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SYNTHESIS, CHARACTERIZATION OF COPPER(II) COMPLEX WITH TETRADENTATE N_2O_2 AND POTENTIAL CONTRIBUTION AS PROMISING ANTIBACTERIAL STUDIES

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Abstract: A square planar Schiff base complex, Cu(H_2L) namely *N,N'*-bis(4-methyl-α-salicylidene) propane-1,3-diaminecopper(II) through the condensation reaction of ligand with copper(II) acetate in the molar ratio of 1:1 in acetonitrile solvent was successfully synthesized. The obtained complex was characterized using elemental analysis of carbon, hydrogen, and nitrogen (CHN), Fourier Transform Infrared spectroscopy (FTIR) and electronic spectra of ultraviolet-visible (UV-vis) spectroscopy. FTIR confirmed the coordinates the metal ion to form mononuclear complex via tetradentate (ONNO) atoms of the phenolic group and azomethine group, respectively. The antibacterial studies of H_2L and its Cu(H_2L) were determined by screening the compounds against selected various Gram (+) and Gram (-) bacterial strains such as *Staphylococcus epidermidis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholera*, *Enterobacter cloacae,* and *Escherichia coli*. Indeed, preliminary results exhibited Cu(H_2L) more active than the free ligand and displayed promising antibacterial activity, while no effect has been observed on strains for H_2L.

Keywords: N_2O_2 Schiff base, Cu(II) complex, spectral studies, antibacterial studies, biological activity.

Introduction

Schiff bases known as substantial class of organic compounds which play an important role in the development of coordination chemistry as they can easily form stable complexes with most of transition metals (Amiri Rudbari *et al*., 2016). It is formed from the reaction of primary amine and carbonyl group under several conditions. In this research, tetradentate N_2O_2 Schiff base ligand is used as it is widely applied in many industries and also biological studies.

Ligands and their metal complexes have different applications in different fields such as bioinorganic chemistry, material science, catalysis, separation and encapsulation processes, formation of compounds with unusual properties and metal–metal interactions (Abu-Dief & Mohamed, 2015; Soroceanu & Bargan, 2022). The most favorable metal that usually used to bind with the ligand is transition metal as it is the most abundance metals and reactive toward biological activity. Copper(II) ion was used in this study as it has vital roles in several biological processes and significantly in the action of different enzymes that catalyze a great variety of reactions (Shebl *et al*., 2017). Copper(II) Schiff base complexes also have been extensively studied because of their biological and pharmaceutical properties as well as in the field of catalysis and magnetism (Aouaidjia *et al*., 2017). It has been emphasized in various review papers that the biological activity of copper(II) complexes is enhanced with the presence of a nitrogen donor ligand (Shebl *et al*., 2017).

In the biological process, there are many types of reactions that can be studied and commonly are anti-bacterial, antifungal, antimalarial, and antivirus (Claudel *et al*., 2020; Malik *et al*., 2018). These common reactions have been focused on due to the demand and problem occurs caused by various kinds of bacteria that can affect health. The Schiff base ligands and metal complexes were tested towards the bacteria in order to study the effectiveness of the compound to inhibit the growth of the bacteria from spreading. This research is focused on synthesis and characterization of copper(II) Schiff base complex [Cu(H_2L)] using spectroscopic and analytical techniques. Then, the complex obtained was further applied for biological test using Agar well-diffusion method on specific strains of bacteria to observe their ability towards the biological study.

Materials and Methods

Materials and Physical Measurements

All chemicals and solvents used for synthesis and analysis were commercially available. FTIR spectrum was recorded using a KBr pellet on FTIR Perkin Elmer 100 spectrometer over a range of (400–4000) cm^{-1}. The percentages elemental analysis of C, H, and N in Schiff base metal complex, Cu(H_2L) was determined using Analyzer Flashea 1112 Series. Electronic absorption spectrum was recorded in methanol at room temperature on Shimadzu UV-1800 UV-Vis Spectrophotometer.

Preparation of Schiff Base Complex, Cu(H_2L)

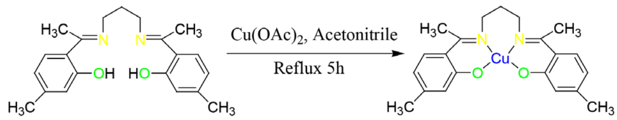
The Schiff base ligand (H_2L) was successfully prepared in our previous work and well characterized as reported in the literature (Che Soh & Shamsuddin, 2013). In a three-necked round bottom flask, Cu(H_2L) was prepared by mixing the synthesized H_2L (1 mmol, 0.34 g) with copper(II) acetate (1 mmol, 0.20 g) in the ratio of 1:1 (Scheme 1). The ligand and copper(II) acetate were dissolved separately in the acetonitrile (10 mL). Copper(II) acetate was then added into the flask containing the ligand solution. The mixture was stirred and refluxed for 5 hours. Then, the obtained dark green solid was separated by filtration and dried overnight in desiccator. The solid was recrystallized, if necessarily, in acetonitrile to obtain the desired pure complex Cu(H_2L). Cu(H_2L) complex: (FW: 399.97 g/mol, yield 78%, m.p.: 252°C). *Anal.* Calculate for $C_{21}H_{24}CuN_2O_2$: C, 63.14; H, 6.01; N, 7.02. Found: C, 62.55; H, 6.10; N, 7.18%. IR (KBr, ν, cm^{-1}): 1609 (s, C=N), 1540 (s, $C{=}C_{aromatic}$), 1151 (m, C-O), 516 (O-Cu), 471 (N-Cu). UV-Vis bands (λ_{max}, nm, (ε, M^{-1} cm^{-1}): $\pi\rightarrow\pi^*$, 224 (43811), 279 (23203), 385 (9764).

Antibacterial Activity Studies

The antibacterial activity of H_2L and its Cu(H_2L) complex were evaluated for six bacteria: *Staphylococcus epidermidis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholera*, *Enterobacter cloacae* and *Escherichia coli* using agar well-diffusion method (Hassan *et al*., 2018). The bacterial culture (50 µL) was swabbed onto Muller Hinton agar plate and labelled accordingly. Each plate contained eight well for eight different concentrations were placed with different concentrations of H_2L and its Cu(H_2L) between the range of 0 µg/µL to 10 µg/µL using two-fold dilution method in DMSO. An antibiotic disc, Oxytetracycline (30 µg) was used as positive control. The plates were incubated at 37°C for 18-24 h, for determination of antibacterial activity of H_2L and its Cu(H_2L), the inhibition zone (in mm) was measured, and the results were compared. Also, the minimal inhibitory concentration (MIC) of H_2L and its Cu(H_2L) were determined (Saadat *et al*., 2022). All experiments were done in triplicate.

Results and Discussion

In the present study, the Schiff base complex, Cu(H_2L) namely *N,N'*-bis(4-methyl-α-salicylidene) propane-1,3-diaminecopper(II) was obtained by condensing an equimolar amount of metal salt and the synthesized H_2L with 1:1 molar ratio (Scheme 1). The mononuclear complex is completely soluble in DMSO. The percentages of C, H, and N were determined experimentally by using CHN analyzer and the data are in good agreement with the proposed formulation with slightly different (±1.0%) compared to the theoretical values.



Scheme 1: Synthetic pathway of Cu(H_2L)

The Cu(H_2L) was successfully spectroscopically characterized using an FTIR and UV-vis. The FTIR spectrum of Cu(H_2L) showed a sharp and strong peak at 1609 cm^{-1}, which was primarily due to the azomethine group ν(C=N). The peak was shifted to the lower values in the case of metal complex compared to the ligand, particularly due to the electron-withdrawing effect on the coordinated metal, which decreases electron density in the imine bond thus leading to a lower frequency (Priya Gogoi *et al.,* 2022) (Es-Sounni *et al.,* 2023). This is further supported by the backward shift in phenolic ν(C-O) at 1151 cm^{-1}, confirming the participation of the phenolic oxygen towards the formation of C-O-Cu bond (Ejidike & Ajibade, 2015). Another peak at 1540 cm^{-1} appeared because of the ν(C=C) of aromatic ring. The appearance new bands at 516 cm^{-1} and 471 cm^{-1} are attributed to the ν(O-Cu) and ν(N-Cu) stretching vibration respectively (Joseyphus & Nair, 2008). The presence of these peaks concludes the formation of Cu(H_2L) with tetradentate H_2L binding through azomethine nitrogen and phenolic oxygen atoms.

The electronic spectrum of Cu(H_2L) was recorded in methanol solution with the range of 200–800 nm (Figure 1). The complex shows three peaks at 224 nm, 279 nm, and 385 nm, which assigned to π-π^* transition of phenolic chromophore at the fist band and the second and third bands corresponding also to π-π^* transitions of imine chromophore. On complexation, these bands were shifted to the lower wavelength region, suggesting the coordination of azomethine nitrogen with Cu(II) ion (Khandar *et al*., 2006).

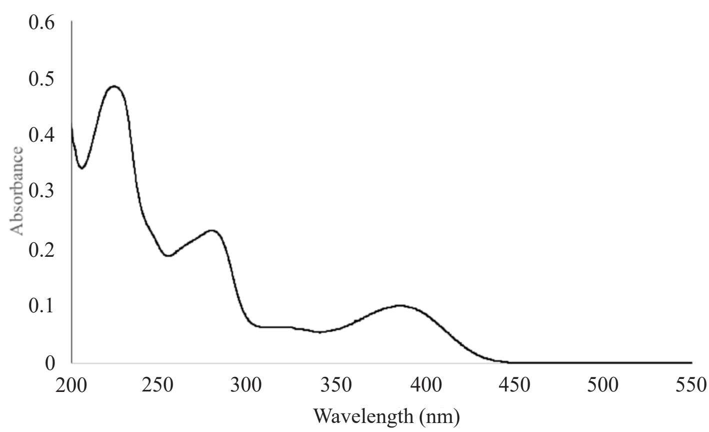


Figure 1: Electronic absorption spectrum of Cu(H_2L) complex in methanol

The antibacterial studies of H_2L (Table 1) and its Cu(H_2L) (Table 2) complex were tested in order to determine the abilities and potential of the synthesized compounds as antibacterial agents, against three Gram-negative bacteria namely *Escherichia coli, Enterobacter cloacae* and *Vibrio cholera*, and three Gram-positive bacteria namely *Staphylococcus epidermidis, Bacillus subtilis* and *Staphylococcus aureus* using well-diffusion method (Figure 2). The obtained results show that antibacterial activity of Cu(H_2L) against the microorganism is better than ligand [H_2L<Cu(H_2L)]. The free ligand H_2L shows no antibacterial activity against all tested bacteria strains. It can be shown that the Cu(H_2L) with zone of inhibition of 17.0±1.2 was found to be more effective against *Enterobacter cloacae*. The Cu(H_2L) is more active compared to free ligand and such enhanced activity of metal chelates is due to the lipophilic nature of the metal ion in complex (Shelke *et al*., 2012). As reported by Overtone, this liposolubility is a significant factor that governs the activity of antibacterial agents in which influenced the cell permeability. Further, the difference in geometry and steric hindrance effect also facilitates the penetration and inhibition of microorganism's cell (Abdel-Rahman *et al*., 2017). However, the Cu(H_2L) was found to be bacteriostatic against *Vibrio cholera* probably due to the different habitats, obtained low to high salinity in either free-floating or biofilm-associated lifestyles (Toulouse *et al*., 2018).

Table 1: The average diameter of inhibition zone (Mean ± SD) for different concentrations of H_2L against six different pathogenic bacteria strains. Oxytetracycline (30 µg) was used as positive control

Concentration (µg/µL)	**Type of Bacteria**					
	E. coli	***V. cholerae***	***E. clocae***	***B. subtilis***	***S. aureus***	***S. epidermidis***
10	0±0	0±0	5±7.1	4.6±6.6	0±0	0±0
5	0±0	0±0	4.7±7.1	4.0±5.7	0±0	0±0
2.5	0±0	0±0	4.3±7.1	7.7±5.6	0±0	0±0
1.25	0±0	0±0	0±0	7.3±5.2	0±0	0±0
0.625	0±0	0±0	0±0	7.7±5.4	0±0	0±0
0.3125	0±0	0±0	0±0	7.3±5.2	0±0	0±0
0.1563	0±0	0±0	0±0	7.0±5.0	0±0	0±0
0	0±0	0±0	3±7.4	5.6±4.0	0±0	0±0
Antibiotic	35	28	30	30	32	40

Table 2: The average diameter of inhibition zone (Mean ± SD) for different concentration of Cu(H_2L) against six different pathogenic bacteria strains. Oxytetracycline (30 µg) was used as positive control

Concentration (µg/µL)	**Type of Bacteria**					
	E. coli	***V. cholerae***	***E. clocae***	***B. subtilis***	***S. aureus***	***S. epidermidis***
10	16.3±1.2	0±0	17.0±1.2	13.6±0.9	11.6±0.9	14.6±0.5
5	14.0±1.0	0±0	16.7±2.6	11.7±0.5	9.7±0.6	12.7±0.5
2.5	14.7±0.5	0±0	15.7±1.7	12.0±0.8	9.7±0.6	10.0±0
1.25	12.7±0.5	0±0	14.0±1.4	11.3±0.5	0±0	0±0
0.625	11.3±1.3	0±0	14.7±0.5	10.3±1.3	0±0	3.3±4.7
0.3125	13.7±0.9	0±0	14.3±0.5	12.0±1.6	0±0	4.0±5.7
0.1563	15.0±0.8	0±0	16.3±1.9	11.7±1.3	0±0	0±0
0	13.0±0.8	0±0	16.0±1.0	9.6±1.7	0±0	0±0
Antibiotic	35	28	30	30	32	40

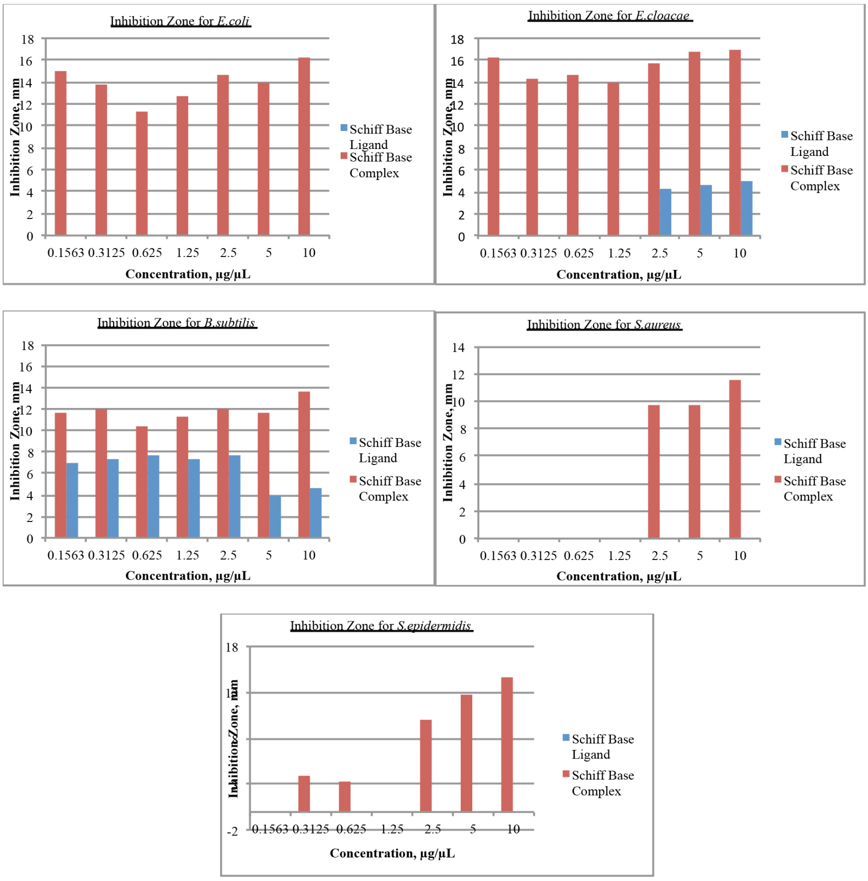


Figure 2: Antibacterial activity of H_2L and its $Cu(H_2L)$

Conclusion

As a conclusion, the Schiff base complex, $Cu(H_2L)$ was successfully synthesized from tetradentate N_2O_2 Schiff base ligand (H_2L) and copper(II) acetate and the complex structural was confirmed by FTIR, CHN, and UV-vis. The H_2L and $Cu(H_2L)$ were employed in bactericidal activities. The antibacterial activity analysis data revealed that $Cu(H_2L)$ has higher values of antibacterial activity than parental synthesized H_2L and it would be the part of future recommendation as this complex potentially used in other biological activities and pharmaceutical field due to the high ability to kill the microbes.

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References

Abdel-Rahman, L. H., Ismail, N. M., Ismael, M., Abu-Dief, A. M., & Ahmed, E. A. H. (2017). Synthesis, characterization, DFT calculations and biological studies of Mn(II), Fe(II), Co(II) and Cd(II) complexes based on a tetradentate ONNO donor Schiff base ligand. *Journal of Molecular Structure*, *1134*, 851-862. https://doi.org/10.1016/j.molstruc.2017.01.036

Abu-Dief, A. M., & Mohamed, I. M. A. (2015). A review on versatile applications of transition metal complexes incorporating Schiff bases. *Beni-Suef University Journal of Basic and Applied Sciences*, *4*(2), 119-133. https://doi.org/10.1016/j.bjbas.2015.05.004

Amiri Rudbari, H., Iravani, M. R., Moazam, V., Askari, B., Khorshidifard, M., Habibi, N., & Bruno, G. (2016). Synthesis, characterization, X-ray crystal structures and antibacterial activities of Schiff base ligands derived from allylamine and their vanadium(IV), cobalt(III), nickel(II), copper(II), zinc(II), and palladium(II) complexes. *Journal of Molecular Structure*, *1125*, 113-120. https://doi.org/10.1016/j.molstruc.2016.06.055

Aouaidjia, F., Messai, A., Siab, R., & Ayesh, A. I. (2017). A new tetranuclear copper(II) complex using a Schiff base ligand: Synthesis, structural, and magnetic studies. *Polyhedron*, *133*, 257-263. https://doi.org/10.1016/j.poly.2017.05.043

Che Soh, S. K., & Shamsuddin, M. (2013). Tetradentate N2O2 chelated palladium(II) complexes: Synthesis, characterization, and catalytic activity towards mizoroki-heck reaction of aryl bromides. *Journal of Chemistry*, *2013*, 1-8. https://doi.org/10.1155/2013/632315

Claudel, M., Schwarte, J. v., & Fromm, K. M. (2020). New antimicrobial strategies based on metal complexes. *Chemistry*, *2*(4), 849-899. https://doi.org/10.3390/chemistry2040056

Ejidike, I. P., & Ajibade, P. A. (2015). Synthesis, characterization, antioxidant, and antibacterial studies of some metal(II) complexes of tetradentate schiff base ligand: (4E)-4-[(2-{(E)-[1-(2,4-Dihydroxyphenyl) ethylidene]amino}ethyl)imino]pentan-2-one. *Bioinorganic Chemistry and Applications*, *2015*, 1-9. https://doi.org/10.1155/2015/890734

Es-Sounni, B., Nakkabi, A., Bouymajane, A., Elaaraj, I., Bakhouch, M., Filali, F. R., el Yazidi, M., el Moualij, N., & Fahim, M. (2023). Synthesis, characterization, antioxidant, and antibacterial activities of six metal complexes based tetradentate salen type bis-schiff base. *Biointerface Research in Applied Chemistry*, *13*(4), 1-14. https://doi.org/10.33263/BRIAC134.333

Hassan, M. A., Omer, A. M., Abbas, E., Baset, W. M. A., & Tamer, T. M. (2018). Preparation, physicochemical characterization, and antimicrobial activities of novel two phenolic chitosan Schiff base derivatives. *Scientific Reports*, *8*(1), 11416. https://doi.org/10.1038/s41598-018-29650-w

Joseyphus, R. S., & Nair, M. S. (2008). Antibacterial and antifungal studies on some schiff base complexes of Zinc(II). *Mycobiology*, *36*(2), 93-98. https://doi.org/10.4489/MYCO.2008.36.2.093

Khandar, A. A., Shaabani, B., Belaj, F., & Bakhtiari, A. (2006). Synthesis, characterization, and spectroscopic and electrochemical studies of new axially coordinated cobalt(III) salen (salen=N,N′-bis(salicylidene)-1,2-ethylenediamine) complexes. The crystal structure of [CoIII(salen)(aniline)2]ClO4. *Polyhedron*, *25*(9), 1893-1900. https://doi.org/10.1016/j.poly.2005.12.001

Knopp, P., Wieghardt, K., Nuber, B., Weiss, J., & Sheldrick, W. S. (1990). ChemInform Abstract: Syntheses, electrochemistry, and spectroscopic and magnetic properties of new mononuclear and binuclear complexes of vanadium(III), -(IV), and -(V) containing the tridentate macrocycle 1,4,7-trimethyl-1,4,7-triazacyclononane (L). crys. *ChemInform*, *21*(23). https://doi.org/10.1002/chin.199023256

Malik, M. A., Dar, O. A., Gull, P., Wani, M. Y., & Hashmi, A. A. (2018). Heterocyclic Schiff base transition metal complexes in antimicrobial and anticancer chemotherapy. In *MedChemComm*, *9*(3), 409-436. https://doi.org/10.1039/c7md00526a

Priya Gogoi, H., Singh, A., Barman, P., & Choudhury, D. (2022). A new potential ONO Schiff-Base ligand and its Cu(II), Zn(II) and Cd(II) complexes: Synthesis, structural elucidation, theoretical, and bioactivity studies. *Inorganic Chemistry Communications*, *146*, 110153. https://doi.org/10.1016/j.inoche.2022.110153

Saadat, A., Banaei, A., McArdle, P., & Jafari, R. (2022). Spectral, structural, and antibacterial study of Copper(II) complex with N2O2Donor Schiff base ligand and its usage in preparation of CuO nanoparticles. *Journal of Chemistry*, *2022*, 1-13. https://doi.org/10.1155/2022/8913874

Shebl, M., Adly, O. M. I., Abdelrhman, E. M., & El-Shetary, B. A. (2017). Binary and ternary copper(II) complexes of a new Schiff base ligand derived from 4-acetyl-5,6-diphenyl-3(2H)-pyridazinone: Synthesis, spectral, thermal, antimicrobial, and antitumor studies. *Journal of Molecular Structure*, *1145*, 329-338. https://doi.org/10.1016/j.molstruc.2017.05.064

Shelke, V. A., Jadhav, S. M., Patharkar, V. R., Shankarwar, S. G., Munde, A. S., & Chondhekar, T. K. (2012). Synthesis, spectroscopic characterization, and thermal studies of some rare earth metal complexes of unsymmetrical tetradentate Schiff base ligand. *Arabian Journal of Chemistry*, *5*(4), 501-507. https://doi.org/10.1016/j.arabjc.2010.09.018

Soroceanu, A., & Bargan, A. (2022). Advanced and biomedical applications of schiff-base ligands and their metal complexes: A review. *Crystals*, *12*(10), 1436. https://doi.org/10.3390/cryst12101436

Toulouse, C., Metesch, K., Pfannstiel, J., & Steuber, J. (2018). Metabolic reprogramming of *Vibrio cholerae* impaired in respiratory NADH oxidation is accompanied by increased copper sensitivity. *Journal of Bacteriology*, *200*(15). https://doi.org/10.1128/JB.00761-17