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**Title:** **Antibacterial Properties of Binuclear Zn(II)-Azomethine Complexes Derived from Diaminodiphenylsulphide Bridged Spacer**

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## Antibacterial Properties of Binuclear Zn(II)-Azomethine Complexes Derived from Diaminodiphenylsulphide Bridged Spacer

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### ABSTRACT

*The current research aims to study the antimicrobial resistance of microbes against once-effective treatments for the development of new drugs. Accordingly, three binuclear Zn(II)-Schiff bases complexes, ZnLa-ZnLc were prepared by the direct reaction of the ligands La-Lc with Zn(II) ions in equimolar ratios bearing salicylaldehyde with OH, NO<sub>2</sub>, and Cl functional groups. The synthesized compounds ZnLa-ZnLc were evaluated against gram-positive (*E. faecalis*, *S. mutante*, and *S. aureus*) and gram-negative (*E. coli*, *S. Typhi*, and *P. aeruginosa*) bacterial strains by agar well diffusion and broth microdilution technology. Minimum inhibitory concentration (MIC<sub>90</sub>) values were calculated using a microplate reader at 550 nm for optimal results. Binuclear Zn(II) complexes showed superior antibacterial activity as compared to their parent Schiff base ligands, which were clearly investigated from their respective MIC values and the diameter of the growth inhibitory zone. Additionally, *E. faecalis* were not active against the Schiff base ligand La-Lc, whereas their complexes ZnLa-ZnLc showed biological activity in an inhibition zone ranging from 9-10 mm to *E. faecalis*. In general, Schiff base 3,4-dihydroxy as well as its metal complex showed excellent antibacterial activity with zone of inhibition (13-26 mm) and (10-28 mm), respectively. Therefore, the synthesized compounds may be promising entities in the field of medicine.*

**Keywords:** azomethines, antibacterial activity, binuclear complexes, broth microdilution, well diffusion, Zn(II)

## INTRODUCTION

The problematic issue of antimicrobial-resistant microorganisms with numerous drugs has engendered the synthesis of innovative compounds for appropriate remedy of microbial infections and is of prime importance in the biomedical field [1]. Extensive synthesis and characterization of Schiff bases and their metal complexes set new benchmarks for their future and widespread applications in biomedicine, biochemistry, and biotechnology [2–4]. Schiff bases, including azomethine, are valuable for a variety of pharmacological activities and are considered versatile pharmacophore. Various research studies have shown that Schiff bases are beneficially associated with the use of anti-inflammatory, antibiotic, antibacterial, anticancer, and anti-HIV [5–8]. In addition, the predicted ability of salicylaldehyde Schiff base ligand to structure a substantial multi-transition metal complex results in the ability to form intermolecular hydrogen bonds between O and N atoms. As it played an important role in the development of the coordination chemistry [9–12]. Various Schiff base metal complexes have been used in catalytic reactions [13–18] as models of biological systems [19, 20]. It is well known that various Schiff base complexes are used for anti-inflammatory, anti-fever, chemical sensation, anti-diabetes, anti-cancer, anti-cancer, anti-HIV, antioxidant, anti-tumor, and anti-fungal effects [1, 21, 22–30].

Furthermore, the antimicrobial properties of Schiff bases in conjunction with halogen groups, as well as, their metal complexes have attracted the researchers interest. Besides, salicylaldehyde derivatives with one or more halogen atoms in aromatic rings exhibit versatile biological activities [19, 20]. Schiff bases containing one or more hydroxyl groups have a special interest because of their antimicrobial properties such as 2,5-dihydroxybenzaldehyde as reported earlier [6].

Zn(II) Schiff base complexes are consistently utilized as superior electroluminescent materials [31, 32], and were extensively used as sensors in supramolecular chemistry [33]. With the biological properties, zinc is an imperative trace metal after iron and contributes significantly, in enzyme catalysis, apoptosis, and neurotransmission [34]. In addition, Zn(II) metal ion in  $d^{10}$  electronic system may result in different coordination number with versatile molecular structure in a given ligand frameworks [31].

However, the potential antimicrobial characteristics of binuclear zinc complexes were scarcely studied and needs to be examined comprehensively. The binuclear complex, which features two metal centers with close but separate ligand compositions, represents an important criterion in the analysis of transition metal systems [35]. Therefore, the synthesis and characterization of binuclear Schiff base zinc complexes for exceptional antibacterial properties are essential.

Enormous applications of Schiff base metal complexes clearly indicates to stimulate the work and attempt amplification of antimicrobial performances through chemical structure modification of ligands [36]. In anticipation of the above analysis, diminidiphenylsulfide is used as the diamine, salicylaldehyde with functional groups such as Cl, OH, and NO<sub>2</sub> is used to combine the two well-established pharmacophores for antibacterial activity by making molecular Schiff base unit. Considering that the dinuclear complex in which the two metals are held close to each other contributes significantly, to the antibacterial activity, the synthesis of the dinuclear Zn (II) coordination compound having remarkable antibacterial properties are promoted. Therefore, apart from being derived from diaminodiphenylsulfide spacers with various salicylaldehyde derivatives, the structural and antibacterial properties of dinuclear zinc complexes have been reported. All the synthesized Zn (II) complexes were evaluated for their antibacterial activities against gram positive (*E. faecalis*, *S. mutant*, and *S. aureus*) and gram negative (*E. coli*, *S. typhi*, and *P. aeruginosa*) bacterial strains, respectively.

## 2. EXPERIMENT

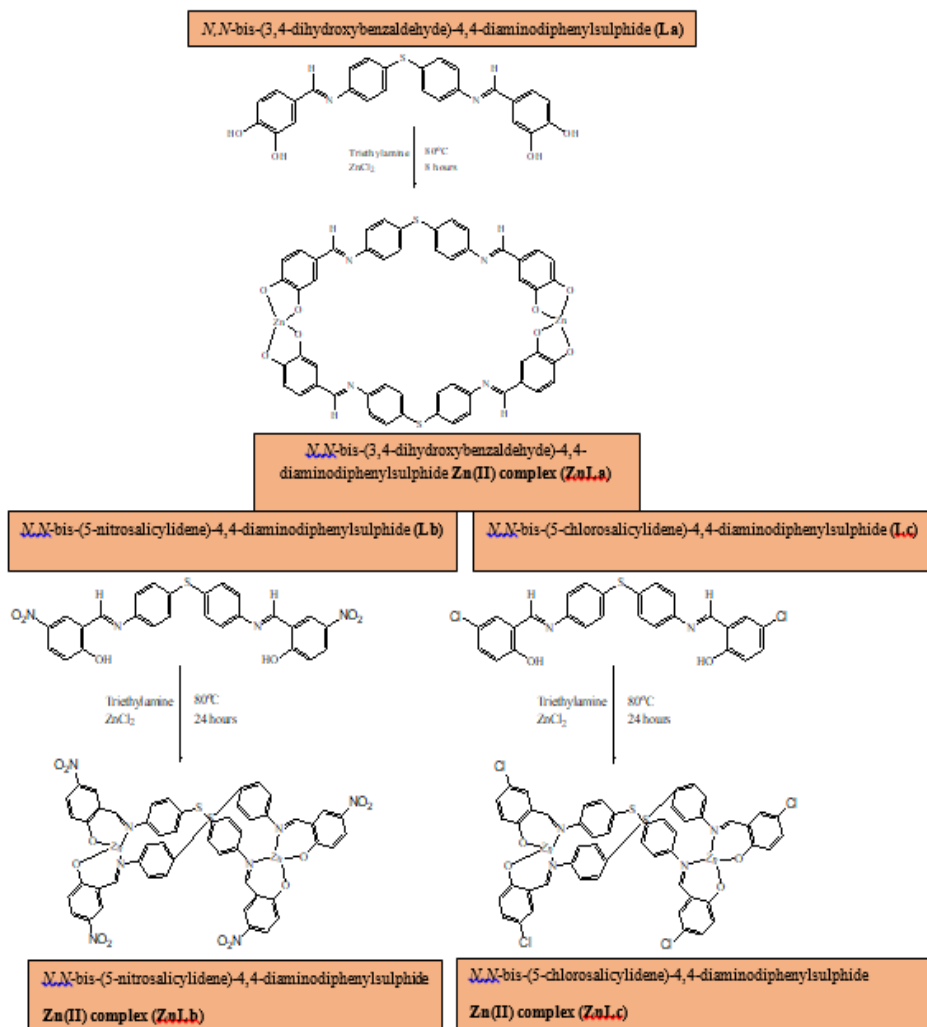
### 2.1. Materials, Methods, and Instrumentation

PANalytical, X'Pert highscore diffractometer with primary monochromatic high intensity Cu-K $\alpha$  ( $\lambda = 1.54184$ ) radiation in the scanning range of 5°–90° was used to determine the crystallinity of the compounds.

### 2.2. Synthesis of Schiff Base Zn(II) Complexes

The azomethine Schiff base ligand (La-Lc) and the respective three binuclear Zn (II) complexes were prepared in previous study [37]. Schiff base ligands were prepared by reacting 4, 4'-diaminodiphenyl sulfide with the corresponding salicylaldehyde derivative by a 1: 2 molar ratio condensation reaction in methanol. In contrast, synthesis of three Zn (II)

metal complexes (ZnLa – ZnLc) was performed with equimolar amounts of ligand and metal salt in methanol in the presence of a few drops of triethylamine, as shown in **Scheme 1**.



**Scheme 1.** Schemetic diagrams for the synthesis of schiff base ligands (La – Lc) and Zn(II) complexes (ZnLa – ZnLc)

### 2.3. Antibacterial Activity

The synthesized compounds were tested for bactericidal activities which was investigated in triplicates and the acquired results were represented as the mean  $\pm$  standard deviation (SD) according to our reported method [38].

### 2.3.1. Agar Well Diffusion Method

Gram-positive and gram-negative bacteria, for instance. *E. coli*, *S. typhi*, *P. aeruginosa*, *E. faecalis*, *S. mutant*, and *S. Aureu* were used to measure the antibacterial activity of compounds (ZnLa-ZnLc) by agar well diffusion method assay [39]. Hence,  $0.144 \times 10^6$  CFU mL<sup>-1</sup> bacteria in 100  $\mu$ l solution was blotted for inoculation on agar plates. A well was created on the agar plate using a sterile cork borer. A stock solution of Zn (II) complex at a concentration of 25 mg / ml was prepared. A 100  $\mu$ l composite solution was added to the wells and diffused into agar for 2 hours. DMSO (100  $\mu$ L) was used as a negative control. The plates were incubated at 37° C for 24 hours. The diameter of the inhibition zone (millimeters (mm)) was used to measure antibacterial activity.

### 2.3.2. Minimum Inhibitory Concentration (MIC) Method

The minimum concentration of compounds that inhibit the visible growth of bacteria can be analyzed using the minimum inhibition concentration (MIC) method. The CLSI M38-A2 was used in combination with a 96-well micro-diluted microplate for MIC. The standard method recommended by CLSI [40, 41] was to test the compound using both micro-dilution methods. Mueller-Hinton (MH) broth was used as blank medium and DMSO broth was used as a negative control for each well. These plates were incubated at 37 ° C for 24 hours with 10  $\mu$ l bacteria, 100  $\mu$ l MH broth, and 100  $\mu$ l compound in the incubator at a final concentration of  $1.0 \times 10^6$  cells / ml. Bacterial growth was measured at (PowerWave 200, Bio-Tek Instruments, and Winooski, VT, USA), microplate reader at 550 nm. The inhibition of 90% bactericidal growth (MIC<sub>90</sub> values) was calculated by using the minimum concentration of test compounds.

### 2.3.3. Minimum Bactericidal Concentration (MBC) Method

The minimum bactericidal concentration (MBS) was determined by the solution of inherent turbidity of compound. MBC was determined by streaking MH agar plates containing aliquots (50  $\mu$ l) of suspension used for MIC from wells with no detectable growth. Agar plates were incubated at 37 ° C for 24 hours [40, 42].

### 3. RESULTS AND DISCUSSION

#### 3.1. Powder XRD Studies

X-ray diffraction patterns of the compound (ZnLa-ZnLc) were recorded in the range of  $5^{\circ}$  - $90^{\circ}$  ( $2\theta$ ). Comparing the XRD pattern of the complexes with the initiating ligands helps determine the crystallinity of the complexes. **Table 1** shows the X-ray powder diffraction data. The X-ray diffraction patterns of the ligand and the Zn (II) metal complex are shown in Fig. 1 and Fig. 2.

##### 3.1.1. Powder XRD Studies of Zn (II) Azomethine Complexes

Upon coordination between La and Zn(II) ion, exhibited five peaks from  $6.931^{\circ}$  and  $23.412^{\circ}$  range, lowered in intensity as well as crystallinity to the parent Schiff base ligand found in the range  $7.856$  and  $24.503^{\circ}$ , more intense. The weaker and broader reflections found in the diffractogram of Lb, indicates its amorphous nature. The ZnLb diffractogram showed three sharp peaks in the range of  $8.242$  and  $14.319^{\circ}$ , indicating its crystalline nature.

In Lc, intense peaks appeared in the range of  $19.26^{\circ}$  and  $33.011^{\circ}$ . Upon coordination to Zn (II) ion in ZnLc showed intense reflections in the range of  $10.210$  to  $17.567$  confirming its crystallinity in phase.

The well-defined sharp peaks in La, Lc, and their respective complexes (ZnLa, ZnLb, and ZnLc) indicated its crystallinity in phase. Whereas, crystallinity of ZnLb is higher to the parent Schiff base after complexation with Zn(II).

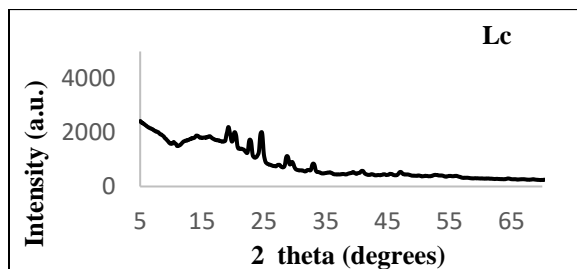
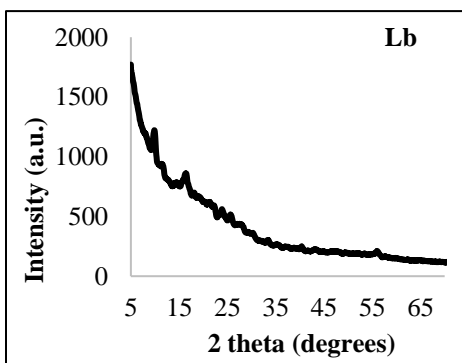
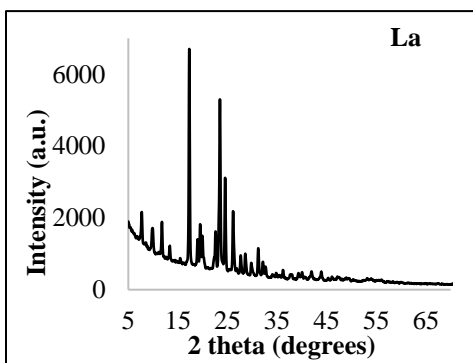
Debye-Scherrer's formula (**Eq. 3.1**) was used to determine the average crystallite size (D).

$$D = \left( \frac{K \times \lambda}{\beta \times \cos \theta} \right) \quad (3.1)$$

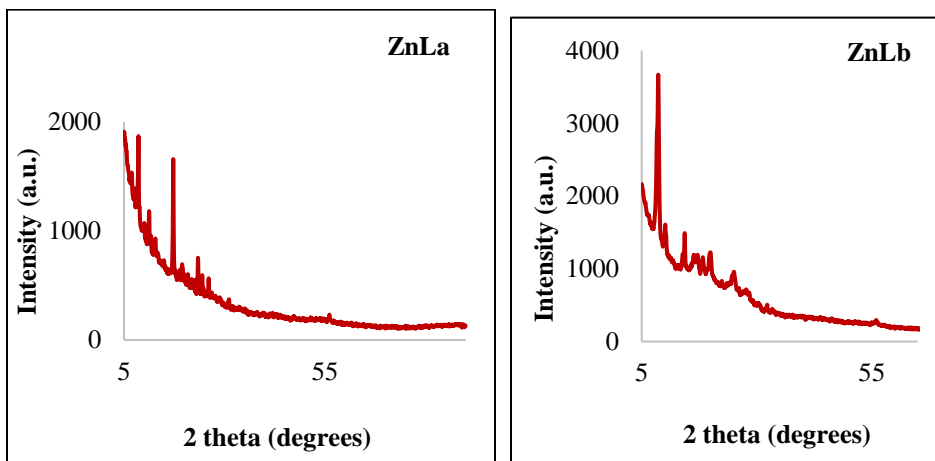
The average crystallite size of Zn(II) complexes are  $42.89$  nm (ZnLa),  $64.19$  nm (ZnLb), and  $158.14$  nm (ZnLc). The sequence found for the crystallinity of the complexes was ZnLc>ZnLb>ZnLa.

**Table 1:** Powder X-ray Diffraction Data of Zn (II) Complexes (ZnLa – ZnLc)

Compd	Peak	2 $\theta$	Intensity (a.u)	d ( $^{\circ}$ Å)	$\beta$
ZnLa	1	6.931	865.89	12.753	0.204
	2	7.660	711.44	11.540	0.103
	3	8.611	1312.36	10.268	0.076
	4	17.256	1213.85	5.138	0.122
	5	23.412	409.01	3.799	0.103
ZnLb	1	8.242	1397.84	10.727	0.127
	2	8.551	2351.90	10.339	0.204
	3	14.319	661.760	6.185	0.102
ZnLc	1	10.210	1749.89	8.656	0.102
	2	12.123	1933.58	6.957	0.127
	3	13.111	1768.92	6.752	0.127
	4	14.692	1408.10	6.029	0.102
	5	17.567	1046.981	5.048	0.102

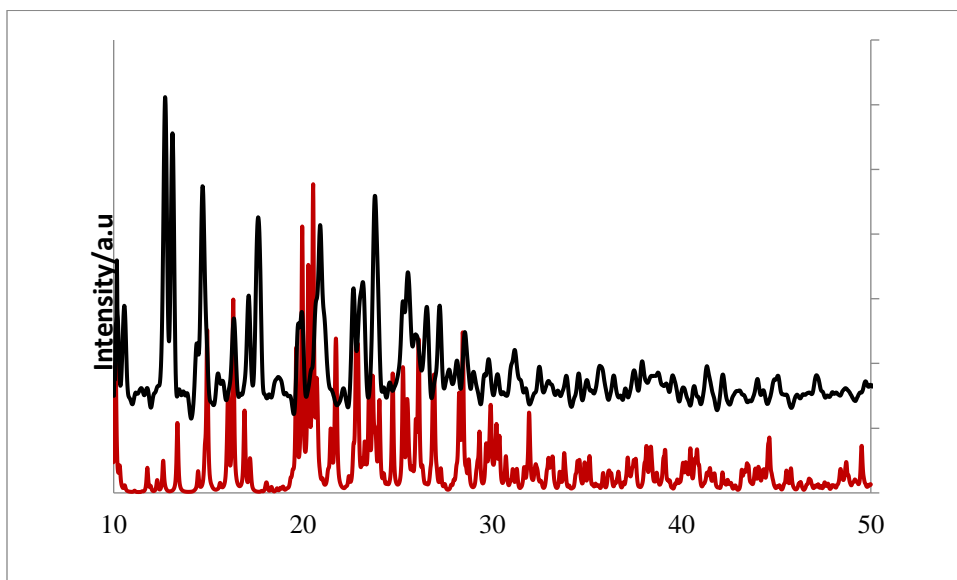






**Figure 1.** X-ray diffraction patterns of ligands (La – Lb) [38] and their corresponding Zn(II) complexes (ZnLa and ZnLb)

**Figure 2** shows the experimental powder X-ray diffraction pattern for the ZnLc complex, which matched well with the simulated pattern calculated from the single crystal structure of complex ZnLc [37], showed that the sample was single-phased and free from impurities.



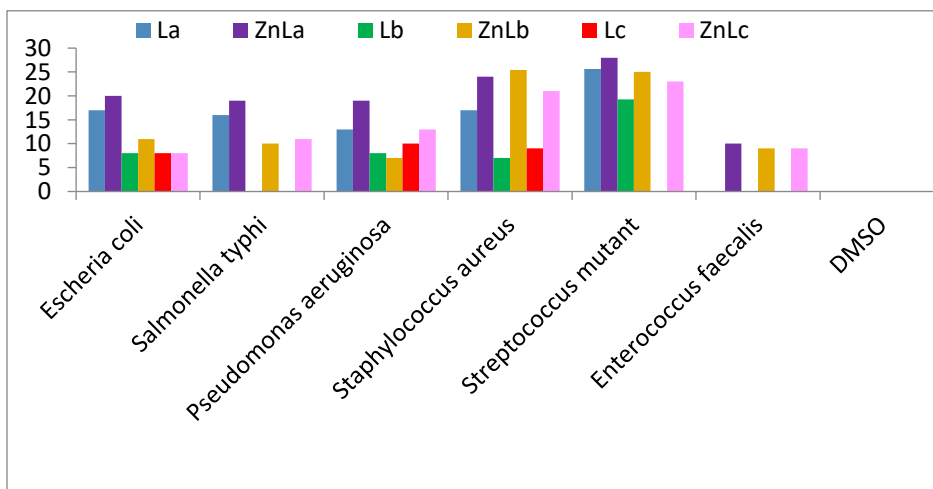
**Figure 2.** X-ray diffraction pattern of ZnLc (Red-Simulated from CIF file; and Black-Experimental)

### 3.2. Antibacterial Studies

The three gram-negative bacteria *Escheria coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and the three gram-positive *Staphylococcus aureus*, *Streptococcus mutants*, and *E. faecalis* were used to assess the antibacterial activity of the Schiff base (La-Lc) and their respective Zn (II) complexes (ZnLa-ZnLc). As reported, the commonly used antibiotic streptomycin was used as a positive control and DMSO was used as a negative control [38]. The diameter of zones of inhibition of antibacterial activity caused by the compounds is given in **Table 2** and the chart presented in **Figure 3**.

**Table 2:** Bactericidal Activity of ligands (La – Lc) [38] as well as (ZnLa – ZnLc) Complexes against Gram-negative and Gram-positive bacterial strains [inhibition zone] in (mm).

Bacteria	La	ZnLa	Lb	ZnLb	Lc	ZnLc
<i>E. coli</i>	17	20	8	11	8	8
<i>S. typhi</i>	16	19	9.0	10	0	11
<i>P. aeruginosa</i>	13	19	8	7	10	13
<i>S. aureus</i>	17	24	7	25.4	9	21
<i>S. mutant</i>	25.6	28	19.3	25	0	23
<i>E. faecalis</i>	0	10	0	9	0	9



**Figure 3.** Bactericidal activity (mm) of ligand (La – Lc) and (ZnLa-ZnLc) complexes against gram-negative and gram-positive strains [inhibition zone]

Several interesting structure-activity relationships between the studied compounds and their bactericidal activities have been observed in the current work. Generally, the Zn(II) complexes exhibited the highest bactericidal activity than the parent ligands.

The introduction of electron donating OH, and electron withdrawing NO<sub>2</sub> and Cl caused alterations to antibacterial properties of the Schiff bases, suggesting the importance of source materials as bactericidal agents. Amongst ligands, La with *m*-OH substituent was the strongest antibacterial agent followed by Lb with *o*-NO<sub>2</sub> moiety and Lc with *o*-Cl moiety, in that particular order. Generally, the antibacterial activities are mostly affected by various electron withdrawing and electron donating nature as well as position of substituents in the phenyl ring. The presence of substituents at the *meta* and *para* positions has been observed by [43] to increase the antibacterial activity, whereas those at the *ortho* position resulted in compounds with lower antimicrobial activity. In the present study, the same trend was observed where the order of antibacterial activity of the Schiff bases based on the type and position of substituents is as follows.

La [*m*-OH] > Lb [*o*-NO<sub>2</sub>] > Lc [*o*-Cl]

La with OH functional groups at the *meta* position, performed remarkably well for leading antibacterial activity. Schiff bases containing one or more hydroxyl groups such as 2,5-dihydroxybenzaldehyde are of special interest because of their enhanced antimicrobial properties as reported earlier by [6].

Enhancement of antibacterial activity of La was observed upon complexation with Zn(II). literature which could be associated with the relatively smaller size of Zn(II) as compared to our reported Ir(III) complexes quenched upon coordination [38]. In numerous complexes, the antibacterial activity has been reported to be similarly enhanced upon coordination with smaller nuclear size of Cu(II) Schiff base complexes amongst the first transition series [44–48].

Zn(II) ions are essential for growth inhibitor effect due to its inherent toxicity [49]. The antibacterial activity of Zn (II) can be explained by two different mechanisms. Direct interaction with the microbial membrane leads to membrane destabilization and increased permeability, and secondly, to nucleic acid interaction and inactivation of respiratory enzymes that cause cell death [50–52].

In addition to the nature and size of metal ions as well as position of substituents, the pharmacological activity is extremely dependent on the donor sequence of the ligands as well as types of chelations [45, 53]. It was observed that the ZnLa with [OOOO] donor atom system exhibited higher antibacterial activity than the reported IrL1 complex [38] with [NNOO] donor atom system, reflecting the importance of bonding motifs of metal centres to ligands.

The antibacterial property of ligand Lb was enhanced by the presence of Zn(II) metal ions. The nature of the ligand Lb with NO<sub>2</sub> substituent at the *ortho* position is one of the key factors for the pharmacological activity of the ZnLb metal complex, where the uncoordinated donor atoms in NO<sub>2</sub> enhanced the activity of the complexes by bonding with trace elements present in microorganisms, inhibiting the growth of the microorganisms [49, 54]. The activity may also be, due to the electron withdrawing property of nitro group in the phenyl ring [6, 55]. Similar enhanced antibacterial activity was also reported for Ir(III) complex of the named ligand [38].

The permeability of the cells of the microorganisms or the difference in the ribosomes of the microbial cells affects the efficiency of different complexes towards different organisms [56]. The absence of outer lipid layer on the cell walls of *Staphylococcus aureus* and *Streptococcus mutant* made them particularly susceptible to the permeation of ZnL2 as well as our reported IrHL<sup>2</sup> [38] resulting in inhibited bacterial growth.

Ligand Lc that contains Cl functional group at *ortho* position was the least bactericidal compound but exhibited enhanced antibacterial activity when coordinated to Zn(II), similar trends were observed in the reported Ir(III) metal ions [38], as similarly observed for Lb. The antimicrobial properties of Schiff bases containing halogen groups and their metal complexes have attracted researchers' interest. Besides, salicyldehyde derivatives with one or more halogen atoms in aromatic rings exhibit versatile biological activities [6, 57].

*Enterococcus faecalis* was not inhibited by any of the investigated ligands, but all Zn(II) complexes exhibited significant antibacterial activity against this microbe. Therefore it seems that Zn(II) ions are quite toxic for *Enterococcus faecalis*, as reported earlier by [58].

The synthesized compounds in the current work exhibited strong activity against gram-positive bacteria, the phenomenon that could be

explained by the fact that gram-positive bacteria possess a permeable cell wall that usually does not restrict the penetration of antimicrobials [59].

The experimental details indicated that the new compound La has shown good inhibition zones of 13-26 mm with a MIC values ranging from 1.9-7.5 mg/ml. *Pseudomonas aeruginosa* and *Streptococcus mutant* were the most sensitive to the Schiff base compound La with a MIC value of 1.9mg /ml. The compound Lb showed promising activity against *Pseudomonas aeruginosa* followed by *Salmonella typhi* with a MIC of 1.9 and 3.8 mg/ml and zones of inhibition ranging from 8.0-19.3 mm. Furthermore, the MIC values for compound Lc against *Staphylococcus aureus* and *Pseudomonas aeruginosa* is 3.8 mg/ml, whereas 7.5 mg/ml against *Escheria coli*, *Salmonella typhi* with inhibition zones ranging from 8-10 mm.

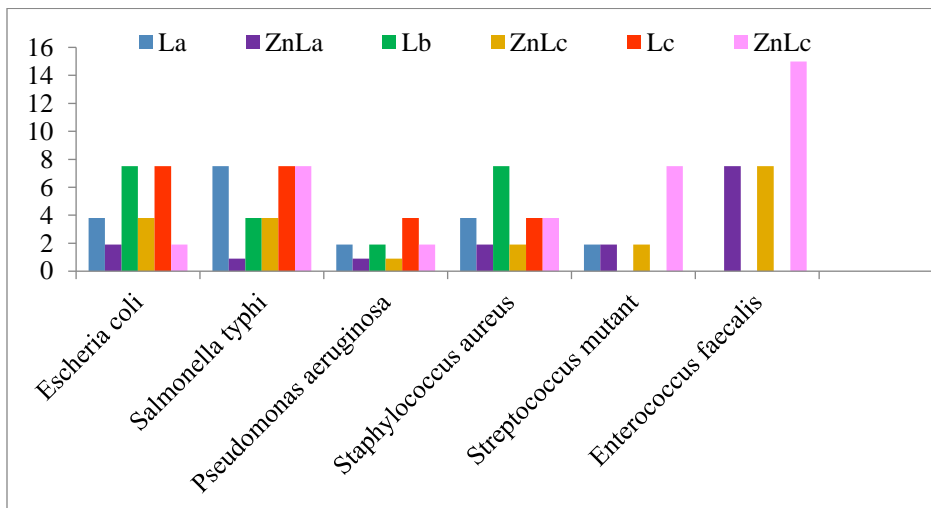
The Zn (II) complexes ZnLa-ZnLc showed high antibacterial activity against both gram-positive and gram-negative bacteria. This is clear from the respective MIC values and the diameter of the growth inhibitory zone. When the activity of the complex test was compared to the stem-Schiff base ligand, it was shown that the activity of the ligand was lower than the activity of those complexes. All test compounds except Lc have promising biological activity against Streptococcus mutants with inhibition zones ranging from 19-28 mm. The complex ZnLa, which has a La-Schiff base ligand and its corresponding dihydroxy moiety, appears to have higher activity than all other ligands and metal complexes. Enterococcus faecalis was not active against the Schiff base ligand La-Lc, whereas their complexes ZnLa-ZnLc has biological activity in an inhibition zone ranging from 9-10 mm to Enterococcus faecalis. Due to its significant activity, the Zn (II) complexes can be used not only to enhance antibacterial activity, but also to overcome drug resistance [60]. Minimum inhibitory concentration MIC<sub>90</sub> and Minimum bactericidal concentration MBC is given in **Table 3** and **Table 4**, and the charts are presented in **Figure 4** and **Figure 5**.

**Table 3:** Minimum Inhibitory Concentration MIC<sub>90</sub> (mg/ml) Values of Ligands (La – Lc) and Their Respective Zn (II) (ZnLa - ZnLc) Complexes Against Gram-negative and Gram-positive Bacteria

Bacteria	La	ZnLa	Lb	ZnLb	Lc	ZnLc
<i>E.coli</i>	3.8	1.9	7.5	3.8	7.5	1.9
<i>S. typhi</i>	7.5	0.9	3.8	3.8	7.5	7.5

Bacteria	La	ZnLa	Lb	ZnLb	Lc	ZnLc
<i>P. aeruginosa</i>	1.9	0.9	1.9	0.9	3.8	1.9
<i>S.aureus</i>	3.8	1.9	7.5	1.9	3.8	3.8
<i>S. mutant</i>	1.9	1.9	NA	1.9	NA	7.5
<i>E. faecalis</i>	NA	7.5	NA	7.5	NA	15

NA: No activity

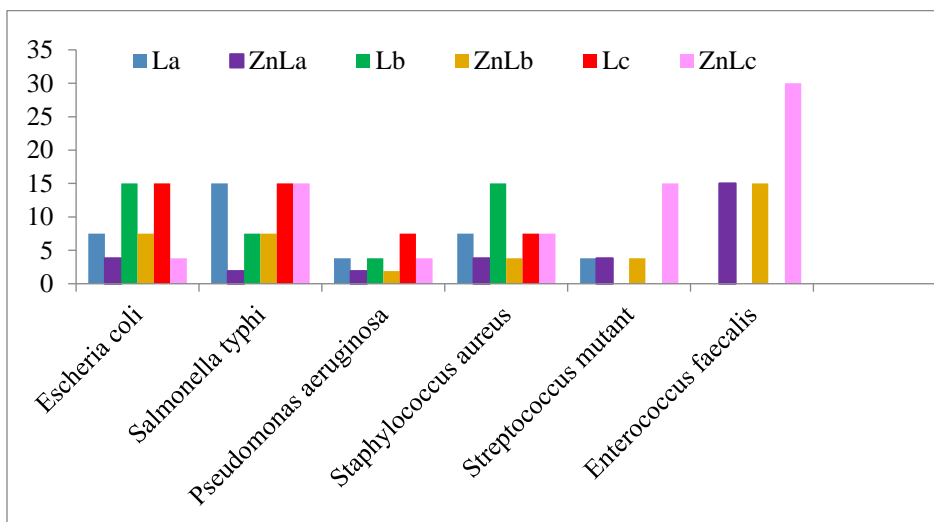


**Figure 4.** Minimum inhibitory concentration MIC<sub>90</sub> (mg/ml) of ligands (La - Lc) and their respective Zn(II) (ZnLa - ZnLc)) complexes against Gram-negative and Gram-positive bacteria

**Table 4:** Minimum Bactericidal Concentration MBC (mg/ml) Values of Ligands (La – Lc) and Their Respective Zn (II) (ZnLa - ZnLc) Complexes Against Gram-Negative and Gram-Positive Bacteria

Bacteria	La	ZnLa	Lb	ZnLb	Lc	ZnLc
<i>E. coli</i>	7.5	3.8	15	7.5	15	3.8
<i>S. typhi</i>	15	1.9	7.5	7.5	15	15
<i>P. aeruginosa</i>	3.8	1.9	3.8	1.9	7.5	3.8
<i>S. aureus</i>	7.5	3.8	15	3.8	7.5	7.5
<i>S. mutant</i>	3.8	3.8	No MBC	3.8	No MBC	15
<i>E. faecalis</i>	No MBC	15	No MBC	15	No MBC	30

NO MBC: No minimum bactericidal concentration value



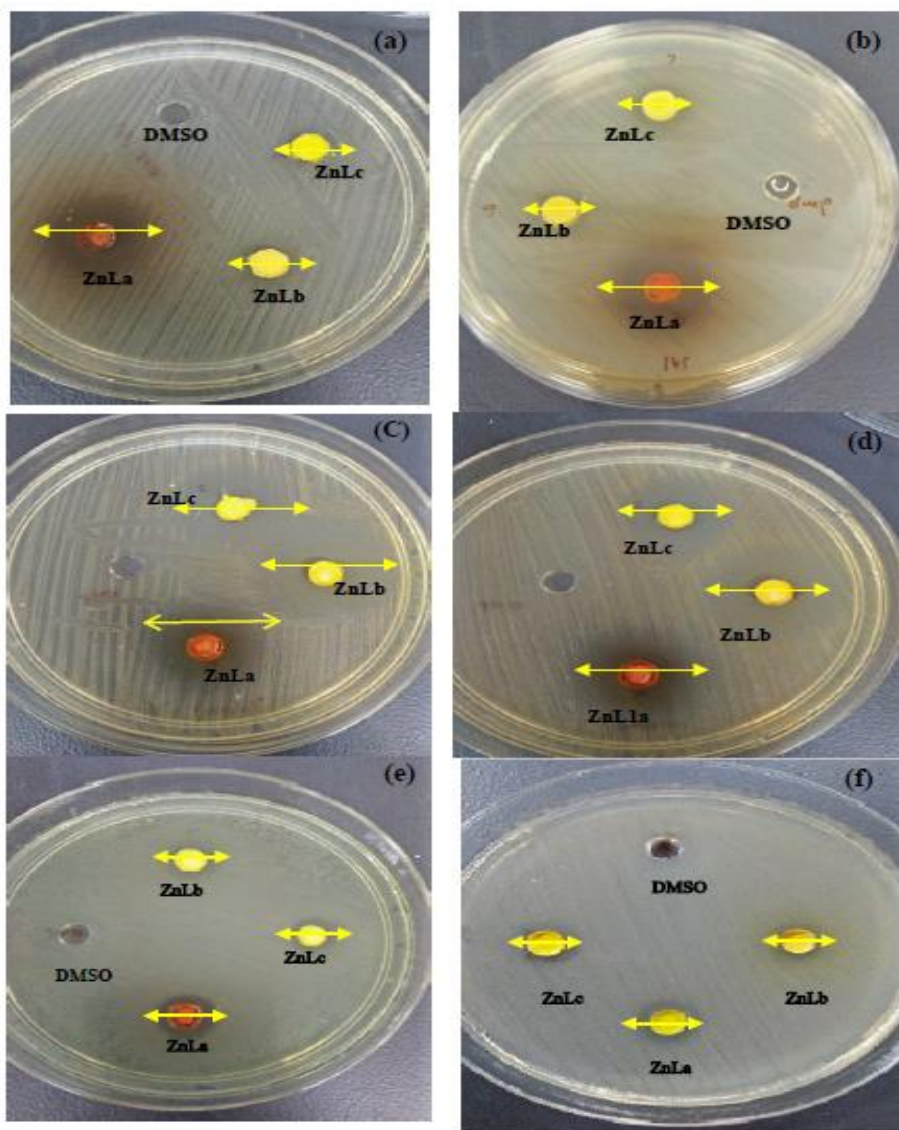
**Figure 5.** Minimum bactericidal concentration MBC (mg/ml) of ligands (La - Lc) and their respective Zn(II) (ZnLa - ZnLc) complexes against Gram-negative and Gram-positive bacteria

**Table 5:** Antibacterial Activity of Positive Control [38](Streptomycin)

Bacteria	Diameter of zone of inhibition (mm)	Minimum Inhibitory Concentration (mg/mL)
<i>E. coli</i>	26 ± 0.89	1.60
<i>S. typhi</i>	29 ± 0.00	3.1
<i>P. aeruginosa</i>	24 ± 0.00	6.30
<i>S. aureus</i>	28 ± 0.57	1.60
<i>S. mutant</i>	32 ± 0.00	0.05
<i>E. faecalis</i>	18 ± 0.51	12.5

### 3.3. Findings and Conclusion

Powder X-ray diffraction and antibacterial activity of the binuclear azomethine Zn (II) complex have been reported in the current research. Previous studies on PXrd showed that La, Lc, and Zn (II) complexes (ZnLa, ZnLb, ZnLc) have high crystallinity due to their strong peaks. The crystallinity of Lb was increased by complex formation with Zn (II) in ZnLb. The crystallinity of the azomethine complexes was found in the order ZnLc>ZnLb>ZnLa.



**Figure 6.** Photographs of Gram-negative and Gram-positive antibacterial test for Zinc (II) complexes (ZnLa-ZnLc) [zone of inhibition (mm)] (a) *Escheria coli* (b) *Salmonella typhi* (c) *Staphylococcus aureus* (d) *Streptococcus mutant* (e) *Pseudomonas aeruginosa* (f) *Enterococcus faecalis*.



Moreover, the binuclear Zn (II) azomethine complex showed significant bactericidal activity against six different bacterial strains and superior antibacterial activity as compared to the parent Schiff base ligand. The order of antibacterial activity of the Schiff bases was based on the type and position of substituents, namely La [*m*-OH] > Lb [*o*-NO<sub>2</sub>] > Lc [*o*-Cl]. In general, Schiff base 3,4-dihydroxy as well as its metal complex showed excellent antibacterial activity with zone of inhibition (13-26 mm) and (10-28 mm), respectively. Furthermore, *E. faecalis* was not inhibited by any of the investigated ligands, although all Zn(II) complexes exhibited significant antibacterial activity against this microbe. Therefore, it was identified that Zn(II) ions were quite toxic for *E. faecalis*. The antibacterial activities of the complexes clearly indicated that the synthesized complexes in this study ZnLa-ZnLc deserve to be established as a promising basis for subsequent advances in antibacterial agents

### Acknowledgements

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