



Dinuclear Co(II) and Zn(II) Azomethine Complexes: Physicochemical and Antibacterial Studies

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ABSTRACT

A symmetrical azomethine ligand L was synthesised from a reaction of *m*-phenylenediamine and *o*-vanillin in 1:2 molar ratio. Dinuclear complexes of Zn₂L₂ and Co₂L₂ have been successfully isolated and characterised through ¹H NMR, IR and magnetic moment. The x-ray crystal structure of Zn₂L₂ showed that the two Zn(II) nuclei were coordinated to two L moieties through the phenolic oxygen and imine nitrogen atoms, forming a slightly distorted tetrahedral geometry around the Zn(II) centres. When coordinated to metal centres, the signature ν(C=N) of Lat 1616 cm⁻¹ experienced a shift towards lower wave numbers of 1573-1613 cm⁻¹. The Zn(II) complex was diamagnetic whereas the Co₂L₂ complex was paramagnetic with 3 unpaired electrons having μ_{eff} = 4.07 B.M. An antibacterial screening against methicillin resistant *Staphylococcus aureus* (MRSA) revealed that the activity of the complexes was more pronounced than that of the uncoordinated L. The complex Zn₂L₂ revealed the lowest MIC value of 0.56 μg/μl MIC, indicating that it was a better bactericides than Co₂L₂ and L, in that order.

Keywords: Antibacterial, azomethine, binuclear, X-Ray crystallography

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INTRODUCTION

Azomethines, also known as Schiff bases, have imine-C=N- as the signature group in their structures. The synthesis is commonly performed through a facile condensation of primary amines with aldehydes or ketones. Their remarkable ability to coordinate with not just one metal centre but many, such as dinuclear (Clarke et al., 1998) and tetra nuclear metal complexes (Torayama, Nishide, Asada, Fujiwara, & Matsushita, 1997) has

triggered much interest in the coordination chemistry research fraternity due to the fascinating chemical structures and architecture of the metal complexes. This generates diverse exploration on the activities of the ligands as well as the complexes such as the study of anti-corrosion (Abdul Ghani, Bahron, Harun, & Kassim, 2014; Ramlee, Abu Bakar, Bahron, Harun, Kassim, & Yahya, 2010) and also bioactivity (Tajuddin, El Hassane, Ramasamy, Yamin, Alharthi, & Bahron, 2017) such as antibacterial and anti-cancer properties. It has been reported that Schiff base ligands exhibit numerous biological activities when coordinated to metals such as cobalt (Amirnasr, Schenk, Gorji, & Vafazadeh, 2001) and zinc (Shakir, Azim, & Parveen, 2006). The complexes are observed to be generally more biologically active than their parent ligands due to the presence of the metal moieties.

Of all the azomethine complexes, symmetrically coordinated salicylaldimines have drawn wide attention as they are very versatile compounds resulting from the flexible synthetic procedures. Changes in their carbonyl and diamine moieties could lead to a radical alteration of behaviour. For example, when using *o*-vanillin as the carbonyl, the character of the metal salicylaldimine complexes as ligands is drastically changed, transforming them from bidentate to exceedingly effective tetradentate ligands (Torayama et al., 1997) that can form stable complexes where coordination takes place through N_2O_4 donor groups (Gaballa, Asker, Barakat, & Teleb, 2007). The use of tetradentate Schiff base complexes is increasingly significant for designing metal complexes related to synthetic and natural O_2 carriers.

Meta-phenylenediamine (MPD) has not been as commonly used as *ortho*-phenylenediamine (OPD) in the synthesis of azomethine ligands due to the distance between the nitrogen atoms of MPD and the molecule (Ramlee et al., 2010), hampering successful chelation to one metal centre within the same ligand. However, this feature enables the MPD derived salicylaldimines to obtain the formation of complexes containing two ligand moieties, that is, dimers, where the two azomethine moieties are bridged by two metal centres (El-Wahab, 2007; Hernández-Molina et al., 1997) forming dimeric dinuclear complexes. The distinctiveness of such behaviour however has rarely been applied and studied in the biological field, unlike complexes derived from the other two aromatic diamines namely, the *ortho*- and *para*- analogues. Dinuclear complexes are suggested to enhance biological activity when compared to mononuclear complexes due to the presence of double equivalence of metal ions (Kaczmarek, Pospieszna-Markiewicz, Kubicki, & Radecka-Paryzek, 2004), that are most often the active site.

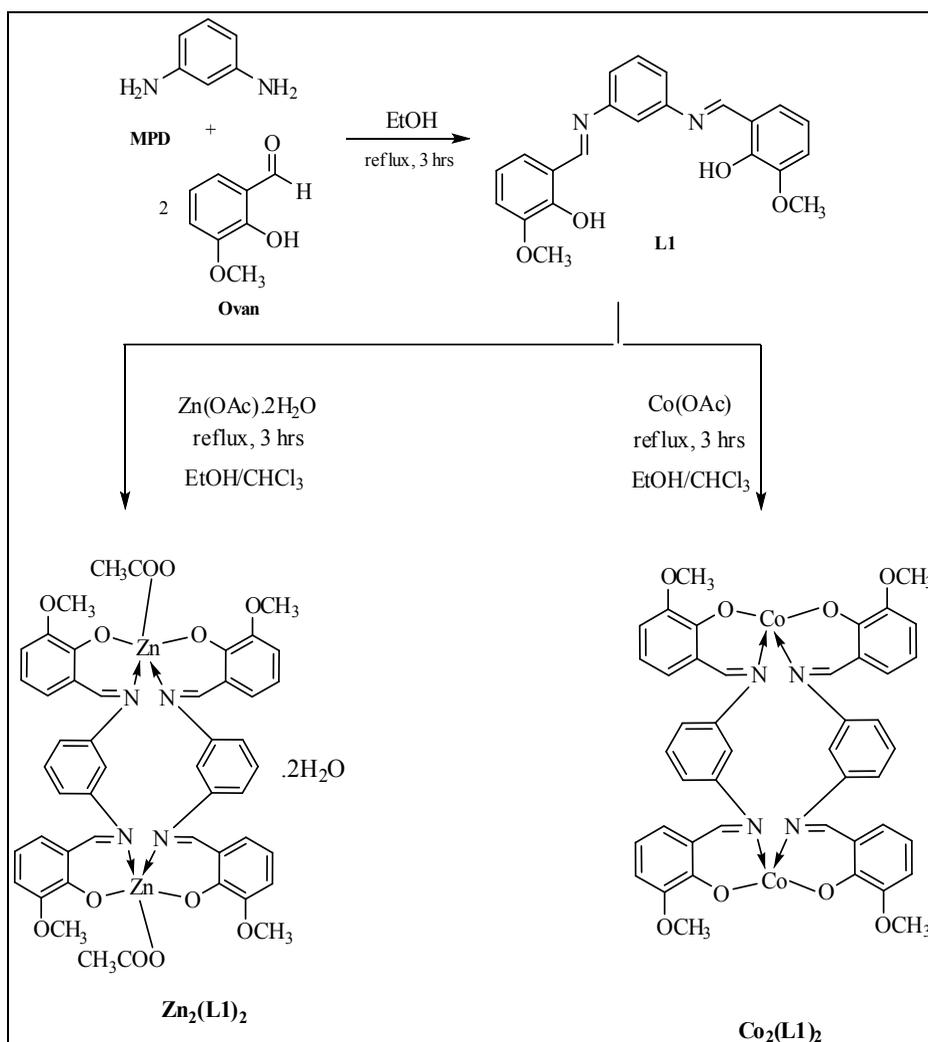


Figure 1. Reaction scheme for the synthesis of L and its dimeric dinuclear Zn(II) and Co(II) complexes

This paper reports the synthesis, characterisation and antibacterial studies of dinuclear Zn(II) and Co(II) complexes of symmetrical Schiff base ligand derived from *m*-phenylenediamine and *o*-vanillin, a salicylaldehyde derivative. The reaction scheme for the formation of the ligand L and its dimeric dinuclear Zn(II) and Co(II) complexes is illustrated in Figure 1.

MATERIALS AND METHODS

Materials

The solvents and reagents used in this research were ethanol, chloroform, dimethylsulfoxide (DMSO), *m*-phenylenediamine, *o*-vanillin (Ovan), cobalt(II) acetate tetrahydrate and zinc(II) acetate dihydrate. All chemicals were of analytical reagent grade purchased from Sigma Aldrich and used as received without prior purification.

Instrumentation

The microelemental analysis for C, H and N percentages were carried out on a Flash EA 110 Elemental Analyzer. Melting points of the ligand and complexes were determined in open capillary tubes using a BÜCHI Melting Point B-545 apparatus. ¹H NMR spectra for the free ligand and its diamagnetic zinc(II) complex were recorded on Bruker Avance 300 MHz as CDCl₃ solutions. Infrared (IR) spectra was recorded on Perkin Elmer 1600 Spectrometer in the 4000-400 cm⁻¹ range. The magnetic susceptibility measurements of the complexes were performed using Sherwood Auto Magnetic Susceptibility Balance.

Synthesis

Synthesis of L ligand. *O*-vanillin and *m*-phenylenediamine were mixed in 2:1 in absolute ethanol, refluxed and stirred under nitrogen for three hours. The orange precipitate which formed was filtered off, rinsed with cold ethanol and dried in-vacuo over blue silica gel.

Synthesis of Co₂(L)₂ and Zn₂(L)₂ complexes. A reaction mixture containing a 1:1 molar ratio of the Co(II)/Zn(II) metal acetate salts with ligand L in chloroform was refluxed and stirred under nitrogen for three hours. The resulting precipitate of brick red Co₂(L)₂ and yellow Zn₂(L)₂ were filtered off, washed with cold ethanol and dried under vacuum in a desiccator over blue silica gel. Recrystallisation was carried out from chloroform to afford reddish-brown and light yellow crystals, respectively. The air-sensitivity of Co₂(L)₂ hampered x-ray crystallography investigation at ambient conditions.

Antibacterial Study

An antibacterial screening against methicillin resistant *Staphylococcus aureus* (MRSA) was carried out using two methods.

Well diffusion method. The anti-bacterial property of L, Co₂(L)₂ and Zn₂(L)₂ was qualitatively evaluated using the well diffusion method as formerly reported by Gaballa Asker, Barakat and Teleb (2007). The samples were dissolved in DMSO with concentration of 10 µg/µl. Positive control experiments were carried out using commercially available standards, streptomycin and chloramphenicol.

Minimum inhibition concentration (MIC). The antibacterial activities of the ligand and complexes were investigated quantitatively using the minimum inhibitory concentration. A serial dilution method protocol was employed as described by Mohtar, Johari, Li, Isa, Mustafa, Ali and Basri. (2009) with slight modification where the microbial inoculums in the microtitre wells were directly exposed to the investigated compounds as pre-prepared solutions with serial concentrations of 4.5-0.04 $\mu\text{g}/\mu\text{lin}$ DMSO. The lowest concentration which completely inhibited visible microbial growth was recorded as the minimum inhibition concentration. Streptomycin and chloramphenicol standards were used as positive controls.

RESULTS AND DISCUSSION

The condensation of the aromatic diamines *m*-phenylenediamine with *o*-vanillin resulted in the formation of the azomethine L: MPD(ovan)₂. Subsequently, the ligand L reactions with Co(II) and Zn(II) metal ions afforded the metal complexes Co₂(L)₂ and Zn₂[(L)₂(OAc)₂].2H₂O. The analytical data and the physical properties are shown in Table 1.

Table 1
Physical properties of L and Its metal complexes

| Sample | Molecular formula | Molecular weight | Colour | Yield (%) | Melting point (°C) | Elemental analysis Found (Theoretical) (%) | | |
|----------------------------------|--|------------------|-----------|-----------|--------------------|--|----------------|----------------|
| | | | | | | C | H | N |
| L | C ₂₂ H ₂₀ N ₂ O ₄ | 376 | Orange | 72.2 | 129-131 | 69.95 (70.20) | 5.61 (5.36) | 7.42 (7.44) |
| Co ₂ (L) ₂ | C ₄₄ H ₃₆ Co ₂ N ₄ O ₈ | 867 | Brick red | 29.2 | >300 | 60.02 (60.98) | 4.31 (4.19) | 6.01 (6.46) |
| Zn ₂ (L) ₂ | C ₄₈ H ₄₆ N ₄ O ₁₄ Zn ₂ | 1033 | Yellow | 16.6 | >300 | 55.18 (55.77) | 4.50 (4.49) | 5.41 (5.42) |

The elemental analysis results of Co₂(L)₂ and Zn₂(L)₂ indicate that both are dimeric dinuclear complexes.

¹H NMR Spectroscopy

The ¹H NMR spectroscopic data of the diamagnetic zinc complex is compared to that of the free L ligand as shown in Table 2.

The shifting of the azomethine carbon proton peaks in the complexes upon coordination indicates that the azomethine N is involved in bonding with Zn. The shift towards the upfield region from 8.7 to 8.2 point out that the azomethine proton has become shielded with this coordination. The absence of the hydroxyl proton in the Zn(II) complex suggests that the hydroxyl groups underwent deprotonation in the complexation leaving the negatively charged oxygen to form ionic bonds with metal centres. Two new peaks are observed on the spectrum of the Zn₂(L)₂ complex, suggesting the existence of two acetate and two free water molecules (Silverstein & Webster, 1998) in the structure.

Table 2
¹H NMR data of L and its Zn(II) complexes

| Sample | ¹ H NMR (ppm) | | | | | |
|----------------------------------|--------------------------|-----------------------|----------------------|---------------------------------------|--------------------------------------|--------------------------------------|
| | Azomethine proton (HC=N) | Hydroxyl proton (-OH) | Aromatic protons | Methoxyl protons (-OCH ₃) | Free water proton (H ₂ O) | Acetate proton (CH ₃ COO) |
| L | 8.7 ^s | 13.5 ^s | 7.5-6.9 ^m | 3.9 ^s | - | - |
| Zn ₂ (L) ₂ | 8.2 ^s | - | 7.1-6.5 ^m | 3.9 ^s | 1.6 ^s | 2.2 ^s |

^s: singlet, ^m: multiplet

Infrared Spectroscopy

The mode of bonding between the ligands and the metal salts in complexes was examined by comparing the IR spectra of the complexes vs. that of the free ligand. Table 3 summarises the pertinent infrared peaks for the three compounds. L shows a strong band at 1616 cm⁻¹ which is the characteristic peak for C=N, indicating successful formation of the azomethine. The peak shifted to lower frequencies of 1573-1613 cm⁻¹ upon complexation with Co(II) and Zn(II), which means that the imine nitrogen atoms are involved in the chelation with the metal ions (Clarke et al., 1998), supporting the ¹H NMR data.

Similarly, the C-O stretching frequencies were observed to have shifted to lower frequencies from 1245 cm⁻¹ in the ligand to a lower values of 12.44 and 1215 cm⁻¹ in Zn₂(L)₂ and Co₂(L)₂, respectively. This indicates the participation of the oxygen atoms belonging to the phenolic hydroxyl group in the coordination to the metal ions (Golcu, Tumer, Demirelli, & Wheatley, 2005). However, the typically weak ν(M-N) and ν(M-O) peaks expected in the 600-400 cm⁻¹ region (Tajuddin et al., 2017) were unable to be detected due to the presence of many fingerprint peaks.

Table 3
 Infrared and magnetic susceptibility data of L, Co₂(L)₂ and Zn₂(L)₂

| Sample | Infrared peaks (cm ⁻¹) | | μ _{eff} (B.M.) |
|----------------------------------|------------------------------------|-------------|-------------------------|
| | C=N stretch | C-O stretch | |
| L | 1616 | 1245 | - |
| Co ₂ (L) ₂ | 1573 | 1215 | 4.07 |
| Zn ₂ (L) ₂ | 1606 | 1246 | - |

Magnetic Susceptibility

Co₂(L)₂ exhibited an effective magnetic moment, μ_{eff}, of 4.07B.M. (Table 3), slightly higher than that of the spin-only magnetic moment of three unpaired electrons (3.88 B.M.). The higher value suggested the presence of some orbital contribution to the magnetic moment values. There are two possibilities for the geometry of the 4-coordinate Co₂(L)₂ viz. tetrahedral or square planar. The presence of three unpaired electrons inferred by μ_{eff} value ruled out square planar geometry that would have only one unpaired electron as illustrated in the crystal field

splitting diagram shown in Figure 2. It is therefore suggested that the geometry of the Co(II) centres were tetrahedral.

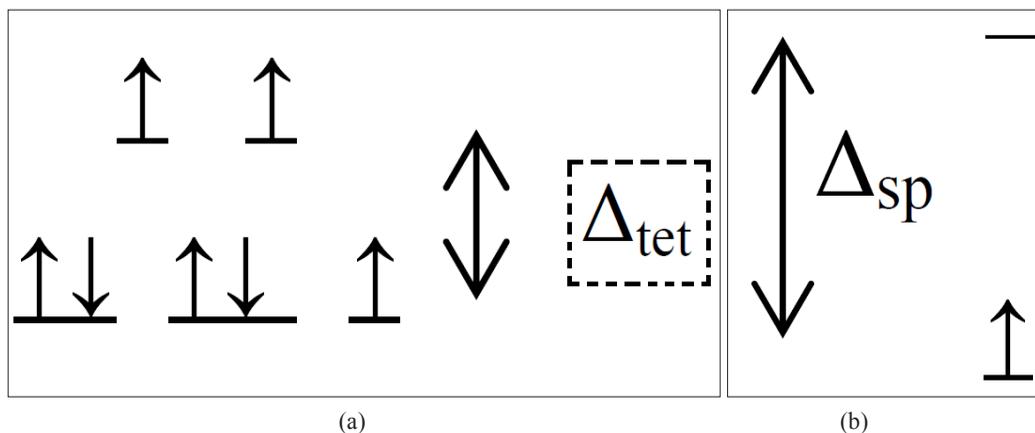


Figure 2. Crystal field splitting diagrams for Co(II) $3d^7$ species in tetrahedral and square planar environments: (a) tetrahedral, 3 unpaired electrons; and (b) square planar, 1 unpaired electron

On the other hand, $Zn_2(L)_2$ showed diamagnetism, consistent with the 0 unpaired electrons of the d^{10} configuration.

Antibacterial Activity

The antibacterial screening against MRSA results revealed that the $Co_2(L)_2$ and $Zn_2(L)_2$ metal complexes were better bactericides than their parent ligand, L. The qualitative method indicated that the cobalt complex, with 15 mm inhibition zone, showed the highest activity (Table 4). However, quantitatively, with the lowest MIC of $0.56 \mu\text{g}/\mu\text{l}$, the zinc complex exhibited a better antibacterial property than the cobalt complexes.

Table 4

Antibacterial activity of L, $Co_2(L)_2$ and $Zn_2(L)_2$ against methicillin resistant *Staphylococcus Aureus* (MRSA)

| Compound | Antibacterial activity against MRSA | |
|-------------|-------------------------------------|-----------------------------------|
| | Inhibition Zone (mm) | MIC ($\mu\text{g}/\mu\text{l}$) |
| L | - | 2.25 |
| $Zn_2(L)_2$ | 12 | 0.56 |
| $Co_2(L)_2$ | 15 | 1.13 |

Complexes are usually more bioactive than their parent ligand due to the presence of metal centres that are said to be the active sites for their bioactivity. Tweedy's chelation theory clarifies that in complexes, as opposed to plain inorganic salts, the polarity of the metal ion is reduced due to the overlapping of the ligand orbitals and partial sharing of positive charge of metal ion with the donor groups. This increases the delocalization of π -electrons over the whole

chelate ring which in turn, augments the lipophilicity and improves the cell permeability of the complexes (Priya, Arunachalam, Manimaran, Muthupriya, & Jayabalakrishnan, 2009).

According to the same theory, once the complexes penetrate into the cells, they can restrict the normal cell processes by blocking the metal binding sites on enzymes of the microorganism or interrupt the respiration process of the cell and thus, stopping the synthesis of protein which retards further growth of the organism (Priya et al., 2009). There are also other contributing factors attributing to the increased bioactivity of metal complexes such as solubility, conductivity and dipole moment due to the presence of metal ions in the system (Emara, 2010).

CONCLUSION

Dinuclear Zn(II) and Co(II) complexes are successfully synthesised and characterised by elemental analysis, infrared spectroscopy, ¹H NMR spectroscopy, melting point and magnetic susceptibility measurements. The metal centres are indicated to adopt tetrahedral geometry, bridging two moieties of the ligand L. From the preliminary screening, it is observed that the Zn(II) complex exhibits better antibacterial properties against methicillin resistant *Staphylococcus Aureus* (MRSA) than its Co(II) analogue and the parent ligand.

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REFERENCES

- Abdul Ghani, A., Bahron, H., Harun, M. K., & Kassim, K. (2014). Schiff bases derived from Isatin as mild steel corrosion inhibitors in 1 M HCl, *Malaysian Journal of Analytical Sciences*, 18(3), 507-513
- Amirnasr, M., Schenk, K. J., Gorji, A., & Vafazadeh, R. (2001). Synthesis and spectroscopic characterization of [CoIII(salophen)(amine)₂]ClO₄ (amine = morpholine, pyrrolidine and piperidine) complexes. The crystal structures of [CoIII(salophen)(morpholine)₂]ClO₄ and [CoIII(salophen)(pyrrolidine)₂]ClO₄. *Polyhedron*, 20, 695-702.
- Clarke, B., Clarke, N., Cunningham, D., Higgins, T., McArdle, P., Cholchúin, M. N., et al. (1998). Transition-metal schiff-base complexes as ligands in tin chemistry. Part 7. Reactions of organotin(IV) Lewis acids with [M(L)]₂ [M = Ni, Cu and Zn; H₂L = N, N'-bis (3-methoxysalicylidene) benzene-1,3-diamine and its -1,4-diamine analog]. *Journal of Organometallic Chemistry*, 559, 55-64.
- El-Wahab, Z. H. A. (2007). Mononuclear metal complexes of organic carboxylic acid derivatives: Synthesis, spectroscopic characterization, thermal investigation and antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 67(1), 25-38.

- Emara, A. A. A. (2010). Structural, spectral and biological studies of binuclear tetradentate metal complexes of N₃O Schiff base ligand synthesized from 4,6-diacetylresorcinol and diethylenetriamine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 77, 117-125.
- Gaballa, A. S., Asker, M. S., Barakat, A. S., & Teleb, S. M. (2007). Synthesis, characterization and biological activity of some platinum(II) complexes with Schiff bases derived from salicylaldehyde, 2-furaldehyde and phenylenediamine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 67, 114-121.
- Golcu, A., Tumer, M., Demirelli, H., & Wheatley, A. (2005). Cd(II) and Cu(II) complexes of polydentate Schiff base ligands: Synthesis, characterization, properties and biological activity. *Inorganica Chimica Acta*, 358, 1785-1797.
- Hernández-Molina, R., Mederos, A., Gili, P., Domínguez, S., Lloret, F., Cano, J., ... & Solans, X. (1997). Dimer species in dimethyl sulfoxide–water (80: 20 w/w) solution of N, N'-bis (salicylideneimine)-m-phenylenediamine (H₂ sal-m-phen) and similar Schiff bases with Cu II, Ni II, Co II and Zn II. Crystal structure of [Co₂ (sal-m-phen)₂]·CHCl₃. *Journal of the Chemical Society, Dalton Transactions*, (22), 4327-4334.
- Kaczmarek, M. T., Pospieszna-Markiewicz, I., Kubicki, M., & Radecka-Paryzek, (2004). Novel lanthanide salicylaldimine complexes with unusual coordination mode. *Inorganic Chemistry Communications*, 7, 1247-1249.
- Mohtar, M., Johari, S. A., Li, A. R., Isa, M. M., Mustafa, S., Ali, A. M., & Basri, D. F. (2009). Inhibitory and resistance-modifying potential of plant-based alkaloids against methicillin-resistant *Staphylococcus aureus* (MRSA). *Current Microbiology*, 59(2), 181-186.
- Priya, N. P., Arunachalam, S., Manimaran, A., Muthupriya, D., & Jayabalakrishnan, C. (2009). Mononuclear Ru(III) Schiff base complexes: Synthesis, spectral redox, catalytic and biological activity studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 72, 670-676.
- Ramlee, N., Bakar, S. N. A., Bahron, H., Harun, M. K., Kassim, K., & Yahya, M. Z. A. (2010, December). Interaction of Schiff bases and their corresponding amines and aldehyde with mild steel surface in 1.0 M hydrochloric acid solution. In *Science and Social Research (CSSR), 2010 International Conference on* (pp. 451-456). IEEE.
- Shakir, M., Azim, Y., & Parveen, S. (2006). Synthesis, characterization of complexes Co(II), Ni(II), Cu(II) and Zn(II) with 12-membered schiff base tetraazamacrocyclic ligand and the study of their antibacterial and reducing power. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 65, 490-496.
- Silverstein, R. M., & Webster, F. X. (1998). *Spectrometric identification of organic compounds* (6th edition). John Wiley and Sons, Inc.
- Tajuddin, A. M., El Hassane, A., Ramasamy, K., Yamin, B. M., Alharthi, A. I., & Bahron, H. (2017). DFT analysis and bioactivity of 2-((E)-(4-methoxybenzylimino) methyl) phenol and its Ni(II) and Pd(II) complexes, *Arabian Journal of Chemistry*, 10, 769-780.
- Torayama, H., Nishide, T., Asada, H., Fujiwara, M. & Matsushita, T., (1997). Preparation and characterization of novel cyclic tetranuclearmanganese(III) complexes: Mn₄(X-salmphen)₆ (X-salmphenH₂ = N,N'-disubstituted-salicylidene-1,3-diaminobenzene (X=H, 5-Br)). *Polyhedron*, 16 (21), 3787-3794.

