

Synthesis, Characterization and Evaluation of Antibacterial Activity of a New Phenylmethylidene Thiourea Derivative and Its Copper (II) Complex

¹Samarasinghe, W.M.P., ^{1*}Sithambaresan, M. and
²Mahendranathan, C.

¹Department of Chemistry, Faculty of Science, Eastern University, Sri Lanka.

²Department of Botany, Faculty of Science, Eastern University, Sri Lanka.

For Correspondence E-mail: *sithambaresan@gmail.com

Abstract: A new thiourea derivative was synthesized by the condensation reactions of thiourea with benzaldehyde in ethanol. The copper complex was prepared from the reactions of the copper chlorides with newly synthesized phenylmethylidene thiourea derivative. The progress of the reaction was monitored by TLC using water - ethanol as solvent for every 30 minutes. The new ligand and its copper complex were characterized by UV- Visible spectroscopy and IR spectroscopy. The melting point of the synthesized ligand and its copper complex were determined and conductivity of the complex was measured. Synthesized ligand and the complex were screened for antimicrobial activity against two bacteria strains *Escherichia coli*, and *Staphylococcus aureus* using agar diffusion disc method.

Keywords: Antibacterial activity, Copper complex, Thiourea derivatives.

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Introduction

Thiourea is an important industrial chemical product and it shows a broad spectrum of biological activities such as anti-bacterial, antiviral, anticancer, anticonvulsant, analgesic and HDL-elevation properties (Asieh Yahyazadeh and Zahra Ghasemi, 2013). Recently, structural studies of active thiourea derivatives have shown that these compounds contain a central hydrophilic part and two hydrophobic moieties forming a butterfly like conformation. This conformation is a part of structure of anti HIV agent (Bielenica *et al.*, 2009). Thiourea derivatives and their metal coordinated compounds display important biological properties such as analgesic (Lorenados Santosa *et al.*, 2018), anti-inflammatory, antitumor, antibacterial (Badiceanu *et al.*, 2010), antifungal, antidepressant, anticonvulsant, anthelmintic, antihistaminic, anesthetic, antitussive anti-tubercular, anti-thyroid and insecticidal properties (Madan *et al.*, 1991). In addition to that certain transition metal complexes of thiourea derivatives have antioxidant activity and catalytic properties as well. Therefore, study of thiourea, thiourea derivatives and their transition metal complexes is very important and an interesting research area in chemistry.

A number of thiourea derivatives has been reported to form complexes with copper and cobalt (Domnguez *et al.*, 2002). Substituted thioureas are known to form stable, neutral coordination compounds with a variety of transition metal ions and some have been structurally characterized (Moloto *et al.*, 2003). Apart from their various applications mentioned, these ligands are of interest because they have three potential coordination sites (Ajibade and Zulu, 2011). These thiourea derivatives are very important in coordination chemistry as ligands which can be coordinated to metal Centre via doth sulfur and nitrogen atoms. These metal complexes are stable compounds and highly colored chelated structures (Mishra *et al.*, 2012). Thiourea derivatives and their transition metal complexes have been known for over a century and are easily synthesized in good yield (Mishra *et al.*, 2012). Metal complexes of thiourea commonly called semi organics include the advantages of both organic and inorganic part of the complex. In order to observe the enhanced complexing behavior of thiourea a number of its extended derivatives coupled with the β - di-ketones have been prepared as the powerful complexion agents (Mishra *et al.*, 2012). These 3-(N-phenyl) thioureapentanone-2 have been noticed to possess a remarkable capacity to coordinate with the transition metals giving rise to highly colored cheated structures.

Thiourea metal complexes display a wide range of biological activities including antibacterial, antifungal properties (Arslan *et al.*, 2009). Metal complexes of ligands containing sulfur as donor atoms are known to possess antifungal and antibacterial activities (Abdullah and Salh, 2010). Thiourea and its derivatives coordinate to several transition metal ions to form most stable complexes and in these complexes thiourea and its derivatives can able to coordinate to metal centres either as neutral ligands, monoanions, or dianions (Jadhao and Rathod, 2012).

The complexes with thiourea derivatives, which have biological activities, have been successfully screened for various biomimetic, antihistaminic, anesthetic, antitussive, analgesic etc. (Badiceanu *et al.*, 2010). The transition metal complexes usually show higher antibacterial activity than their constituent ligands. Such increased activity can be explained based on Overtones concept (Anjaneyulu and Prabhakara Rao, 1986) and the tweedy chelation theory. Metal based drug design was initiated by the discovery of cisplatin as anticancer agent after the successful studies of antibacterial activity of certain metal complexes. Recent researches have proved that those Cobalt, Copper, Zinc and Iron complexes of diisoprophylthiourea have a broad spectrum of antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Basicillus cereus*, *Staphylococcus aureus* and *Bacillus pumilus* (Ajibade and Zulu, 2011).

Infectious diseases are a major problem to the health especially in developing countries. Due to the rapid development of resistance to the existing antibacterial drugs, infectious diseases caused by bacteria remain as a major world health problem Even though the vast majority of bacterial diseases have been brought under control with available knowledge. Thus metal based antibacterial agents can be used to control the pathogenic bacteria. With the vast knowledge of Research in the field of Coordination Chemistry and Bio-Inorganic Chemistry along with the Computational Chemistry, these metal based coordination complexes of thiourea derivatives can be developed further as more effective antibacterial agents with lack of side effects in future.

The present investigation is aimed at synthesis and characterization of the thiourea derivative by condensation of thiourea with benzaldehyde. Furthermore, synthesis, characterization and

evaluation of antibacterial activity of the Copper (II) metal complex with the thiourea derivative.

Material and Methods

Chemicals

All the chemicals and solvents used for the synthesis of ligand and metal complex were of analytical grade and highest purity. Thiourea, benzaldehyde, cupric chloride and ethanol were purchased from Himedia Laboratories (Pvt) LTD, India and Hemas International (Pte) LTD.

Synthesis of the Ligand (L_1)

Benzaldehyde (0.08 mol) was dissolved in 15 ml of hot 95% ethanol and it was mixed with thiourea (0.04 mol) dissolved in 15 ml of hot 95% ethanol. Glacial acetic acid (5 drops) was added in to the mixture and then it was refluxed for 3 hours and 30 minutes. The progress of the reaction was monitored by TLC using water-ethanol as solvent for every 30 minutes.

Then the reaction mixture was allowed to cool and it was kept for one week without any disturbance. The formed crystal was filtered and washed with water and recrystallized from methanol. Formed crystal was dried and kept in the desiccator. The single crystals were obtained for the ligand. The reaction scheme for the synthesis of ligand is shown in Figure 1.

Figure 1. Reaction scheme for synthesis of ligand

Synthesis of Metal Complex with Ligands and Copper (II) (C_1)

Hydrated copper (II) chloride (0.175 g) was dissolved in 25 ml of ethanol and it was added to the hot ethanol solution of the ligand (0.504 g). Then the solution was stirred under reflux for 2 hours. The reaction mixture was allowed to cool and it was kept for 3 days. Then the green colored crude formed was collected by filtration. It was washed with cold methanol and recrystallized by methanol. The reaction scheme for the synthesis of complex is shown in Figure 2.

Figure 2. Reaction scheme for synthesis of metal complex

Instructions used for Characterization of the Ligand and the Metal complex

The ligand and the metal complex were characterized by using the UV-Visible (UV/VIS SPECTROPHOTOMETER: BK-D580) in range (150-600) nm of 1000 ppm solution in ethanol at RT and IR (THERMO SCIENTIFIC NICOLET IS10-spectrometer) spectroscopy in the range (500-4000) cm^{-1} . Melting point of ligand and metal complex were determined by using Gallenhampt melting point apparatus. The conductivity of metal complex was measured with ethanol solution by using PL-700 AL multi parameter at RT at the Department of Chemistry, Faculty of Science, Eastern University, Sri Lanka.

The methods used to evaluation Antibacterial Activity

Bacterial Culture: Pure bacteria cultures of *Staphylococcus aureus* and *Escherichia coli* were brought from teaching hospital, Batticaloa. Each culture was sub cultured in nutrient agar (NA) medium and incubated for 24 hrs. in the incubator at 37°C during the studies. This was done at the Department of Botany, Faculty of Science, Eastern University, Sri Lanka.

Antibacterial activity: Antimicrobial activity of synthesized metal complex and ligand were determined by standard agar disc diffusion method. Nutrient agar medium was prepared and sterilized in autoclave at 121 °C (15 lbs/sq.in) for 2 hours. Then it was poured in to petri-plates carefully in the laminar flow in the Department of Botany, EUSL. Agar surface of each plates was streaked by a sterile glass spreader with the respective bacterial culture.

Then 6mm sterile whatman paper discs impregnated with 1000 ppm, 500 ppm, 250 ppm and 125 ppm concentrated solutions of the ligand and the metal complex were placed on surface the above inoculated agar plates at labeled places as A, B, C and D respectively. The plates were allowed to standby for 1 hours. Then each plate was labeled with including culture name, test solution and date. 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. Ethanol was used as solvent control. Amino-penicillin (Amoxicillin) was used as reference antibacterial agent. The tests were carried out in triplicates. Then the mean diameter of inhibition zone was recorded. The inhibition zones produced by the ligand and the metal complex were compared with the reference.

Results and Discussion

Determination of melting point and conductivity

Color of the formed compounds, the melting point (MP), conductivity measurements, yield percentage were given in the Table 1. Melting points obtained for the ligand and the metal complex were different and complex has higher melting point than that of ligand. Therefore, complex is more stable than the ligand due to the coordination. Conductivity measurement was taken for the metal complex (Table: 1). It exhibits that the metal exists as Cu^{2+} .

Table 1. Physical properties and yield percentage of synthesized compounds

Compound	Color	MP ($^{\circ}\text{C}$)	Yield Percentage (%)	Conductivity ($\mu\text{s}/\text{cm}$)
L ₁	White	184	82.26	-
C ₁	Green	268	74.78	192

Analysis of UV- Visible spectra

The UV-Visible spectra are associated with the electron transition between the electronic energy levels. The UV-Visible Spectra of the ligand and the metal complex are displayed in Figure 3.

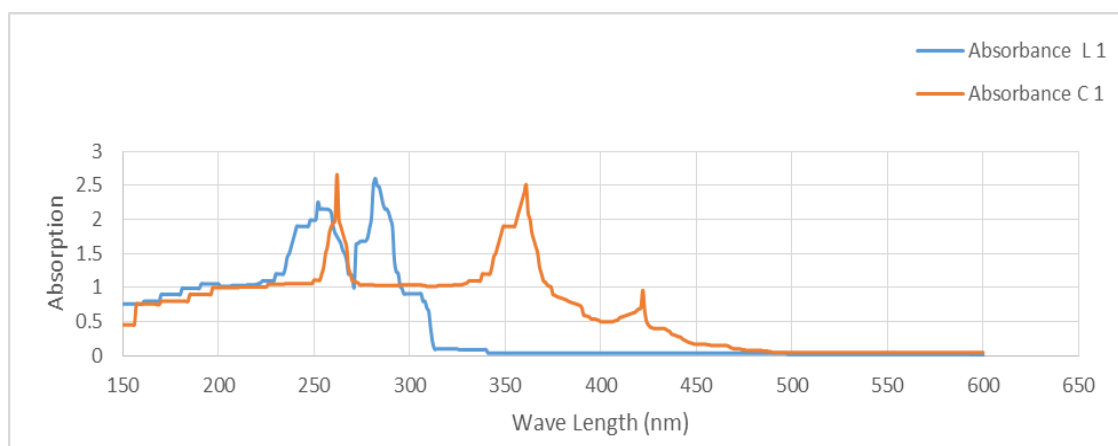


Figure 3. UV-Visible spectra for ligand and complex

The electronic spectra of the free ligand exhibited the high intense absorption peaks at 252nm and 282nm assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition respectively (Yusof *et al.*, 2015). In common transition metal complexes, the color consequences of light absorptions are due to the transfer of electron from an orbital primarily on ligand to one primarily located orbital on the metal Centre. In the electronic spectra of the formed complex, the intraligand transition are shifted as a result of coordination to the metal Centre.

There are 2 strong absorption peaks at 252 nm and 282 nm in the UV-Visible spectrum of the ligand whereas in the UV-Visible spectrum of the complex, there are two high intense absorption peaks at 262 nm, 360 nm and a weak absorption peaks at 422 nm. Shifting of these peaks clearly reveals that the ligand is coordinated to the metal centre.

Analysis of IR spectra

In order to conform to coordination sites of ligand to the metal ion in the complex, the IR spectral data of the ligand and the metal complex were analyzed on the basic of careful comparison of band shifts. The IR spectra of the ligand and the metal complex are given in

Figure 4 and 5 respectively. The IR Spectrum of the free ligand was obtained over a spectral range of $(4000-500) \text{ cm}^{-1}$. In the IR Spectrum of the free ligand, the absence of the absorption band at around 1740 cm^{-1} associated with -CHO aldehyde group stretching vibration indicates the loss of the aldehydic group of benzaldehyde during the formation of ligand by condensation reaction of benzaldehyde with thiourea.

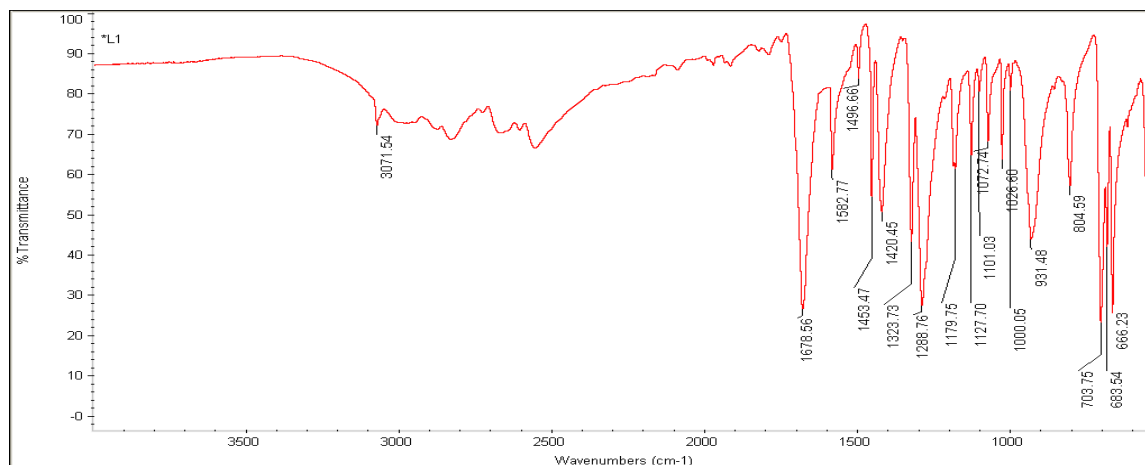


Figure 4. IR Spectrum of ligand

The absence of the two absorption bands above 3100 cm^{-1} associated with the primary amino group stretching vibration indicates the loss of the primary amino group of thiourea during the formation of ligand by condensation reaction of benzaldehyde with thiourea. Absences of the absorption bands associated with the free aldehyde group and primary amino group is strong evidence to the formation of the ligand.

The characteristic IR bands for the ligand: IR (cm^{-1}) 3071.54 (C-H), 1678.56 (C=N), 1582.77 (C=C), 1288.76 (C=S), 1026.60 (C-N).

The strong band at 1678.56 cm^{-1} associated with the stretching vibration of the C=N bond in the IR spectrum of the free ligand, shifted towards the lower wave number region (at 1605.94 cm^{-1}) in the IR spectrum of the metal complex indicates the participation of the -C=N- nitrogen atom in coordination to the metal centre. The C=N stretching frequency appears at 1288.76 cm^{-1} in the IR spectrum of the free ligand was shifted to the lower wave number region (1241.15 cm^{-1}) indicating the involvement of the >C=S Sulphur atom during coordination to the metal Centre. It clearly indicates the coordination of N atom and S atom to the metal Centre.

The characteristic IR bands for the metal complex: IR (cm^{-1}) 3041.90 (C-H), 1605.94 (C=N), 1573.64 (C=C), 1241.15 (C=S), 1018.01 (C-N).

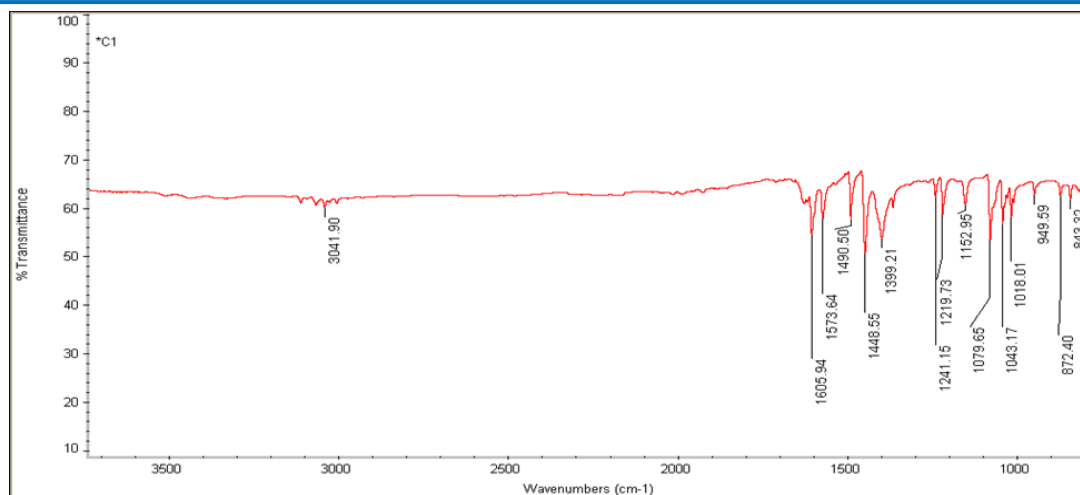


Figure 5. IR Spectrum of metal complex

Antimicrobial Screening

The synthesized and characterized ligand and the metal complex were tested to evaluate their antibacterial activity against on the two bacteria strains *Escherichia coli*, and *Staphylococcus aureus* using agar diffusion disc method. Antibacterial activities of the test compounds against *Staphylococcus aureus* is shown in Figure 6.

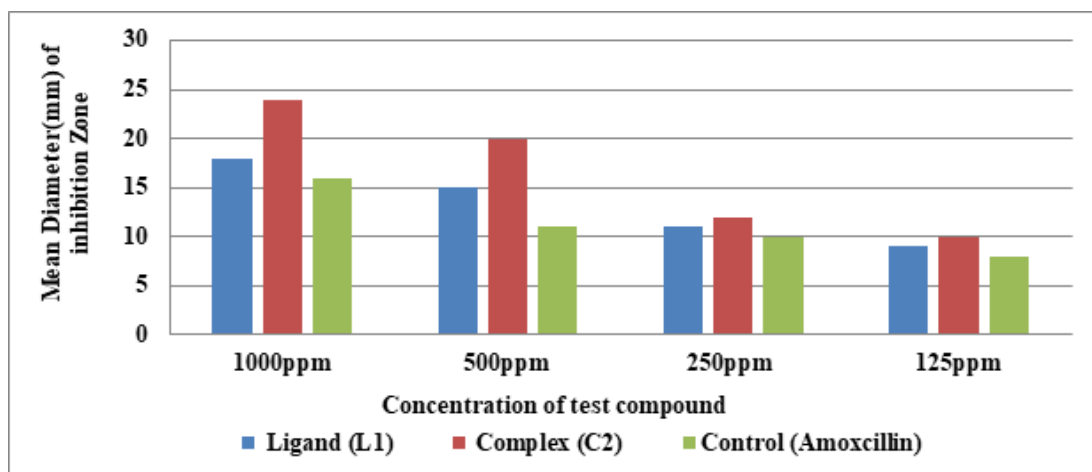


Figure 6. Antibacterial activity against *Staphylococcus aureus*

Antibacterial activity against *Staphylococcus aureus* than the ligand and the amoxicillin at all tested concentrations. The ligand shows the high antibacterial activity against *Escherichia coli* than the amoxicillin at all tested concentrations.

Antibacterial activities of the test compounds against *Escherichia coli* is shown in Figure 7.

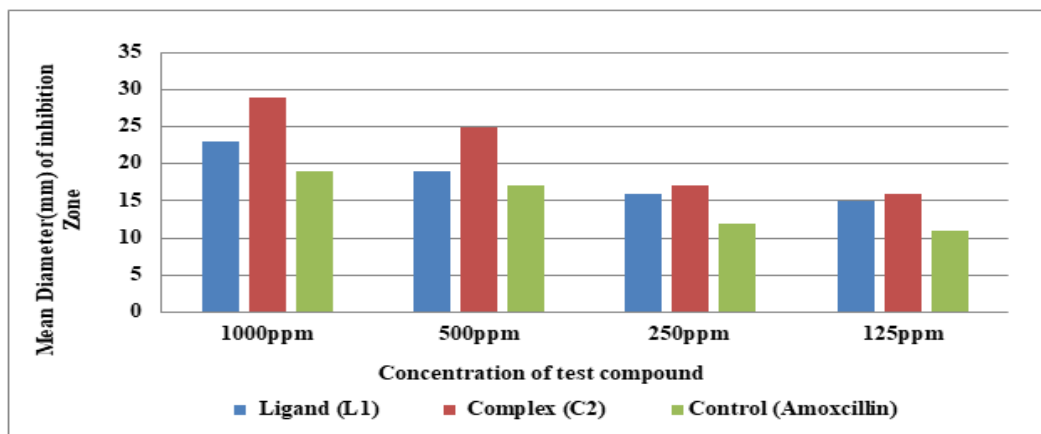


Figure 7. Antibacterial activity against *Escherichia coli*

The formed metal complex shows higher antibacterial activity against *Escherichia coli* than that of the ligand and the amoxicillin at all tested concentrations. The ligand itself also shows higher antibacterial activity against *Escherichia coli* than that of amoxicillin.

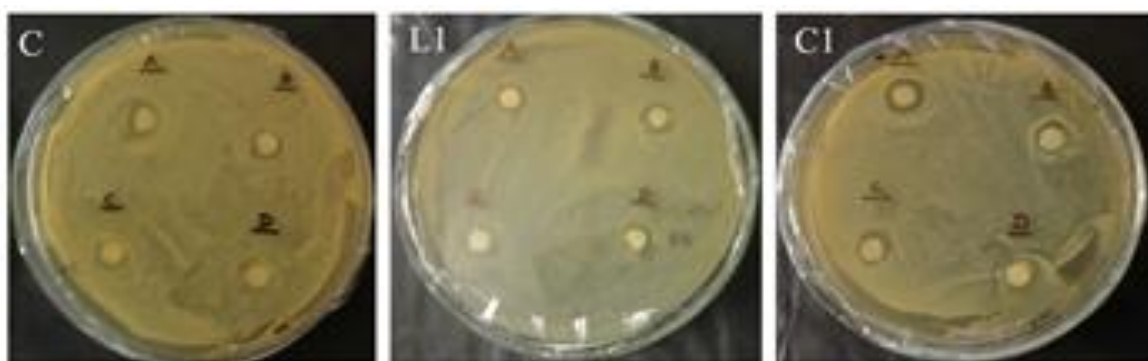


Figure 8. Zone of inhibition of growth of *E. coli* C- Control, L1- Ligand and C1- Complex

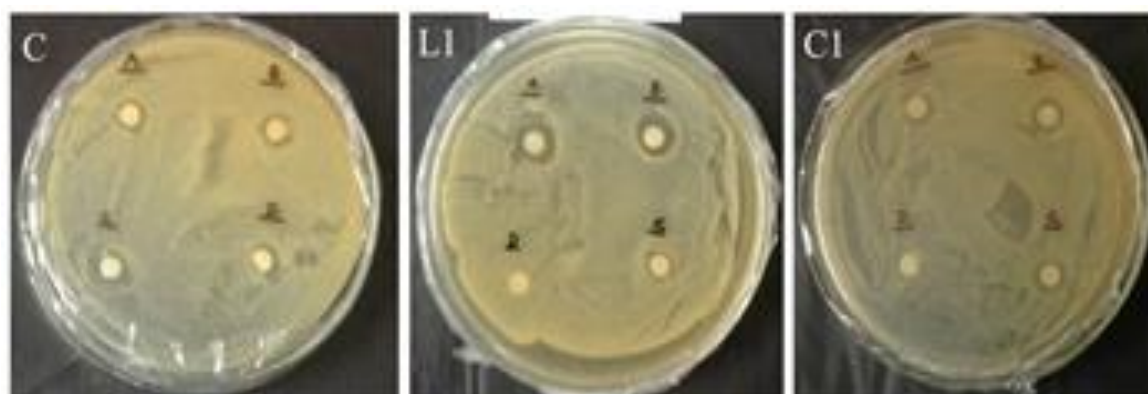


Figure 9. Zone of inhibition of growth of *S. aureus* C- Control, L1 -Ligand and C1- Complex

Conclusion

The phenylmethylidene thiourea derivative was successfully synthesized as a ligand by condensation reaction between benzaldehyde and thiourea in good yield. The single crystal was observed for the ligand. The formed ligand was characterized well by UV-Visible, IR and melting point determination. The copper complex of thiourea derivative was successfully

synthesized in considerable yield. The formed metal complex was characterized and coordination sites were confirmed by using UV- Visible and IR spectroscopy. Furthermore, 2:1 electrolytic behavior of the metal complex was attributed by the measurement of its conductivity. The synthesized ligand and its metal complex were tested to evaluate their antibacterial activity against two bacteria strains *Escherichia coli* and *Staphylococcus aureus* using agar diffusion disc method. The highest antibacterial activity was observed in the complex than in ligand against test bacteria and the higher inhibition zones were observed against the gram negative *Escherichia coli* than gram positive *Staphylococcus aureus*.

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