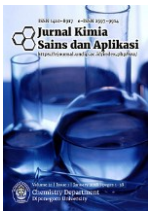
ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 28 (5) (2025): 267-273

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa



Synthesis and Antibacterial Testing of Cu(II)-3-Picolylamine Complexes

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https://doi.org/10.14710/jksa.28.5.267-273

Article Info

Article history:

Received: 20th March 2025
Revised: 22nd June 2025
Accepted: 24th June 2025
Online: 10th July 2025

Keywords:
synthesis; complex characterization; Cu(II) complex; 3-picolylamine; antibacterial activity

Abstract

The synthesis and characterization of a novel Cu(II)-3-picolylamine complex were successfully carried out, aiming to explore the structural properties and antibacterial potential. The complex was synthesized by reacting $CuSO_4.5H_2O$ with 3-picolylamine in a 1:4 molar ratio, yielding a dark blue precipitate (78.14% yield). Characterization techniques, including UV-Vis spectroscopy, FTIR, thermal analysis (TG/DTA), magnetic susceptibility, and powder XRD, confirmed the formation of the complex with the proposed formula $[Cu(3\text{-picolylamine})_4]SO_4.5H_2O$. The complex exhibited a square planar geometry around the Cu(II) ion, coordinated through nitrogen donor atoms of the ligand, with the sulphate ion acting as a counter ion. Thermal analysis revealed a two-stage decomposition process, with the release of five water molecules at 55–130°C and ligand decomposition at higher temperatures. Magnetic susceptibility measurements indicated paramagnetic behaviour with an effective magnetic moment of 1.86 BM, consistent with a d^9 configuration. Despite its well-defined structure, the complex showed no antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853 at all concentrations up to 1000 ppm. The lack of activity was attributed to reduced lipophilicity and the presence of hydrophilic counterions, hindering bacterial cell wall penetration.

1. Introduction

In recent years, the synthesis of complex compounds has become a pivotal point of effort in developing novel advanced materials and therapeutics. A complex compound consists of a central atom surrounded by donor molecules known as ligands, which can be neutral or charged ions. The complex compound formation is conditioned by the properties of the ligands, namely alkalinity, bonding, and chelating ability. The synthesis of these compounds also relies on reaction prerequisites, such as those of solvent type, solvent volume, temperature, pH, and the ratio of ligands to metals being reacted [1].

Transition metals are frequently used in the synthesis of complex compounds due to their varied chemical properties. The metal complex provides several benefits in catalysis, optics, electroluminescent devices, as well as biological applications such as anticancer, antibacterial, and antifungal agents [2]. Copper(II) is selected as the central atom in complexes mainly due to its abundance, environmental friendliness, and effectiveness against resistant bacteria, with a lower toxicity risk compared to toxic heavy metals, namely, lead (Pb) and mercury (Hg) [3]. Copper(II) is also noted for high complex stability when being coordinated with various ligands, as indicated in the Irving-Williams series: Ca(II) < Mg(II) < Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) [4].

Copper (II) complex can adopt various geometries such as square planar, tetrahedral, square pyramid, and octahedral. For instance, Zandvakili *et al.* [4] reported a square planar Cu(II) complex with deferasirox pyridine, where the metal center coordinates with two nitrogen

atoms (from triazolidine and pyridine) and one oxygen atom (from a hydroxyl group). In contrast, El-Gammal *et al.* [5] demonstrated that the (E)-4-(3-cyano-4,6-dimethylpyridin-2-ylamino)-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide ligand forms an octahedral Cu(II) complex, involving two oxygen and one nitrogen donor atoms, along with two water molecules and a nitrate ion. Additionally, Ragab *et al.* [6] observed a tetrahedral structure in a Cu(II) complex with a 2-oxoindole-derived ligand, coordinated via nitrogen, sulfur, oxygen atoms, and an acetate ion.

Meanwhile, Bergamini *et al.* [7] described a square pyramidal Cu(II) complex with a pyridine hydrazone ligand, coordinated through two nitrogen atoms, one oxygen atom, and two chloride ions. These variations highlight the critical role of ligand design in. Cu(II) complex containing nitrogen donor atoms exhibits distinctive characteristics and diverse structures. For example, copper (II) complex containing the ligand 3,5-dimethylpyrazole adopts a square planar geometry, where the primary amine nitrogen donor coordinates with the Cu(II) ion, enhancing complex stability and determining its antibacterial activity [8].

The heteroatomic compound 3-picolylamine (Figure 1), though structurally simple, serves as an effective ligand for Cu(II) complexes due to its dual nitrogen donors (pyridine ring and amine group). The small yet rigid pyridine ring enhances lipophilicity, while the flexible amine side chain promotes hydrophobic interactions. These features collectively improve membrane penetration by reducing polarity, thereby facilitating bacterial cell wall uptake and enhancing antibacterial activity [9].

Prior studies have synthesized Cu(II) complexes with picolylamine derivative ligands. Synthesis of Cd(II) and Zn(II) complexes with 3-picolylamine resulted in square planar and tetrahedral geometries, in which the metal ions are linked to the ligand via two nitrogen pyridine atoms and two SCN^- ions [10]. The study of Cu(II) complex with derivative 2-picolylamine Schiff base ligand (*ortho* amine group), showed the absence of antibacterial activity towards *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, due to ligand steric effect in *ortho* position that complicate the bond forming of Cu(II) and protein functional group and bacterial DNA namely –SH and $–PO^{3-}$ [11].

Another study that synthesized Cu(II) complex with derivative 4-picolylamine Schiff base ligand (*para* amine group), also demonstrated tenuous antibacterial activity, on account of the substituent group in the *para* position that enlarges the ligand molecule, making it difficult to penetrate the small pores of the bacterial cell wall [12].

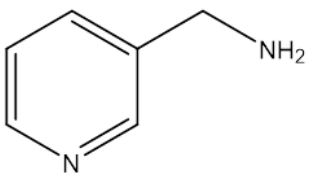

Figure 1. Structure of 3-picolylamine

Therefore, this study aims to synthesize Cu(II) complex with 3-picolylamine (containing a meta-positioned amine group) as a novel compound. The meta-positioned amine group is expected to minimize steric effects and enhance bacterial cell penetration, potentially improving antibacterial activity. This work aims to characterize the complex's structural and electronic properties and evaluate its antibacterial efficacy against *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853. This study advances the understanding of metal–ligand coordination and highlights the potential of the synthesized complex as an effective antibacterial agent.

2. Experimental

2.1. Materials

3-picolylamine (Sigma-Aldrich, 99%), $CuSO_4.5H_2O$ (Merck, 99%), methanol (Merck, 99,8%), dimethyl sulfoxide (Merck, 99%), distilled water, HNO_3 (Merck, 69%), dried KBr, $CuCl_2.2H_2O$ (Merck, 99%), $Cu(NO_3)_2.3H_2O$ (Merck, 99%), $NiCl_2.6H_2O$ (Merck, 98%), $CoSO_4.7H_2O$ (Merck, 99%), $CoCl_2.6H_2O$ (Merck, 98%), $FeSO_4.7H_2O$ (Merck, 99%), NaCl (Merck, 99.5%), Mueller Hinton Agar (MHA), paper disk (diameter 6 mm), Gentamicin 10 µg/disk, *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853.

2.2. Synthesis of Cu(II)-3-picolylamine Complex

The complex was synthesized following the methodology of Sobola *et al.* [11] with several modifications. The preparation started by dissolving $CuSO_4.5H_2O$ (0.249 grams; 1 mmol) and 3-picolylamine (0.4326 grams; 4 mmol) separately in methanol (10 mL). Subsequently, the $CuSO_4.5H_2O$ solution was continuously added to the 3-picolylamine solution. The solution was refluxed at 60°C with constant stirring for 20 minutes, yielding a dark blue precipitate. The product was washed with methanol, filtered, and finally dried in a vacuum desiccator overnight.

2.3. Characterization

The indication of complex formation and electronic spectra was determined using a UV-Vis spectrophotometer (Lambda 25, PerkinElmer) with DMSO as the solvent. Copper content was determined by Atomic Absorption Spectrophotometry (Shimadzu AA-6650) after HNO_3 digestion and dilution in 0.05 N HNO_3. Thermal analysis was conducted using TG/DTA (Diamond PerkinElmer) from 25–600°C at a heating rate of 10°C/min under nitrogen

Fourier Transform Infrared Spectroscopy (FTIR, Prestige-21 Shimadzu) was employed to identify functional groups, using KBr pellets in the range of 400–4000 cm^{-1}. The magnetic moment was measured using a Magnetic Susceptibility Balance (MSB, Auto Sherwood Scientific 10169). Electrical conductivity of the complex was evaluated using a conductometer (Jenway CE 4071) in DMSO at a concentration of 1×10^{-3} M. The intensity and d-spacing of the complex were analyzed using powder X- ray diffraction (XRD, D8 Advance Bruker).

2.4. Antibacterial Activity Testing

The antibacterial testing of the Cu(II)-3-picolylamine complex, 3-picolylamine ligand, and $CuSO_4.5H_2O$ was determined against *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853 employing the paper disk diffusion method [13]. The bacteria were obtained and sub-cultured by the microbiology laboratory of the Medical Faculty of Universitas Sebelas Maret. Each sample was dissolved in DMSO (negative control) and prepared in varying concentrations of 1000, 500, and 250 ppm.

Gentamycin served as a positive control. The bacterial colonies were suspended in a test tube containing 10 mL of 0.9% NaCl. The suspension was homogenized and its turbidity was equalized to the 0.5 McFarland standard. The antibacterial assay involved applying 15 µL of the complex, ligand, and $CuSO_4.5H_2O$ solution onto paper disks of 6 mm diameter, followed by placement into MHA and incubation at 37°C for 24 hours. The clear zone of inhibition diameter was observed with a calliper to evaluate the antibacterial efficacy [13].

3. Results and Discussion

3.1. Complex Synthesis

The synthesis of the Cu(II)-3-picolylamine complex, using a metal-to-ligand molar ratio of 1:4, produced 0.547 g (78.14% yield) of dark blue precipitate. The formation of the complex was confirmed by the UV-Vis maximum absorption wavelength shifting from 815 nm to 751 nm (Figure 2). This shift occurred due to the coordinated H_2O molecules on the Cu(II) ion being exchanged by the 3-picolylamine ligand, which has higher ligand strength compared to H_2O. The absorption wavelength shifted toward shorter wavelengths is also evidently similar in the electronic spectra of the $[Cu(MeCN)_4]ClO_4$ complex, with a notable transition from 823 nm to 450 nm [14].

3.2. Characterization of Complex

3.2.1. Copper Content Analysis

The measured copper content in the Cu(II)-3-picolylamine complex was 8.92 ± 0.05%. Experimental copper content was compared with calculated theoretical values to evaluate possible complex stoichiometries, as presented in Table 1. Using AAS, the Cu(II)-3-picolylamine complex was estimated to have the chemical formula Cu-(3-picolylamine)$_4$.SO_4.nH_2O (n = 5 or 6).

Table 1. Theoritical copper content of Cu(II)-3-picolylamine

Complex formula	Molecular weight (g/mol)	Cu content theoretically (%)
Cu(3-picolyl)$_4$.SO_4.$5H_2O$	682.171	9.32
Cu(3-picolyl)$_4$.SO_4.$6H_2O$	700.171	9.08

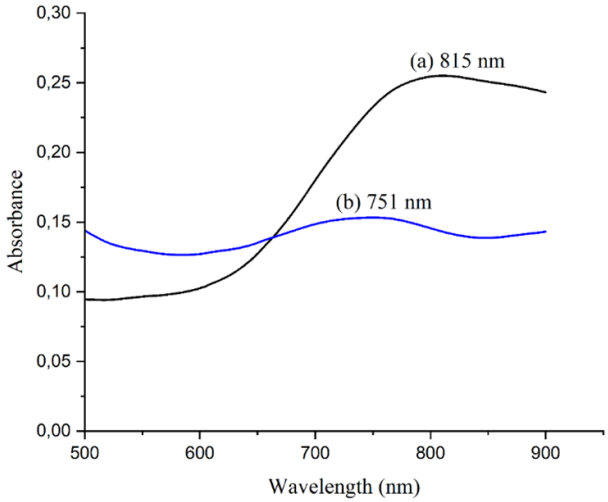

Figure 2. Electronic spectra of (a) $CuSO_4.5H_2O$, (b) Cu(II)-3-picolylamine in DMSO

3.2.2. Thermal Analysis

The thermal analysis and calculation (Supplementary data) were obtained by calculating the percentage of weight loss, based on the estimated molecular formula of the complex obtained from AAS analysis: Cu(3-picolylamine)$_4$.SO_4.nH_2O (n = 5 or 6). The thermogram of the complex in Figure 3 displays two stages of weight loss. The first stage, with a weight loss of 13.45% (calculated: 13.14%), occurred between 55–130°C and corresponds to the release of five water molecules (H_2O). The second decomposition stage exhibited a sharp decrease in weight until it stabilized at a temperature of 477°C, indicating the decomposition of the ligands, followed by the formation of a final CuO residue that was stable at high temperatures [5].

This thermogravimetric analysis is similar to the study of Neumann *et al.* [10], where the Cd(II) complex with the 3-aminomethylpyridine ligand underwent two stages of mass decomposition. The first stage of the complex compound lost 32% by weight in the temperature range of 50-130°C, indicating the release of H_2O molecules as crystal water. The 3-aminomethylpyridine ligand began to decompose at a temperature of 133-269°C, accompanied by the formation of metal oxide residues. Hence, resulting in the molecular formula of the complex is Cu(3-picolylamine)$_4$.SO_4·$5H_2O$ with a molecular weight of 682.171 g/mol.

3.2.3. Molar Conductivity

The electrical conductivity measurements of the Cu(II)-3-picolylamine complex with several standard solutions in DMSO (1.10^{-3} M) at room temperature are presented in Table 2. Molar conductivity values of the Cu(II)-3-picolylamine complex are close to those of $CuSO_4.5H_2O$, $CoSO_4.7H_2O$, and $FeSO_4.7H_2O$. This indicates that the complex contains two ions with a 1:1 ratio of cation to anion charge, with the SO_4^{2-} ion acting as a counter ion (not coordinated with the Cu^{2+} ion). Hence, the estimated molecular formula for the Cu(II)-3-picolylamine complex is $[Cu(3\text{-picolylamine})_4]SO_4.5H_2O$.

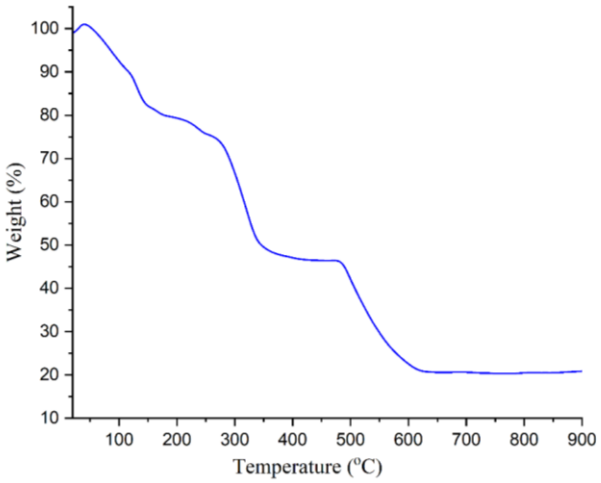

Figure 3. Thermal analysis spectra of the Cu(II)-3-picolylamine complex

Table 2. The electrical conductivity measurements of the Cu(II)-3-picolylamine complex with several standard solutions in DMSO (1×10^{-3} M)

Solution	Λ_m ($S.cm^2.mol^{-1}$)	cation:anion	Number of ions
DMSO	-	-	-
NaCl	6	1:1	2
$CuSO_4.5H_2O$	5	1:1	2
$CoSO_4.7H_2O$	7	1:1	2
$FeSO_4.7H_2O$	6	1:1	2
$NiCl_2.6H_2O$	65	2:1	3
$CuCl_2.2H_2O$	29	2:1	3
$CoCl_2.6H_2O$	52	2:1	3
$Cu(NO_3)_2.3H_2O$	86	2:1	3
Cu(II)-3-picolylamine	**9**	**1:1**	**2**

3.2.4. Infrared Spectra

The FTIR spectra examination of the ligand and the Cu(II)-3-picolylamine complex (Figure 4) reveals a shift in the absorption bands of functional groups in the 3- picolylamine ligand upon complexation with Cu(II). As summarized in Table 3, the ν(N-H stretch) shifts from 3354 - 3156 cm^{-1} to 3400 cm^{-1} (N-H asymmetric) and 3219 cm^{-1} (N-H symmetric), indicating the coordination of the 3-picolylamine ligand to the Cu(II) ion via the nitrogen donor atoms. This shifting is further supported by a new absorption band at 443 cm^{-1}, which corresponds to the Cu-N bond. The higher frequency of NH_2 shifting compared to C=N bonds suggests that the central Cu(II) ion would coordinate with the nitrogen atom of the primary amine group [11]. A similar experiment was reported for the complex [CuCl(ethyl-3-(4-acetyl-3-hydroxy-5-(phenylamino)thiophen-2-yl)-3-oxopropanoate)].H_2O) where the N-H stretching band shifted from 3324 cm^{-1} to 3352 cm^{-1} and Cu-N was confirmed with new absorption at 470 cm^{-1} [15].

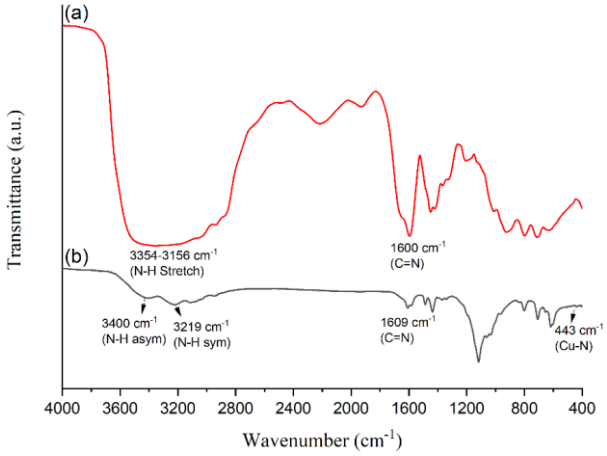

Figure 4. The FTIR spectra of (a) ligand, (b) Cu(II)-3-picolylamine complex

Table 3. Functional group absorption bands from FTIR spectra of the ligand and Cu(II)–3-picolylamine complex

Functional group	Theoretical range (cm^{-1})	Reference	Experiment wavelength (cm^{-1})	
			Ligand	Complex
N-H stretching	3500-3100	[16]	3354-3156	3400 3219
C=N	1617-1592	[17]	1600	1609
Cu-N	437-388	[12]	-	443

3.2.5. Electronic Spectra and Magnetic Moment

The calculation result of the effective magnetic moment (μ_{eff}) of the Cu(II)-3-picolylamine complex is 1.86 BM, indicating paramagnetic properties, which is close to the spin-only value for a single unpaired electron. The observed magnetic moment (1.86 BM) aligns with reported values for square planar Cu(II) complexes, such as the Cu(II)-*m*-phenylenediamine complex (1.70 BM, square planar) [18]. This supports our proposed square planar geometry, where the Cu(II) coordinates with four nitrogen donor atoms in a mononuclear arrangement.

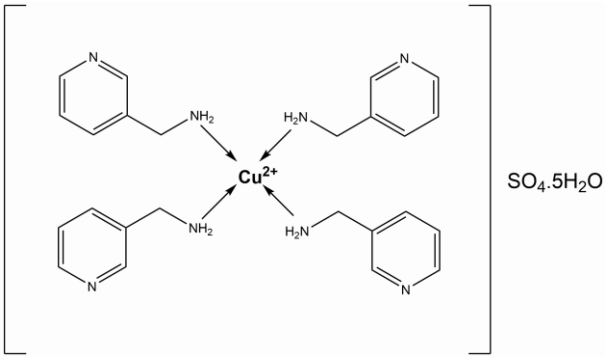

Figure 5. Suggested structure of [Cu(II)-3-picolylamine)$_4$]$SO_4.5H_2O$ with square planar geometry complex

The Cu(II)-3-picolylamine complex exhibits a single absorption peak at 751 nm with a molar absorptivity (ε) of 340.66 L·mol^{-1}cm^{-1} corresponding to the d-d ($d_{z^2} \rightarrow d_{x^2y^2}$) transition of Cu(II) in square planar geometry. A similar study was made for the complex bis[N-n-propyl-5-chloro-2-oxy-κO-benzylideneimine-κN-(1-)]copper(II) with a square planar geometry that exhibited an absorption band at 600 nm which and d-d electronic transitions characteristic [19]. The suggested complex structure is shown in Figure 5.

3.2.6. Crystal System of Complex

The powder X-ray diffraction results of [Cu(II)-3-picolylamine)$_4$]SO_4.5H_2O complex and $CuSO_4$.5H_2O are shown in Figures 6 and 7. These figures reveal that the diffraction peaks of the complex are distinct from those of $CuSO_4$.5H_2O, indicating that the complex has formed and is crystalline. The crystal system of the complex can be estimated by comparing the d-spacing (d(Å)) of the three highest peaks of the complex (Table 4) alongside those of other complexes with known crystal systems. d- spacing values are related to the lattice parameters a, b, and c; if these parameters are similar to those of a known structure, the corresponding d-spacing values will also be similar. It is estimated that the crystal system of this complex is analogous to that of the reference compound [18]. The d(Å) values of the three highest peaks of the [Cu(II)-3-picolylamine)$_4$]SO_4.5H_2O complex are similar to those of other complexes with a triclinic crystal system [20, 21].

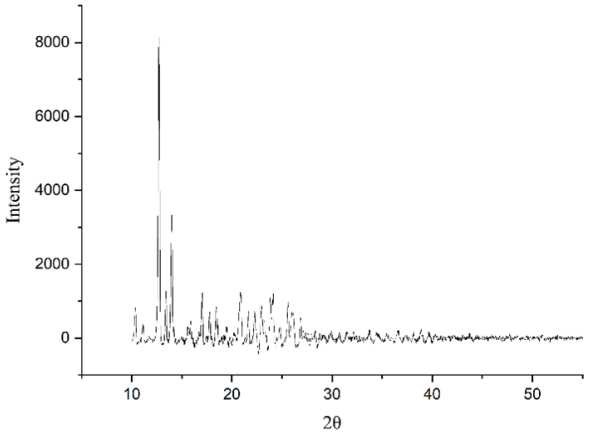

Figure 6. X-ray diffractogram of Cu(II) complex

Table 4. The three highest peaks of the X-ray diffractogram

Compound	2θ (°)	d (Å)
$CuSO_4$.5H_2O	18.745	4.730
	23.963	3.710
	22.253	3.991
Cu(II)-3-picolylamine	12.707	6.961
	13.996	6.322
	24.101	3.689

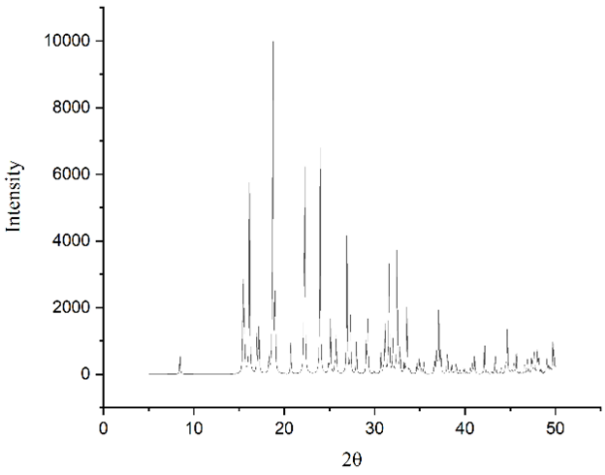

Figure 7. X-ray diffractogram of $CuSO_4$.5H_2O

3.3. Antibacterial Activity Testing

Contradicting to the hypothesis, the testing for antibacterial activity on $CuSO_4$.5H_2O, ligand, and the complex [Cu(3-picolylamine)$_4$]SO_4.5H_2O revealed no inhibition effect on *S. epidermidis* and *P. aeruginosa* at each concentration of 250, 500, and 1000 ppm. This testing result is indicated by the absence of inhibition zones around the discs (Figure 8). In contrast, the positive control, Gentamycin, showed inhibition zones of 14.09 mm for *S. epidermidis* and 23.15 mm for *P. aeruginosa* (Table 5). The lack of antibacterial activity is likely due to decreased lipophilicity and the presence of hydrophilic counterions (SO_4^{2-}), as evidenced by the compound's solubility being limited to polar aprotic solvents (DMSO). This reduced lipid solubility hinders cellular penetration through bacterial membranes [22, 23, 24].

This observation is consistent with the findings of Nurdiana *et al.* [18], who reported that Cu complexes with N-donor atoms and NO_3^{2-} as counterion showed no significant antibacterial activity against *S. epidermidis* and *P. aeruginosa*. However, the finding contradicts the antimicrobial study by Shiekh *et al.* [25] of Cu(II) with a mixed thia-aza-oxo macrocycle Schiff base ligand and sulphate anion that showed rather weak inhibition activity, most likely due to the thickness of agar medium and inoculum size. The report referred to the decrease in inhibition potency as the thickness of agar medium and inoculum size increase.

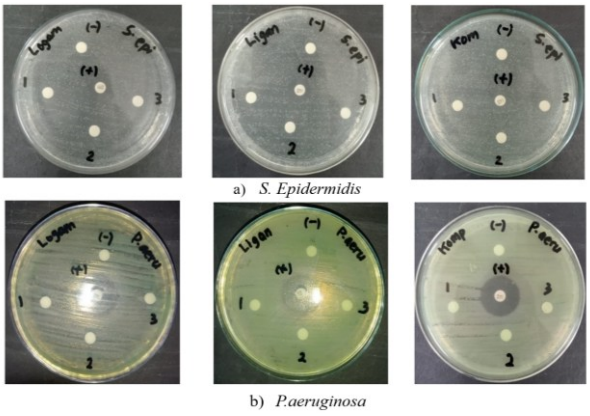

Figure 8. Antibacterial activity testing of (a) *S. epidermidis* and (b) *P. aeruginosa*

Table 5. Inhibition zone diameter measurement

Compound	Conc. (ppm)	Inhibition zone diameter (mm)	
		S. epidermidis	*P. aeruginosa*
$CuSO_4.5H_2O$	1000	-	-
	500	-	-
	250	-	-
3-picolylamine	1000	-	-
	500	-	-
	250	-	-
Complex Cu(II)	1000	-	-
	500	-	-
	250	-	-
Gentamycin		14.09	23.15

Note: Diameter of the paper disk = 6 mm; negative control = DMSO; "–" indicates no inhibition zone observed.

4. Conclusion

The complex in this particular study was synthesized effectively by refluxing $CuSO_4.5H_2O$ along with 3-picolylamine at a 1:4 mole ratio. The synthesis produced a dark blue precipitate with 95% purity. The complex formula is $[Cu(II)\text{-}3\text{-picolylamine})_4]SO_4.5H_2O$, where Cu^{2+} is coordinated through the N donor atom of the ligand and SO_4^{2-} ion acting as a counter ion (not coordinated with the Cu^{2+} ion). The complex exhibits a square planar geometry with a triclinic crystal system. The complex shows a maximum absorption wavelength at 751 nm and is paramagnetic with an effective magnetic moment (μ_{eff}) of 1.86 BM. Under the tested conditions, the complex showed no measurable antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853. However, this does not rule out potential activity under modified conditions or against other bacterial strains. Future studies could explore ligand modifications—such as the introduction of a hydrophobic tail—to enhance lipophilicity and improve antibacterial efficacy

Acknowledgement

The authors gratefully acknowledge the financial support 'Hibah Penelitian PNBP UNS 2025 Contract number: 371/UN27.22/PT.01.03/2025' provided by Sebelas Maret University.

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