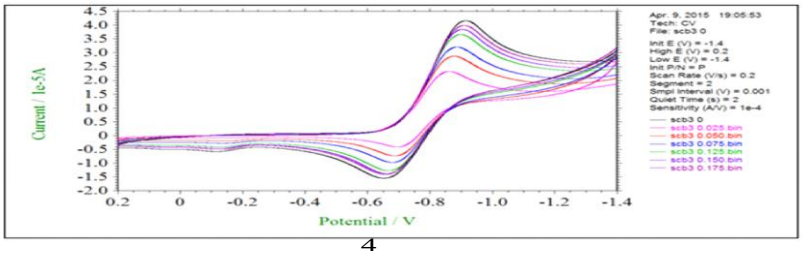
Table-2: Cyclic voltammetric results of Schiff bases ($1x10^{-3}$M) Medium: aqueous dimethyl foramamide (40%v/v): at pH = 4.1.

Schiff Base	Sweep rate (mv/sec)	Cathodic peak potentials (Epc)	Cathodic peak currents (ipc)	Anodic peak potentials (Epa)	Anodic peak currentsm(ipa)
		I	I	I	I
S YN-AMP	0.010	0.71	0.80	0.46	0.35
	0.020	0.77	1.13	0.52	0.5
	0.050	0.83	1.8	0.58	0.8
	0.100	0.89	2.5	0.64	1.1
	0.200	0.95	3.6	0.7	1.5
	0.500	1.04	5.7	0.8	2.5

The anodic peak and cathodic peak potentials($E\ p_a - E\ p_c = \Delta\ Ep > 60/n$ mV) at 25^0 C. The above mentioned facts clearly rules out the possibility of a fast electron transfer which is characteristic of a reversible behaviour. The linear plot of ip_c versus $\upsilon_{1/2}$ suggests the diffusion-nature of the electrode process.



3



4

Figures : 3 &4. Cyclic Voltamograms of single and Overlay plots of Schiff base

The Schiff base exhibit one anodic peak in all sweep rates. The number of polarographic reduction waves in DC polarography and number of cathodic peaks in CVM studies are one and the same. However an additional anodic peak was noticed in Schiff base under experimental conditions. Based on experimental results in DC polarography and CVM studies the following mechanism has been proposed for Schiff base. The cathodoic peak was ascribed to two electron reduction of azomethine group to amine stage. The anodic peak was attributed to 2e oxidation of primary alcohol to aldehyde functional group. The oxidation of- CH_2OH to –CHO peak either one step or in two steps[16]. Polarographic results of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol Briton Robinson buffer solutions ($1x10^{-3}$ mM) in Aqueous DMF (40% v/v) medium was shown in (Table:3).

Table:3 Polarographic results of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol Briton Robinson buffer solutions ($1x10^{-3}$ mM) Medium: Aqueous DMF (40% v/v).

pH	$E_{½}$ vs SCE	Limiting Current i_l(µA)
2.1	0.45	3.5
3.1	054	3.5
4.1	0.63	3.5
5.1	0.72	3.4
6.1	0.81	3.3
7.1	0.90	3.2
8.1	0.99	3.1
9.1	0.99	3.1
10.1	0.99	3.1

Table 4: Effect of Mercury Column height (h) on the limiting current (i_l) of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol ($1x10^{-3}$ M) Medium: aqueous dimethyl foramamide (40%v/v).

Mercury column height (h)(cm)	pH=4.1		pH=8.1	
	First wave		First wave	
	i_l (µA)	$i_l/\sqrt{h}$	i_l (µA)	$i_l/\sqrt{h}$
80	4.0	0.440	3.6	0.40
70	3.70	0.440	3.30	0.40
60	3.20	0.42	2.91	0.38
50	3.00	0.42	2.78	0.38

Effect of Mercury Column height (h) on the limiting current (i_l) of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol ($1x10^{-3}$ M) in aqueous dimethyl foramamide (40%v/v) Medium:was shown in (Table:4). Polarographic characteristics and kinetic parameters of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol ($1x\ 10^{-3}$ mm)in Aqueous dimethyl farmamide (40% v/v) Medium in Table:5.

Table 5: Polarographic characteristics and kinetic parameters of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol ($1x\ 10^{-3}$ mm)in Medium : Aqueous dimethyl farmamide (40% v/v).

pH	$-E_{1/2}$/pH (Mv)	α_{na}	No. of protons p	D X 10^{-6} $cmsec^{-1}$	I* X 10^3	K^0fh cm sec^{-1}	ΔG* k cal $mole^{-1}$
2.1	0.087	0.47	0.69	3.02	4.2	$5.18x10^{-4}$	10.20
4.1	0.087	0.52	0.76	2.01	3.4	$2.76x10^{-4}$	10.78
6.1	0.087	0.65	0.96	1.01	2.4	$9.97x10^{-5}$	11.34
8.1	0.087	0.74	1.10	0.53	1.7	$10.4x10^{-5}$	12.01
10.1	0.087	0.74	1.10	0.53	1.7	$10.4x10^{-5}$	12.01

The test Schiff base 4-((1-hydroxy-2-methylpropan-2-ylimino)methyl)-2,6-dimethxyphenol shows significant antibacterial activity against pathogenic bacterial strains Bacillus cereus,Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus were shown in *figure;5.*

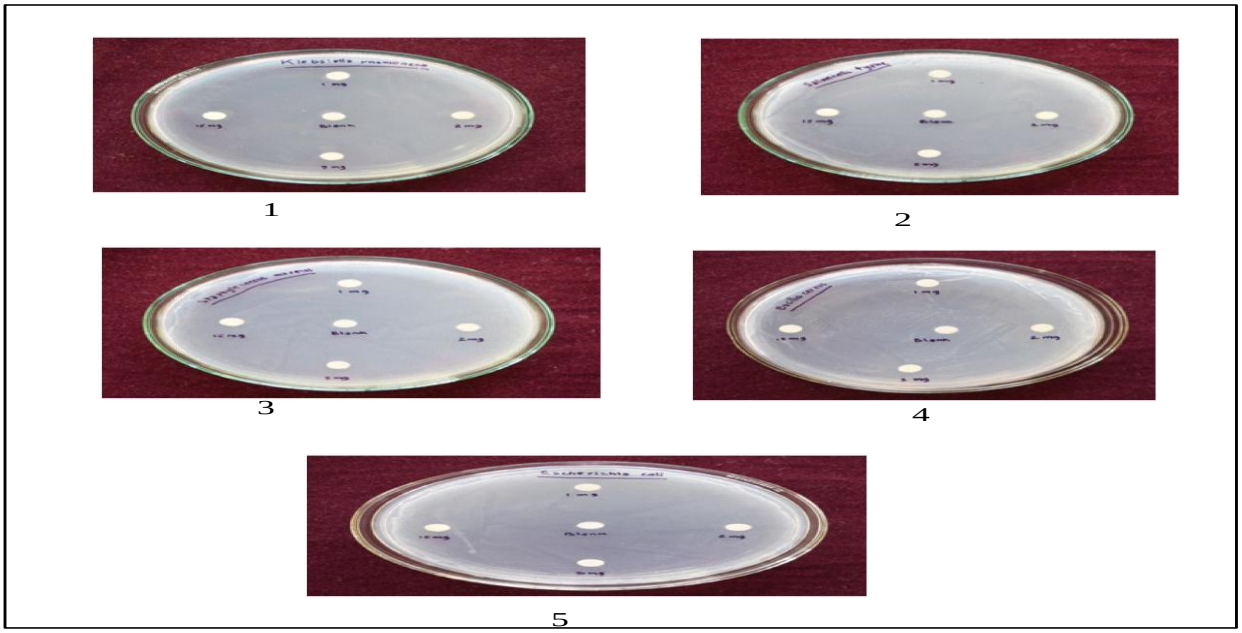


Figure 5: Plate showing antibacterial activity of Schiffs base against 1. Klebsiella pneumonia, 2. Salmonella typhi, 3. Staphylococcus aureus, 4. Bacillus cereus and 5. E. Coli.

Table 6: Results of anti bacterial activity of 4-((1-hydroxy-2-methylpropan-2-ylimino)methyl)-2,6-dimethxyphenol.

S.No	Micro-organism	Inhibition zone (mm^{-1}) Concentrations mg/ml (dissolved in DMSO)				Standard µg/disc
		1	2	5	15	
1	*Bacillus cereus*	7	8	8	10	22^{A}
2	*Escherichia coli*	6	7	7	7	22^{T}
3	*Klebsiella pneumoniae*	8	10	9	11	23^{T}
4	*Salmonella typhi*	6	8	8	10	22^{A}
5	*Staphylococcus aureus*	6	8	8	11	23^{K}
A- *Ampicillin*, K-*Kanamycin*, T-*Tetracycline*						

The test sample showed inhibition zones ranged between 7-11mm against test pathogens. The analysis of the data (Table:-6) revealed that, test sample had a profound inhibition effect against Klebsiella pneumonia whereas it is fail to inhibit the growth of Escherichia coli.

5.0. CONCLUSION

In this present investigation we are reported kinetic parameters (D, K^{0}fh cm sec^{-1} , ΔG* k cal $mole^{-1}$)of 4-((1-Hydroxy-2-Methyl Propan-2-Ylimino) Methyl)-2,6,Dimethxy Phenol (1x 10^{-3} mm) in Aqueous dimethylformamide (40% v/v) Medium. The test sample showed inhibition zones ranged between 7-11mm against pathogens. We hope this study is helpful to know coordination ability of this compound with metals and interaction of with other molecules.

6.0. Funding acknowledgement

The author is very thankful to Human Resource Development Group Council of Scientific and Industrial Research [HRDG- CSIR] SRF fellowship, Grant no. (09/383(0054)/2017-EMR-I).

7.0. Conflict of interest

The author had no conflict of interest.

8.0. REFERENCES

1. Czelaw Belzecki and Tadeusz Urbanski. Roczniki Chem. 32, 769 – 78 (1958); Cf. C.A. 53, 7166 C; Am. Chem. Soc. Abst., 1959; 53: 10188 d, 1088 e.
2. Krishna Murthy, K.S. R. Krishna Mohan Rao and N.V. Subba Rao. J. Indian Chem. Soc., L, 1973; 213.
3. H.G. Garg and S.S. Joshi, *J. Indian Chem. Soc*., 1960; 37: 626.
4. J.W. Ross, R.D.Demars and I. Shain. *Anal. Chem*., 28: 1768(2956).
5. L. Meites. *Polarographic Techniques*. Second Edn., Interscience, New York, 1967; 139.
6. G.C.Gerritsen and W.E.Dulin. *Diabetes*, 1965; 14: 507.
7. D.L.Smith, A.A.Forist and W.E.Dulin. *J. med. Chem*., 1965; 8: 350.
8. L. Meites. *Polarographic Techniques*. Second Edn., Interscience, New York, 1967; 139.
9. G.C.Gerritsen and W.E.Dulin. *J. pharmacol. Exptl. Therap.*, 1965; 150: 491.

10. Tadeuz Urbanski, Czeslaw Belzecki, Bazena Chechelska, Jadwiga Chylinska, Halina Dabrowska, Jerzy Falecki, Daniela Gurne, Leszek Halski, Stanislaw Malinowski, Barbara Skowronska Serafinowa, jerzy Zylowski, Stefan Slopek, Irena Kamienska, Jan Venulet, Mieczyslaw Janowiez, Krystyna Jakimowska, Alicta Urbanska and Anatol Kuzniecow (inst. Gruzlicy, Warsaw). Gruzlica, 1958; 26: 889 – 917 Am. Chem. Soc. Abstracts, 1959; 53: 4568.
11. H.G. Garg and Chandra Prakash. *Indian J. Chem*, 1971; 9: 801.
12. J.B. Wright, W.E. Dulin and J.H.Markillie. *J. med. Chem*, 1962; 7; 102.
13. L. Meites and Y. Israel. *J. Am. Chem. Soc.*, 1961; 83: 4903.
14. Khan F. Electrochemical study and dependence of transition state' in Co(II) and Ni(II) complexes with some antibiotics and cephalothin. EcletQuim, 2007; 32(3): 73-83. doi: 10.1590/S0100-46702007000300010.
15. Meites Louis, Israel Yecheskel. The calculation of electrochemical kinetic parameters from polarographic current-potential curves. J Am Chem Soc., 1961; 83(24): 4903-6. Doi: 10.1021/ja01485a008.
16. Ramanjaneyulu K, Malleswara Reddy P, Murthy Naik Vkrishna, Babu K Sudhakar. Stability studies ofcadmium (ii) presence of 2-(3, 4-Dimethoxybenzylidene Amino)-2-methyl propane-1-ol by usingdc polarography in 40% v/v methanol-water syste. ThePharmacolInnov J., 2018; 7(12): 47-53. ISSN (E): 2277-7695 ISSN (P): 2349-8242.