Synthesis and Characterization of Copper(II) Complexes of Salicylaldehyde Semicarbazone and 1,10 Phenanthroline and their Antibacterial Activities

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Abstract— Copper(II) complexes of Salicylaldhyde semicarbazone and 1,10 Phenanthroline were synthesised and characterized using physio-chemical and spectroscopic methods such as Infra-red spectra, electronic spectra, conductivity and melting point measurements. The anti-bacterial activities of metal-complexes and ligands were evaluated using standard agar well diffusion method. Pure bacteria cultures of *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) were used to check the anti-bacterial activities of all compounds. Anti-bacterial activities were compared by measuring the diameter of zone of inhibition and amoxicillin was used as reference. All compounds showed significant antibacterial activity in different range against gram Positive & gram Negative bacteria.

Index Terms— Copper(II) complexes, Salicylaldhyde, semicarbazone, 1,10 Phenanthroline, anti-bacterial activities, spectroscopic methods, metal-complexe
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1 Introduction

Semicarbazones are a class of compounds obtained from the condensation of semicarbazide with suitable aldehyde or ketone. Many of these compounds possess a wide spectrum of biological activity including activity against tuberculosis[1], leprosy [2], bacterial[3] and viral infections[4], psoriasis[5], and malaria[6]. Salicylaldhyde semicarbazone is formed by the condensation of "$-NH_2$" group of 2nd position to the low electron dense carbonyl carbon and "-C=O" group of salicylaldehyde (shiff base formation). It is described below in scheme (fig. 1.1).

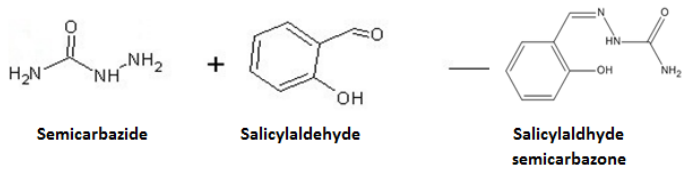


fig 1.1 - Scheme of formation of Salicylaldehyede Semicarbazone

A group of vanadium complexes of salicylaldehyde semicarbazone derivatives were reported recently for their selective potency on human kidney TK 10 tumour cells[7].The results obtained with this study showed that modification of the semicarbazone backbone could have a significant effect on the cytotoxicity of the complexes.

Anti-bacterial activities on Mn (II) complexes of various Schiff bases such as Salicylaldehyde ethylenediamine, Salicylaldehyde Orthoaminophenol, Salicylaldehyde Semicarbazone and salicylaldehyde o-phenylene diamine along with NH_4SCN ligand were reported [8].

1, 10-Phenanthroline (phen) is a rigid, planar, hydrophobic, heteroaromatic system whose nitrogen atoms are marvellously situated to act cooperatively in cation binding. These structural features resolve its coordination towards metal ions and it act as strong bi-dentate ligand.

1,10 Phenanthroline is an inhibitor of metalloprotease, with one of the first observed instances reported in carboxypeptidase A [9]. Inhibition of the enzyme occurs by removal and chelation of the metal ion required for catalytic activity, leaving an inactive apoenzyme. Phenanthroline targets mainly zinc metalloprotease with a much lower affinity for calcium [10].

Copper complexes of 1,10 phenanthroline and its derivatives are able to target DNA and have been used as DNA nuclease as foot printing agents [11,12]. Modification of 1,10 phenanthroline copper complex has resulted in the discovery of a series of anticancer agents casiopeinas [13] and one of the complexes has been shown to induce apoptosis of murine leukemia cell lines [14].

The *in-vitro* antibacterial action of 1, 10 phenanthroline has been demonstrated on several species of bacteria. Copper complexes and metal complexes of Salicylaldehyde Semicar-

bazone and 1,10 phenanthroline exhibit numerous biological activities include anti-tumour, anti-batcterial, DNA binding and anti-fungal.

In the present study, we focus on synthesis of copper(II) metal complexes by using Salicylaldehyde Semicarbazone and the copper(II) complex of 1,10 phenanthroline and investigate the antibacterial activity of each metal complexes and ligand against selected bacteria.

2 Materials & Methodology

2.1 Materials

Semicarbazide hydrochloride (analytical grade), sodium acetate trihydrate, salicylaldehyde, copper nitrate trihydrate and 1,10 Phenanthroline monohydrate (analytical grade) were used without further purification.
Methanol (GRP), Ethanol (95%), IMS, Dichloromethane (WINLAB GRG 98%) and DMSO (BDH lab, England 99%) were used as solvents. Nutrient agar medium (Include-Peptone, Agar, sugar, marmite) was used to check anti-microbial activity.
Ligand 1: Salicylaldhyde semicarbazone was used.
Ligand 2: 1,10 Phenanthroline was used

2.2 Methodology

Synthesis of ligand (L1)
Semicarbazide hydrochloride (11.2g) and sodium acetate trihydrate (16.8 g) were dissolved in distilled water (100 ml) with continuous stirring. The aqueous solution of salicylaldehyde (10.6 ml) was added drop wisely with vigorous stirring until white color suspension was observed. Then the mixture was refluxed for one hour. This mixture was cooled to room temperature. The product was filtered and recrystallized in water [15].

Synthesis of Copper complex- P1
Salicylaldehyde semicarbazone (0.008 mol) was dissolved in dichloromethane (40 ml) in a three neck round bottomed flask (250 ml) using heating mantle with magnetic stirrer and thermo-couple. A solution of $CuNO_3.3H_2O$ (0.008 mol) in methanol (25 ml) was added drop wise to the solution of H_2SAL with constant stirring. It was refluxed for 3 hours at 40 °C. The reaction mixture was filtered and cooled to room temperature. The solution was allowed for slow evaporation. Single crystals were collected, recrystallized and dried in vacuum [16].

Synthesis of Copper complex- P2
A dichloromethane solution of $CuNO_3.3H_2O$ (0.008 mol, 30 ml) was taken in a three neck round bottomed flask (250 ml) using heating mantle with magnetic stirrer and thermo-couple. A methanol solution of 1, 10 Phenanthroline monohydrate **-L2** (0.016 mol, 30 ml) was added to the above solution drop wisely. Reaction mixture was refluxed for 2 hours at 40 °C. The reaction mixture was filtered and cooled to room temperature. This solution was kept for slow evaporation. Single crystals were collected, recrystallized and dried in vacuum.

2.2 Physical Measurements

Melting points of the ligand and products was measured using Gallenhamp melting point apparatus. The Infrared spectroscopic data was recorded on Thermo Scientific Nicolet IS10 spectrometer in the range of 4000-400 cm^{-1}. It was carried out in University of Sri Jayewardenepura, Sri Lanka.
Electronic spectra were recorded on Double beam scanning UV/VIS Spectrophotometer: BK-D580, in the range of 190-1000 cm^{-1}, by using 10 mm Quarts cuvette and solvent DMSO.
Conductivity measurements were carried out in PL-700AL multi parameter using conductivity probe, all products were dissolved in DMSO. Conductivity measurements were taken at the room temperature (28.3°C). This was carried out at the Department of Chemistry, Eastern University, Sri Lanka.

2.4 Antimicrobial Screening

Media used: Nutrient broth and Nutrient agar were used.

Bacterial Culture: Pure bacteria cultures of Staphylococcus aureus and Escherichia coli were brought from Batticaloa teaching hospital and they were sub cultured in nutrient agar medium and incubated for 24 hours in the incubator at 37 °C.

Antibacterial activity: Antimicrobial activity of synthesized metal complex and ligands were determined by standard agar well diffusion method 18, 19. Nutrient agar medium was prepared and petri-plates were prepared carefully in the laminar flow in the Department of Botany, EUSL. Agar surface of each plate was streaked by a sterile cotton swab with the respective bacterial culture. Then 5 mm diameter wells were punched in the agar surface using a sterile cork-borer and 30 µL (600 ppm, 300 ppm, 150 ppm and 75 ppm concentrations of metal-complexes and ligand in DMSO solution) of the sample solutions were poured into the wells.

The plates were allowed to standby for 30 minutes to 1 hour. The plates were incubated for 18-24 hours at 37 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Amino-penicillin (Amoxicillin) was used as reference antibacterial agent. These tests were carried out in triplicates.

3 RESULTS AND DISCUSSION

3.1 Materials

The conductivity measurements were taken in DMSO at room temperature. L1 and L2 have very low conductivity value while their respective metal complexes P1 and P2 have high melting point values. According to the conductivity data given

in **Table 3.1**, we can clearly say that NO_3^- anion may be present outside the co-ordination sphere.

The metal complexes P1 and P2 have high melting point values than their respective ligands L1 and L2. According to the melting point data given in **Table 3.2**, higher values of melting points of P1 & P2 than their respective ligands confirm the formation of metal complexes.

Table 3.1- The conductivity values of the complexes and ligands.

Compound	Melting point (°C)
L1	228
L2	117
P1	306
P2	298

Table 3.2- Melting point of the ligands and complexes.

Compound	Conductivity (µs/cm) at 28.3 °C
L1	1.0
L2	1.0
P1	199.9
P2	182.4

3.2. Infrared Spectral Studies

The IR bands are most useful for the determination of compounds as well as co-ordination pattern.
The IR spectrum of complexes (P1 and P2) and ligands (L1 and L2) show some characteristic bands and shifts. IR spectroscopic assignments for the ligands and metal complexes are shown in fig. 3.1, fig. 3.2, fig. 3.3 and fig. 3.4.

According to **fig. 3.1** and **fig. 3.2** : Sharp band of 1682 cm^{-1} responsible for C=O stretching frequency of semicarbazone is shifted to 1666 cm^{-1}. Usually the IR stretching frequency of C=O group is reduced due to the lengthening of C=O bond length, because of donation of lone pair of electrons of oxygen atom of carbonyl group to the metal centre during chelation. Sharp band around 1583 cm^{-1} responsible for C=N stretching frequency of shiff base region is shifted to 1597 cm^{-1}. These observations clearly describe the formation of the metal complex- P1 and the ligand-L1 is chelated to the metal centre via hydroxyl oxygen, carbonyl oxygen and azomethyl nitrogen.

According to **fig. 3.3** and **fig 3.4**: The IR band at 3372 cm^{-1} responsible for –OH stretching frequency due to the presence of H_2O of 1,10 Phenanthroline monohydrate is absent in the product-P2 due to the loss of -OH hydrogen during co-ordination via O atom of the hydroxyl group of the ligand. The IR band at 1645 cm^{-1} of C=C stretching frequency of the ligand is shifted to 1625 cm^{-1} in the product. IR band around 3045 cm^{-1} responsible for the C-H stretching frequency of the aromatic ring of the ligand is shifted to 3060 cm^{-1}. The IR values, of C-H bending vibrations 622 $cm^{-1}$and 691 cm^{-1} observed for phenanthroline are shifted to 651 $cm^{-1}$and 720 cm^{-1}. These shifts reveal that each of the two nitrogen atoms of phenanthroline ligands donate a pair of electrons to the central copper metal forming a co-ordinate covalent bond.

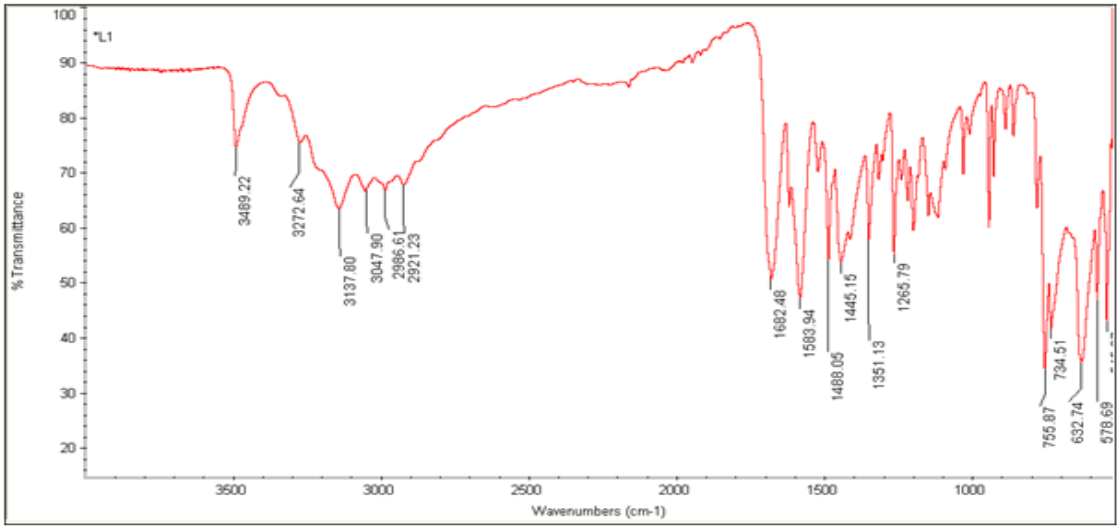


fig. 3.1 - The IR spectroscopic data of ligand-L1.

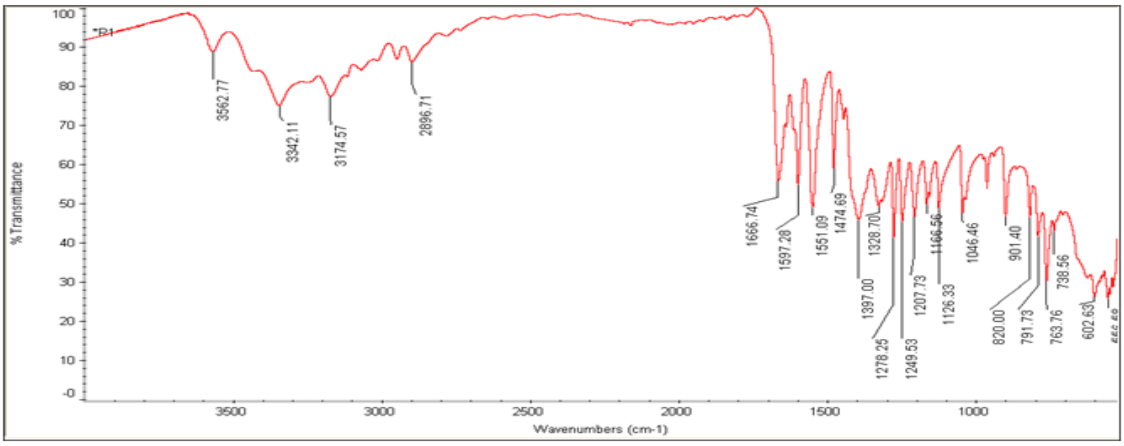


fig. 3.2- The IR spectroscopic data of metal complex-P1

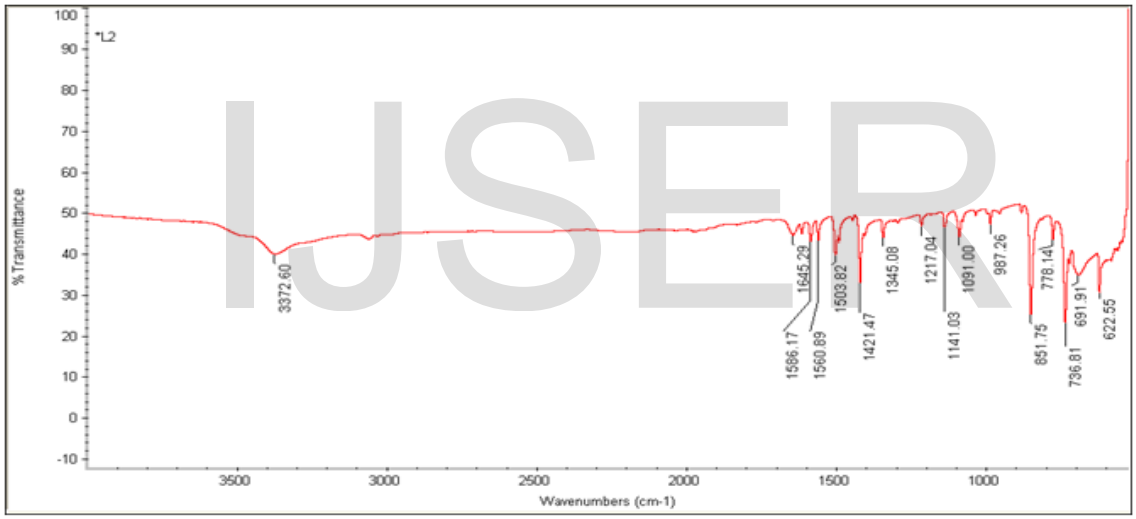


fig. 3.3 - The IR spectroscopic data of ligand-L2.

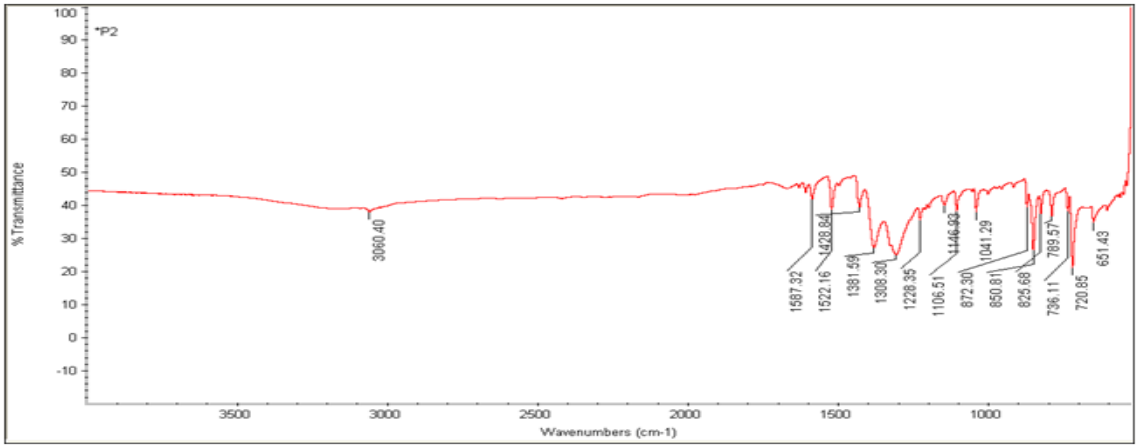
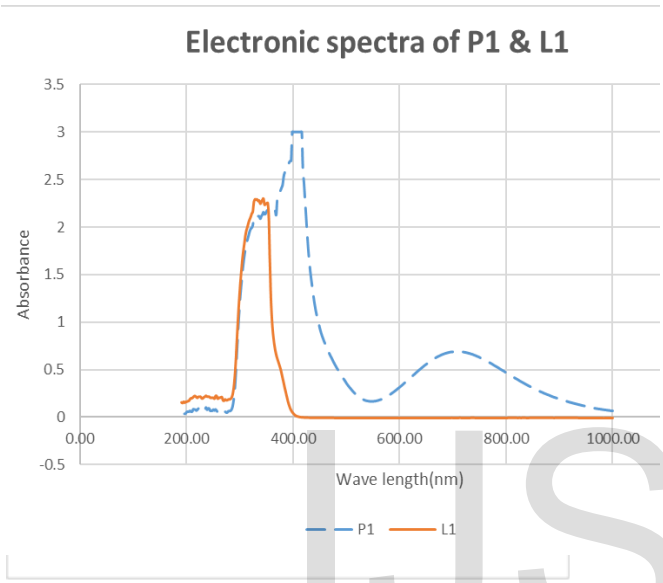


fig. 3.4 - The IR spectroscopic data of metal complex-P2

3.3. Electronic Spectral Studies

The electronic absorption band in the spectra of the ligand-L1, ligand-L2, metal complex-P1 and Metal complex-P2 were recorded in DMSO solution. Both ligands L1 and L2 have λ_{max} values in UV region, but the metal complexes P1 and P2 have λ_{max} values in both UV region and visible region.
Electronic spectra of the L1, L2, P1, & P2 are shown below in the **fig. 3.5** and **fig. 3.6**



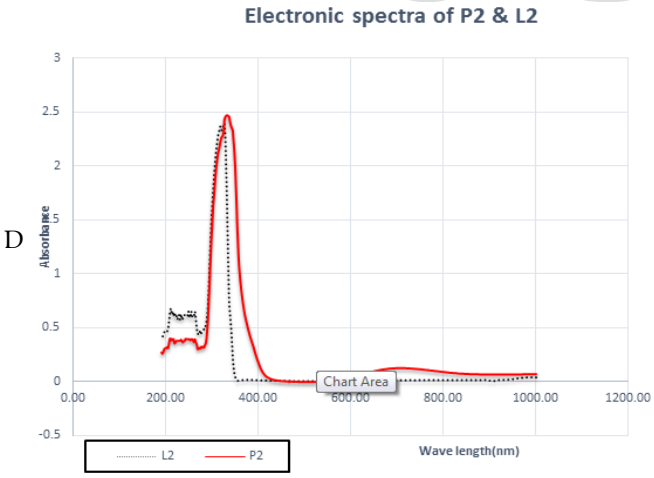


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fig. 3.5 shows the electronic absorption of copper (II) complex-P1 with its respective ligand-L1. Red shift with hyper-chromism has been observed for the P1 with its respective ligand L1. These observations clearly describe the formation of the copper(II) complex-P1. fig. 3.6 shows the electronic absorption of copper(II) complex-P2 with its ligand-L2. Red shift with hyper-chromism has been observed for the P2 with its respective ligand-L2. These observations clearly describe the formation of the copper(II) complex-P2.

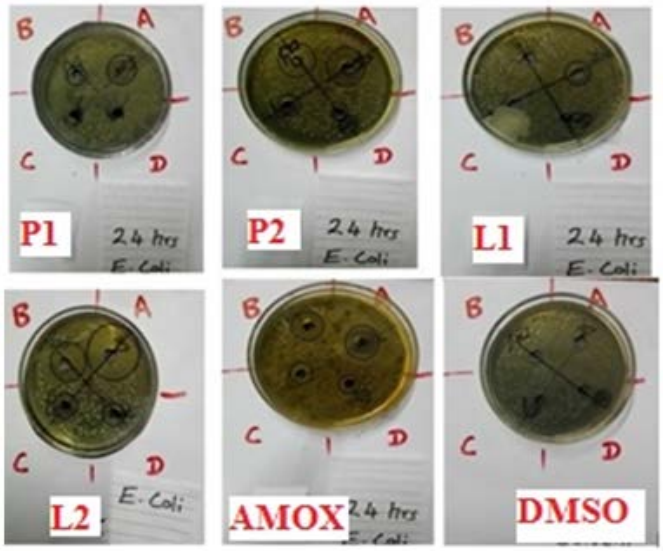

Fig 3.7 - Zone of inhibition for the compounds and reference anti-bacterial drug against *Escherichia coli*

3.3. Anti-microbial Screening

Table 3.3 and **3.4** show the diameter of inhibition zone of ligands, copper(II) complex and reference anti-bacterial drug against gram negative bacteria (Escherichia coli) as well as gram positive bacteria (Staphylococcus aureus). **fig. 3.7** and **fig. 3.8** show zone of inhibition for the compounds and reference anti-bacterial drug. DMSO was used as solvent control, it does not show any inhibition zone and thus no anti-bacterial activity.

fig. 3.6 - Electronic spectra of P2 and L2

Table 3.3 - Diameter of zone of Inhibition for *Escherichia coli*

Compounds	Diameter of Inhibition zone(mm) for ***Escherichia coli*** for different concentrations of compounds			
	600 ppm	**300 ppm**	**150 ppm**	**75 ppm**
L1	8±1	-	-	-
L2	35±1	26±1	17±1	10±1
P1	12±1	8±1	-	-
P2	26±1	21±1	15±1	10±1
Amoxicillin	24±1	20±1	16±1	12±1

(Values are mean+/_ SD of three replicates)

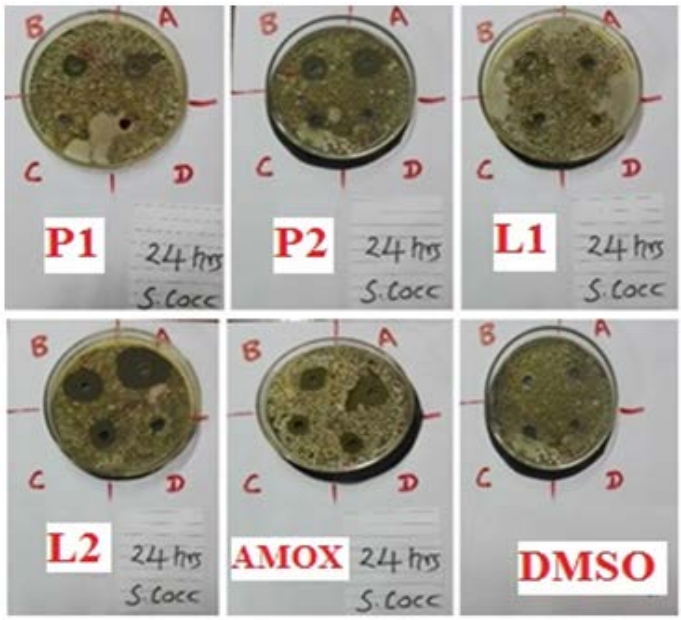

Fig 3.8 - Zone of inhibition for the compounds and reference anti-bacterial drug against ***Staphylococcus aureus***

According to the experimental results, anti-bacterial capacity against Escherichia coli is- L2 > P2 > Amox > P1 > L1.

Table 3.4 - Diameter of zone of Inhibition for *Staphylococcus aureus*

Compounds	Inhibition zone(mm) for ***Staphylococcus aureus*** for different concentrations of compounds			
	600 ppm	**300 ppm**	**150 ppm**	**75 ppm**
L1	9±1	-	-	-
L2	31±1	24±1	12±1	8±1
P1	24±1	16±1	7±1	-
P2	20±1	12±1	7±1	-
Amoxicillin	22±1	16±1	13±1	10±1

(Values are mean+/_ SD of three replicates)

According to the experimental results anti-bacterial capacity against
Staphylococcus aureus is- L2 > P1 > Amox > P2 > L1.

The metal complex of copper(II)-P1 exhibit high anti-bacterial activity against gram positive bacteria than gram negative bacteria. The metal complex of copper (II)-P2 exhibit high anti-bacterial activity against gram negative bacteria than gram positive bacteria.

1, 10 Phenanthroline is strong bi-dentate ligand; we have observed 1,10phen alone act as high anti-microbial agent. This is because; it may act as metalloprotease or deactivate the enzymes of the micro-organisms 17. But in metal complex it already in co-ordination with metal cation so it may not want to find another lone pair accepter.

But in the case of metal complex of copper (II) with salicylaldehyde semicarbazone, it shows high antibacterial activity than its ligand (L1). So, we can say metal complex show enhanced biological activity than its ligand.

4 CONCLUSIONS

In this work, we have successfully synthesised copper(II) complexes of Salicylaldhyde semicarbazone and 1,10 Phenanthroline. Formed complexes were characterized by using FTIR, UV visible spectral studies, melting point and conductivity measurements.

The significant bands and their shifts observed in IR spectroscopy confirm the co-ordination of ligands to metal and formation of the complex. Red/Blue shift and hyper/hypochromism shift of the products with respective ligands show the formation of the complexes. Conductivity studies suggest that the complex have nitrate (NO_3^-) ion outside the co-ordination sphere of the metal complex.

Anti-bacterial activity of metal complex and ligands were evaluated; all the complexes and ligands exhibit antibacterial activity in different range. However, copper (II) complexes showed higher anti-bacterial activity than commercially available antibacterial drug (amoxicillin).

1,10 Phenanthroline and metal complex-P1 show higher antibacterial activity against Staphylococcus aureus (gram Positive) while the other metal complex-P2 shows less activity than the standard anti-bacterial drug. However, 1,10 Phenanthroline and metal complex-P2 show higher antibacterial activity against Escherichia coli (gram negative bacteria) whereas the metal complex-P1 shows less activity than standard anti-bacterial drug. Salicylaldhyde semicarbazone shows less anti-bacterial activity among all compounds against gram Positive & gram Negative bacteria. L2 possesses high antibacterial activity against gram Positive & gram Negative bacteria than all other compounds and standard used.

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