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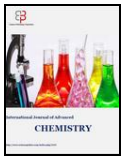

Synthesis, characterization, antimicrobial, analgesic and CNS studies of Schiff base cu(II) complex derived from 4-choro-o-phenylene Diamine

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Abstract

Schiff base ligand and its Cu (II) Complex had been synthesized by the condensation reaction of 4-chloro-o-phenylene diamine and 2-hydroxy acetophenone. The Structure and spectral properties of ligand and complex were confirmed by UV, FT-IR and ^{1}H NMR Spectroscopy. The spectral properties showed that it was a square planar geomentry with a tetradentate ligand. The Schiff base Cu (II) Complex was subjected to antimicrobial studies. In this paper I have taken to discuss these three bactericidal organisms such as Bacillus subtilis, Streptococcus viridians and Staphylococcus epidermidis on the test compounds. Schiff base Copper (II) complex were screened by employing the Disc Diffusion method. A concentration gradient (5, 10, 20 and 30μg/ml) of each compound was put into study. From the study, it was observed that it showed a maximum zone of inhibition. The Schiff base complex were subjected to analgesic studies and it showed a significant increase in analgesic activity when compared with normal saline. The Schiff base complex was subjected to CNS studies and it showed a depressant activity when compared with standard drug chlorpromazine.

Keywords: *Schiff Base, 4-Chloro-O-Phenylene Diamine, Electronic Spectroscopy, Petri Plates and CNS.*

1. Introduction

Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported by Schiff [1] in 1864 (Schiff 1864 p. 118). The common structural feature of these compounds is the azomethines group with a general formula RHC=N-R1, where R and R1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also knows as imines or azomethines. Several studies [2-8] showed that the presence of a lone pair of electrons in a sp^2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance (Schiff et al 1869 & B.C. Baguley et al. 1984 p. 937-943). Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are generally excellent chelating agents,[9-12] especially when a functional group like –OH or –SH is present close to the azomethine group so as to form a five or six member ring with the metal ion. Versatility of Schiff base ligand and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable (E. Saripinar et al. 1989 & O. Lumme et al. 1995 p. 1553) Schiff bases have been known since 1864 when Hugo Schiff reported the condensation of primary amines with carbonyl compounds. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed([13]-[16]) (A. Kriza et al. 2000 & H.S. Seleem et al. 2003).Schiff bases resulted from aromatic aldehydes ortho-substituted with a hydroxyl group have initially arouse the researchers interest because of their ability to act as bidentate ligands for transitional metal ions ([17]-[20]) (Hosseini et al. 2000 & Mahajan et al. 2003). Later, in studies concerning quantitative structure-antitumor activity relationship of a series of Schiff bases derived from variously substituted aromatic amines and aldehydes, it has been shown that azomethines from salicylaldehydes gave the best correlation [21], [22]. (Cozzi, P.G 2004 & M. Sönmez et al. 2003)Schiff bases of salicylaldehydes have also been reported as plant growth regulators [23] (Yurt et al. 2004 p. 420–426) and antimicrobial [24] (M. Weitzer et al. 2005 p. 248) or antimycotic [25] (M. Berber et al. 2005 p. 101) activity. Schiff bases also show some analytical applications [26]. (K.Y.El-Baradie et al. 2005 p. 677) Schiff Bases are characterized by the -N=CH- (imine) group which imports in elucidating the mechanism of transamination and rasemination reaction in biological system ([27]-[30]). (Ashassi-Sorkhabi et al. 2005) Schiff bases are active against a wide range of organisms for example; Candida Albicans, Escherichia coli Staphylococcus aureus, Bacillus polymxa, Trychophyton gypseum, Mycobacteria, Erysiphe graminis and Plasmopora viticola.

Schiff bases have been reported in their biological properties, such as, antibacterial, antifungal activities [32] (Estari Mamidala 2010 p. 380-384). Their metal complexes have been widely studied because they have anticancer and herbicidal applications. O-phenylenediamine Schiff bases show clinical properties. They were reported to possess antiviral, anti-HIV, antiprotozoal and antihelmintic activities. They also exhibit significant analgesic

activity, apart from other pharmacological properties. This paper presents the condensation reaction of 4-chloro-o-phenylene diamine and 2-Hydroxy acetophenone and Schiff base copper (II) Complex were prepared and further study were investigated.

2. Materials and methods

2- Hydroxyacetophenone, 4-chloro-o-phenylene diamine, copper (II) chloride was purchased from sigma Aldrich. The solvents were analar grade. For studying the antibacterial activity of the newly synthesized Schiff base complex the following chemicals were used. The Peptone was purchased from nice chemicals Private limited. The Beef Extract was obtained from Merck Limited. The Sodium Chloride was purchased from Reachem Laboratory Chemicals Private Ltd. The Agar-Agar Type I was obtained from Himedia Laboratories. The Incubator was obtained from in lab Equipments Private Ltd. The solvents used were ethanol, methanol and THF

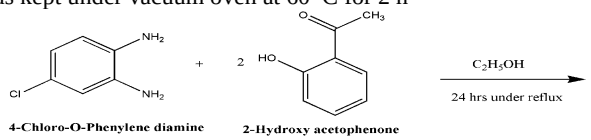
The following materials were used for the analgesic and CNS activity of Schiff base complex Albino mice (15-35 g), Syringe 1 ml, Glass Van Tuberculin – BCG Borosilicate glass.

The Percentage of carbon, hydrogen, nitrogen chlorine,copper,oxygen contents were analyzed using carlo Erba 1108 model elemental analyser using sulphanilamide as a reference standard. The metal content present in the complexes was estimated as given in the book [10] (Arthur Israel Vogel, 1989). The UV spectra of title compound and its complex were recorded in the conventional region (200-800 nm) using DMSO as solvent. The UV spectral measurements were done in the BL 198 Biospectrophotometer. The UV spectral study helps to decide the absorption (λ_{max}) of Schiff base ligand and complex. The infra-red spectra of the compounds were recorded in the conventional region (400-4000cm^{-1}) as KBr pellets. The infra-red spectral measurements were done using FT-IR-Shimadzu spectrometer. The IR spectral study helps to decide the mode of coordination of the ligand to the metal. The NMR spectroscopy for the Schiff base ligand is recorded in BRUKER (300MHz) instrument using DMSO as solvent. The Laminar air flow Chamber was used for studying the antibacterial studies. The analgesiometer (Besto) were used for determining the analgesic activity. Digital actophotometer were used for determining the CNS activity

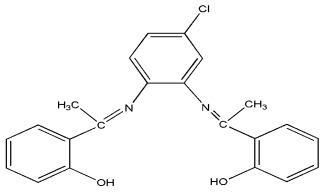
3. Experimental

3.1. Synthesis of Schiff base ligand: 2-[N-(4-chloro-2-{[1-(2-hydroxyphenyl) ethylidene] amino} phenyl) ethanimidoyl] phenol

Initially 2-hydroxy acetophenone (2 mmol) in ethanol was kept under magnetic stirring. It was then added to ethanolic solution of 4-chloro-o-phenylene diamine (1 mmol) in the ratio 1:2. The mixture was refluxed for 8-12 h. The Product as solid mass started appearing after 8 h. The Precipitate was filtered and washed with water, methanol followed by diethyl ether. The Purified product was kept under vacuum oven at 60^{O}C for 2 h



4-Chloro-O-Phenylene diamine 2-Hydroxy acetophenone

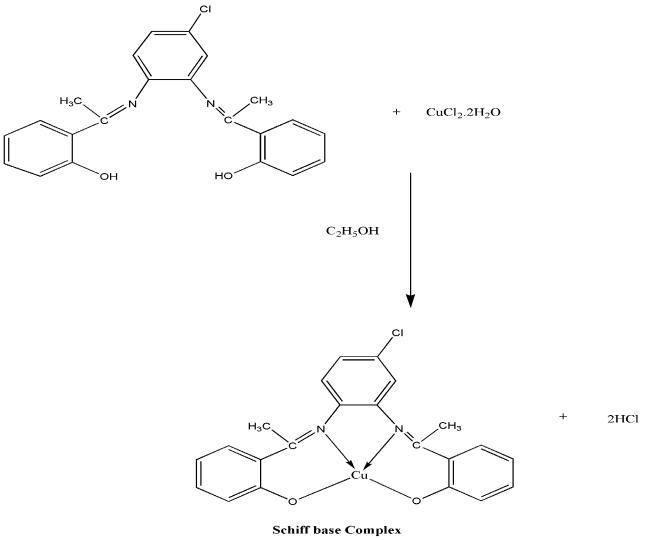


2-[*N*-(4-chloro-2-{[1-(2-hydroxyphenyl)ethylidene]amino}phenyl)ethanimidoyl]phenol

Yield – 72% reddish brown powder Molecular Formula: $C_{22}H_{19}ClN_2O_2$ Molecular Weight 378.85 Elemental Analysis Calculated: C(69.75%) H (5.05%) Cl(9.36%) N(7.39%) O(8.45%); found (%) C (69.42%) H (5.01%) Cl(9.21%) N(7.21%) O(8.38%) ^{1}H NMR (300 MHZ 1% DMSO/D_2O): 6.76-7.45(m Ar-H) 0.9(s -CH_3) 5 (s -OH); IR(KBR) (cm^{-1}) 2924.52 (Aromatic C-H) 2854.13 (Aliphatic C-H) 1241.93(Aliphatic C-C) 1454.06(Aromatic C=C) 751.38(C-Cl) 1626.03 (C=N) 1297.86 (C-O) The Ligand intermolecular hydrogen bonding OH reduces from 3640 to 3368.07 cm^{-1} UV- VIS(λ_{max} nm) transition 300 nm Critical temperature – 987.97 K critical pressure 18.81 Bar

3.2. Synthesis of Schiff base Cu (II) complex

Schiff base ligand (1 mmol) dissolved in ethanol was kept under magnetic stirring and Copper (II) Chloride (1 mmol) dissolved in ethanol were added in the ratio 1:1. The mixture was refluxed for 8 hrs. The Precipitate was filtered and washed with water, ethanol, acetone and diethyl ether. The green products obtained were recrystallized from tetrahydrofuran (THF) and methanol. The Purified product was kept under vacuum oven at 60^{O}C for 2 h



Schiff base Complex

Green powder: Yield 64% Molecular Formula : $C_{22}H_{17}ClCuN_2O_2$ Molecular weight 440.38 Elemental analysis calculated: C(60%) H (3.89%) Cl (8.05%)Cu (14.43%)N(6.36%) O(7.27%) found (%) C(59.62%) H (3.61%) Cl(8.01%) Cu (14.39%) N (6.29%) O (7.25%) IR(KBR) (cm^{-1}) 2923.56 (Aromatic C-H) 2853.17 (Aliphatic C-H) 1207.22(C-C) 1402(Aromatic C=C) 754.03(C-Cl) 1606.41(C-N) 480 (Cu-O band) 425 (Cu-N band).The Complex OH The peak obtained at 3413.39 cm-1 is due to water of hydration present out of the coordination sphere. UV - VIS (λmax nm) transition 470 nm.

4. 4. Results and discussion

4.1. Elemental analysis

From the elemental analysis, it is clear that observed micro analytical data (C, H and N) of the compounds are closely comparable with theoretically calculated C, H and N Values. The elemental analysis for ligand Calculated: C(69.75%) H (5.05%) Cl(9.36%) N(7.39%) O(8.45%); found (%) C (69.42%) H (5.01%) Cl(9.21%) N(7.21%) O(8.38%) and for complex Elemental analysis calculated: C(60%) H (3.89%) Cl (8.05%)Cu (14.43%)N(6.36%) O(7.27%) found (%) C(59.62%) H (3.61%) Cl(8.01%) Cu (14.39%) N (6.29%) O (7.25%).

4.2. Electronic spectroscopy

The electronic spectra of the ligand and complex in UV-Vis region were obtained in DMSO Solutions using a Shimadzu UV-1601 Spectrophotometer in the range of 200-800 nm. The electronic spectra of Ligand and Complex were recorded in DMSO and given as Figure 1 and 2.

The observed λmax values are used to predict the geometry around the central metal ion in the complex. The electronic spectra of Ligand show similar absorption bands and obtain a 290 nm. It is shown in Figure 1. These bands shows the presence of n →n* and π → π* transitions of their azomethines chromophore group and aromatic ring. But in the Spectra of complexes, slightly shifts are observed in the position and intensity of these bands as compare to that of ligand which might be due to the coordination of metal with the ligand. In addition, the charge transfer transition due to metal to ligand π -back bonding may also contribute to these absorption bands (below 400 nm) in the complex investigated [37] (R. Antony et al 2012, p. 14-18).

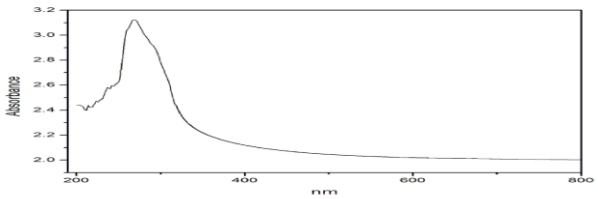


Fig. 1: UV-Visible Spectroscopy of Ligand

An additional absorption band is observed above 400 nm in the electronic spectra of the complex. The Electronic Spectra of Schiff base Copper (II) complex shows this characteristic band at 470 nm which suggests the square planar geometry around Cu (II) Centre. The Electronic Spectra of Schiff base Copper (II) complex shows this characteristic band at 470 nm due to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition (It is shown in Figure 2)

It suggests the square planar geometry around Cu (II) Centre respectively. It confirms complex formation. The corresponding UV visible spectra are shown in the following figure. λ max for ligand and its Copper (II) complex are 300 nm and 470 nm respectively. It confirms complex formation [37] (R. Antony et al 2012, p. 14-18).

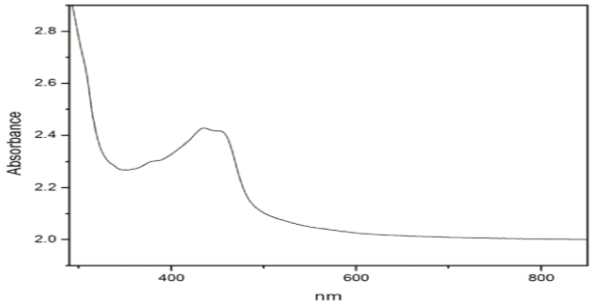


Fig. 2: UV-Visible Spectroscopy of Complex

4.3. Vibrational spectroscopy

The infra-red spectra were recorded by using 1% of the sample on KBR pellet with 16 scans and 2cm^{-1} resolution in a Jasco FT-IR/4100 Spectrophotometer equipped with ATR accessory in the range of 4000-400cm^{-1}.The FT-IR Spectrum of Ligand and Complex are Shown in Figure 3 and 4.

The FT-IR spectra of ligand Stretching obtained at 2924.52cm^{-1} shows the presence of presence of Aromatic C-H group. The FT-IR band at 2854.13 cm^{-1} shows the presence of Aliphatic C-H group. The peak obtained at 1626.03cm^{-1} shows the presence of C=N group. The peak obtained at 1454.06cm^{-1} shows the presence of aromatic C=C group. The peak obtained at 751.38 cm^{-1} shows the presence of C-Cl group. The peak obtained at 1241.93 cm^{-1} shows the presence of C-C group. The peak obtained at 1297.86 cm^{-1} shows the presence of C-O group. The Ligand intermolecular hydrogen bonding OH reduces from 3640 to 3368.07 cm^{-1} [31] (Francis A. Carey 2008 p. 539). It is shown in figure 3.

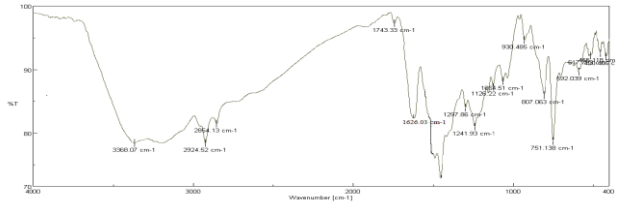


Fig. 3: FT-IR Spectroscopy of Ligand

The FT-IR Spectra of Complex obtained at 2923.26cm^{-1} shows the presence of aromatic C-H group. The peak obtained at 2853.17 cm^{-1} shows the presence of aliphatic C-H group. The peak obtained at 1606.41 cm^{-1} shows the presence of C=N group. In the FT-IR Spectra of the complex, it is expected that coordination of Nitrogen centre to the metal ion would reduce the electron density in the azomethines link and shift the C=N Stretching frequency to the lower wave number. The Shift in C=N Stretch is found in all complex which shows the successful coordination of azomethines nitrogen to the metal (Copper) centre. The peak obtained at 1207.22 cm^{-1} shows the presence of C-C group. The peak obtained at 754 cm^{-1} shows the presence of C-Cl group. The peak obtained at 1402 cm^{-1} shows the presence of aromatic C=C group. The Peak obtained at 480cm^{-1} shows the presence of Cu-O band and Peak obtained at 425 cm^{-1} shows the presence of Cu-N band [31] (Francis A. Carey 2008 p. 539). It is shown in Figure 4.

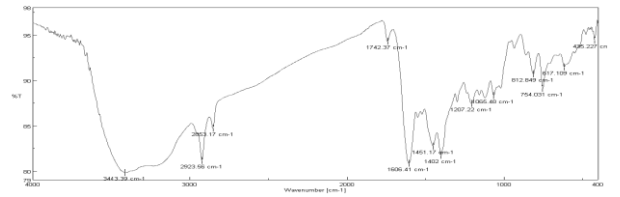


Fig. 4: FT-IR Spectroscopy of Complex

4.5. FT- NMR spectroscopy

The ^{1}H NMR spectroscopy for the Schiff base ligand in 1% DMSO/D_2O was analyzed with TMS as Standard. The Structure of Ligand is characterized from the assignments of observed chemical shifts to the corresponding protons. The multiplet obtained at 7.3 ppm corresponds to aromatic ring attached to nitrogen moiety. The multiplet obtained at 6.76, 6.85, 7.12, 7.45 Corresponds to aromatic Phenolic ring. A singlet obtained at 0.9 ppm corresponds to the presence of $–CH_3$ group. A singlet obtained at 5ppm corresponds to the presence of OH group associated with the ligand. ^{1}H NMR Spectrum of ligand does not show any proton signals for nitrogen, where their values are expected in higher chemical shift than 10 ppm this may be due to the low solubility of ligand in the solvent [31]. (Francis A. Carey 2008 p. 539)

4.6 Antibacterial activity

Antibacterial studies against Gram positive Bacillus subtilis, Streptococcus viridians and Staphylococcus epidermidis by disc diffusion method

Sterilized nutrient agar medium was poured into sterilized Petri plates. The Petri plates were allowed to stand for some time, until the agar medium gets same time, until the agar medium get solidified, the bacterial cultures (Bacillus subtilis, Staphylococcus epidermidis, Streptococcus viridians.)

After that the bacterial culture medium was lawned on the surface of the medium using a sterilized cotton swab. The newly synthesized compound was loaded on sterilized discs in different concentrations. These discs were carefully placed on the surface of the medium with the forceps. The Petri plates were incubated for

16-18 hrs at 37^0C in inverted position. After that the zone of inhibition was measured in mm ([33]-[37]) (B. Parimala Devi et al. 2010 p. 1-16)

The maximum zone of inhibition of 17, 21 and 19mm were observed with Bacillus subtilis, Streptococcus viridians and Staphylococcus epidermidis for the concentration of 30µg/ml represented in Table 1. (Anil Kumar Sharma et al. 2011, p. 380-384) It is shown in Figure 5 to Figure 10.

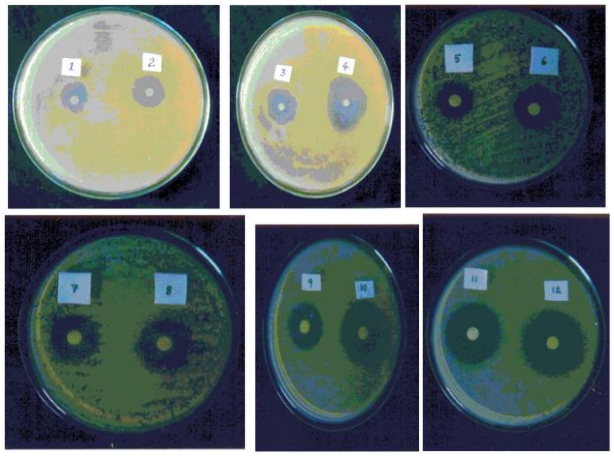


Fig. 5-10: Antimicrobial Activity

Table 1: (Antimicrobial Activity)

Amount in µg/ml	Marked as	Zone of inhibition in mm
Bacillus subtilis		
5	1	12
10	2	15
20	3	16
30	4	17
Streptococcus viridians		
5	5	15
10	6	18
20	7	20
30	8	21
Staphylococcus epidermidis		
5	9	11
10	10	14
20	11	16
30	12	19

4.7. Analgesic activity

The doses of Schiff base Cu (II) Complex are prepared with a concentration of 20mg/ 10ml. The doses were given depending upon the body weight of the animal [38] (Meenakashi Agarwal 2013 p. 258-269).

Table 2: Analgesic Activity of Schiff Base Cu (II) Complex

Animal body weight(g)	Drug and dose	Basal reading (sec)					Reaction time after treatment (sec)				
		15	30	60	90	120	15	30	60	90	120
34.83		1	1	2	1	1	2	1	1	1	1
31.45	Control 1ml saline	1	1	1	2	1	1	1	1	2	1
30.19		1	1	1	1	1	1	1	1	2	1
Mean		1.00	1.00	1.33	1.33	1.00	1.33	1.00	1.00	1.66	1.00
29.18		1	2	2	1	2	2	2	3	4	4
25.16	Test drug (20 mg in 10 ml)	1	2	1	1	1	2	3	3	5	4
27.56		1	1	1	1	1	3	3	3	4	4
Mean		1.00	1.66	1.33	1.00	1.33	2.33	2.66	3.00	4.33	4.00
% of analgesic activity							42.9	62.4	66.7	61.7	75.0

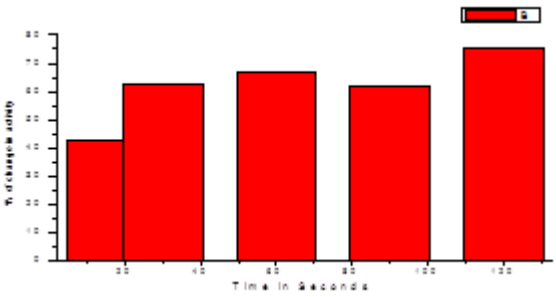


Fig. 11: % of Change in Activity with Time

4.8. CNS activity

The CNS activity was studied using albino mice through oral route using canula insertion via mouth. The scores from the digital actophotometer were tabulated before and after drug administration [39] (NM. Goudgaon et al. 2014 p. 64-68)

Table 3: CNS Activity of Chlorpromazine

Animals body weight (g)	Drug	Dose mg/kg	Actophotometer activity in 10 minutes		
			Before treatment	After treatment	% Change in activity
36.18			190	30	84.21
34.28		30	240	198	17.5
35.10	Chlorpromazine	mg/10	232	48	79.31
35.93		ml	172	70	59.30
36.55			210	54	74.28
				Mean	62.9

Table 4: CNS Activity of Schiff Base Cu (II) Complex

Animals body weight (g)	Drug	Dose mg/kg	Actophotometer activity in 10 min		
			Before treatment	After treatment	% Change in activity
36.18			170	60	64.70
34.28	Schiff	30	225	201	10.66
35.10	base	mg/10	214	53	75.23
35.93	Derivative	ml	164	90	45.12
36.55			203	74	63.54
				Mean	51.85

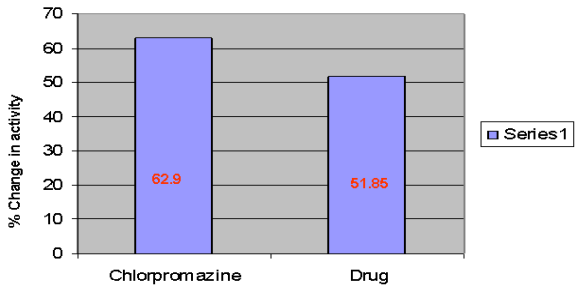


Fig. 12: Comparison of Chlorpromazine with Schiff Base Derivative

5. Conclusion

2-hyroxy acetophenone and 4 – chloro-o-phenylene diamine were refluxed in ethanol and the product is 2-[N-(4-chloro-2-{[1-(2-

hydroxyphenyl) ethylidene] amino} phenyl) ethanimidoyl] phenol and its complexes were prepared. The product was confirmed by IR, ^{1}H NMR and UV Studies. UV visible spectra (λmax) for ligand and its Copper (II) complex are 300 nm and 470 nm respectively. It confirms complex formation and forms a Square Planar geomentry. In FT-IR Spectra of ligand the stretching obtained at 1626 cm^{-1} which reduces to 1606 cm^{-1} which shows the successful coordination of nitrogen to the copper centre. In ^{1}H NMR Spectroscopy for ligand the multiplet obtained at 6.76-7.45 ppm corresponds to aromatic hydrogen. A singlet obtained at 0.9 ppm corresponds to the presence of $–CH_3$ group. A singlet obtained at 5ppm corresponds to the presence of OH group associated with the ligand. The product was subjected to antibacterial activity and it shows a good antibacterial activity and maximum zone of inhibition was calculated. The maximum zone of inhibition of 17, 21 and 19 mm was observed with Bacillus subtilis, Streptococcus viridians, and Staphylococcus epidermidis for the concentration of 30µg/ml. The Schiff base complex were subjected to analgesic studies and it shows a significant increase in analgesic activity when compared with normal saline and obtained 42.9%62.4%, 66.7%, 61.7% and 75% with different time intervals. The Schiff base complexes were subjected to CNS studies and it showed a depressant activity when compared with standard drug chlorpromazine.

Acknowledgements

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