

Determination of Antibacterial Activity of 5-Bromosalicylidene-Aniline, 5-Bromosalicylidene-4-Nitroaniline and Their Cobalt (II) Complexes

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Received March 18, 2024; Revised April 20, 2024; Accepted April 27, 2024

Abstract Antibacterial resistance is a serious global health problem in human beings. Emergence and increase in number of drug resistant and multidrug resistant microorganisms is at an alarming rate. Thus, there is a pressing need for the development of novel antibacterial drugs with promising activity, which can potentially address this resistance. Schiff bases with 5-bromosalicylidene-aniline (BA), 5-bromosalicylidene-4-nitroaniline (BN) and their cobalt (II) complexes had been synthesized but their antibacterial activity has not been done. In this work, the antibacterial activity of 5-bromosalicylidene-aniline, 5-bromosalicylidene-4-nitroaniline Schiff bases ligands and their cobalt (II) complexes was investigated against two gram-positive bacteria (*S. aureus* & *L. monocytogenes*) and one gram-negative bacteria (*E. coli*). Their zones of inhibition were compared to the standard drug (gentamycin). All the test compounds displayed antibacterial potential but none of them had antibacterial activity higher than that standard. 5-bromosalicylidene-4-nitroaniline showed higher activity amongst the ligands; this could be due to substitution of aniline with a nitro group in its structure. Cobalt (II) complexes exhibited higher antibacterial activity in comparison to their corresponding Schiff base ligands. 5-bromosalicylidene-4-nitroaniline cobalt (II) complex showed the highest zones of inhibition amongst all the test compounds.

Keywords: Antibacterial activity, azomethine nitrogen, 5-bromosalicylaldehyde, 5-bromosalicylidene-aniline, 5-bromosalicylidene-4-nitroaniline

Cite This Article: Karithi J. Muketha, Gichumbi M. Joel, Michura J.G. Anne, and Ombaka Ochiengi, "Determination of Antibacterial Activity of 5-Bromosalicylidene-Aniline, 5-Bromosalicylidene-4-Nitroaniline and Their Cobalt (II) Complexes." *American Journal of Infectious Diseases and Microbiology*, vol. 12, no. 2 (2024): 23-28. doi: 10.12691/ajidm-12-2-1.

1. Introduction

5-bromosalicylidene-aniline is a Schiff base ligand formed by condensation of 5-bromosalicylaldehyde and aniline through reflux process while 5-bromosalicylidene-4-nitroaniline is a Schiff base ligand formed through condensation of 5-bromosalicylaldehyde and 4-nitroaniline [1]. Nitro group is an electron withdrawing group. Other previous publications have reported that substitution of an electron withdrawing group enhances the reactivity of the chemical compound due to reduction in electron density in chemical structure [2]. Schiff base ligands and their metal complexes display many biological activities. This could be due to the presence of an azomethine nitrogen bond in Schiff base ligand [3]. The lone pair of electrons in the azomethine nitrogen bond is responsible for antibacterial activity [4]. This is because they form hydrogen bonds with the target receptor site in biological molecules.

Coordinating the ligand to a transition metal improves the

antibacterial activity by reducing the electron density in the ligand's chemical structure. Substituted salicylaldehyde moieties have shown a considerable activity against antibacterial agents [5]. In this work, 5-bromosalicylidene-aniline and 5-bromosalicylidene-4-nitroaniline and their respective cobalt (II) complexes were investigated for their antibacterial activity against two gram-positive bacteria (*S. aureus* & *L. monocytogenes*) and one gram-negative bacteria (*E. coli*). The performance was compared with that of a standard drug used commercially in the market (gentamycin). Figure 1-4 shows the structure of the above-named ligands and their corresponding cobalt (II) complexes.

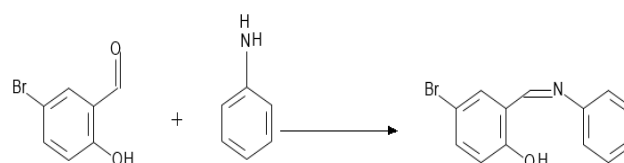


Figure 1. Synthesis of 5-bromosalicylidene-Aniline

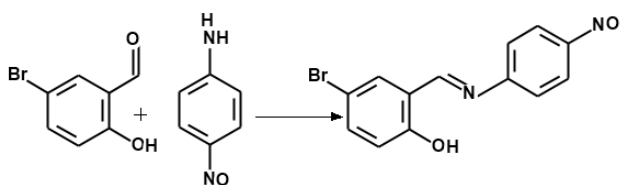


Figure 2. Synthesis of 5-bromosalicylidene-4-Nitro-Aniline

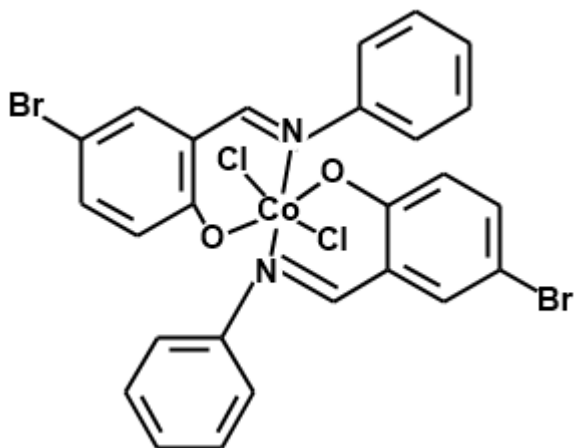


Figure 3. 5-bromosalicylidene-Aniline cobalt (II) complex synthesized

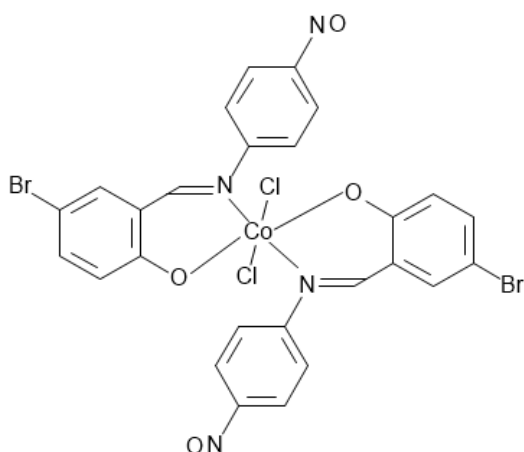


Figure 4. 5-bromosalicylidene-4-Nitro-Aniline cobalt (II) complex synthesized

Objective

To determine the antibacterial potency of 5-bromosalicylidene - aniline, 5-bromosalicylidene - 4-nitroaniline and their cobalt (II) complexes.

2. Materials and Methods

2.1. Experimental Material

All reagents, starting materials and solvents were of analytical grade. They were obtained from reputable sources: aniline, 5-bromosalicylaldehyde and 4-nitroaniline were bought from Sigma-Aldrich., ethanol and DMSO was obtained from ASPET school supplies LTD, bacterial strains were purchased from KEMRI while nutrient agar and McFarland standard was obtained from ASPET School supplies LTD.

2.1.1. Experimental Site

This research was done at Chuka University located at Chuka town in Tharaka Nithi County along Meru-Nairobi highway. Synthesis of the ligands and their cobalt (II) complexes, FT-IR and UV-VIS spectroscopy characterization was done at Chuka University chemistry laboratory while NMR characterization was done university of Nairobi- Chiromo campus. Determination of antibacterial activity was done at animal science laboratory Chuka University.

2.2. Antibacterial Screening

The three resistant bacteria were gram stained to confirm their identity before antibacterial susceptibility test was done. They were cultured under solid Mueller Hinton agar media. They were Autoclaved in sterilized paper (no.1, thickness, 180 μ m) discs of diameter 6 mm, then soaked in the desired concentration of the compounds synthesized [6].

The stock solution of concentration 1000ppm of the prepared ligand and their metal complexes was prepared by dissolving the test compound (20mg) into 20ml of DMSO. From this stock concentration other concentration of 500ppm, 250ppm and 125ppm was prepared by dilution with DMSO as shown section 2.2.1.

2.2.1. Dilution of Stock Concentration

The other three concentrations of 500ppm, 250ppm and 125 was determined using the dilution formula:

$$C_1V_1=C_2V_2$$

Whereby,

C_1 is the initial concentration.

V_1 is the initial volume.

C_2 is the final concentration.

V_2 is the final volume

Three reagent bottles of equal volume were cleaned and labelled 500ppm, 250ppm and 125ppm respectively. Then 5 ml stock solution was withdrawn from the stock solution and transferred to the bottle labeled 500ppm. Thereafter, 2.5ml and 1.25ml of stock solution was withdrawn again from stock solution and then transferred to reagent bottle labelled 250ppm and 125ppm respectively. To top up the solution in each reagent bottle to a total volume 10ml; volume of 5ml, 7.5ml and 8.75ml of DMSO solvent was added to reagent bottle labelled 500ppm, 250ppm and 125ppm respectively.

2.2.2. Anti-bacterial Testing

The three resistant bacteria one gram negative (*E. coli*) and two gram positive (*S. aureus* and *L. monocytogenes*) were sub cultured under Mueller Hinton media. They were inoculated using sterile cotton wool stick; the bottle containing bacteria was opened and its mouth passed over the flame as well to avoid its contamination from the air. The sterile cotton wool stick was inserted into the bottle containing resistant bacteria strain and then swabbed through petri dish containing solidified Mueller Hinton agar, the petri dish was covered and then left for bacteria to grow. The bacteria were also cultured under DMSO (negative control) and Gentamycin (manufacturer concentration) as a positive control for comparison with

the synthesized ligands and their cobalt complexes. Filter Paper (no.1, thickness, 180 μm) discs of diameter 6 mm was autoclave sterilized and then soaked in synthesized compounds at desired concentration. The paper discs were placed carefully in the petri dishes containing solidified Mueller Hinton media inoculated with the *E. coli*, *S. aureus*, *L. monocytogenes* separately and then incubation for 24 hours at 37°C. The diameter of inhibition was measured in millimeter after 24 hours of incubation [7].

2.3. Data Collection

The test compounds impregnated paper discs were placed in agar seeded petri- dishes containing nutrients with the test bacteria strains and incubated for 24 hours. Their diameter of zones of inhibition was measured independently using ruler and their values recorded as shown in appendix 20.

2.3.1. Data Analysis

Data collected experimentally was analyzed by using descriptive and inferential statistics. The former was done through computational of mean and standard deviation on response variable. Analysis of variable (ANOVA) was used to test the significant differences of the inhibition zones of test compounds and the means were separated using least significance differences (LSD) at $\alpha \leq 0.05$ [8].

3. Results and Discussion

3.1. Antibacterial activities of the Synthesized Schiff Bases and Their Complexes

The bacterial susceptibility on gram-positive bacterial (*S. aureus* and *L. monocytogenes*) and one gram-negative bacteria (*E. coli*) towards the two synthesized Schiff base and their cobalt complexes were judged based on their zones of inhibition.

All the Schiff base ligands and their complexes showed zones of growth inhibition at all concentration. This indicate that all the test compounds were microbiologically active. This could have been due to presence of nitrogen (N) and oxygen (O) atoms in the Schiff base ligand and the complexes in their structure.

Heteroatoms such as N and O increases the biological activity of the compound as reported by [4]. The publication reported that N and O induces opposite electronic effects hence affecting the bioactivity of the compound. However, there were no inhibition zones observed on the negative control (DMSO) meaning that the solvent had no effects on antibacterial activity observed. The zones of inhibition were solely on the test compounds that were dissolve in it.

3.1.1. Antimicrobial Activity of Synthesized BA, BN and Their Complexes against Escherichia Coli, Staphylococcus Aureus, and Listeria Monocytogenes

The results on zones of inhibition obtained are presented in Table 1. Statistically Significant differences ($p \leq 0.05$) in antimicrobial activity were observed among the different

compounds (Synthesized BA, BN and their Complexes) against *E. coli*, *S. aureus* and *L. monocytogene*.

At a concentration of 1000ppm, the zones of inhibition for *E. coli* ranged from 11.32 mm (BA) to 21.90 mm (Gentamycin). For *S. aureus*, the range was from 13.33 mm (BA) to 22.9 mm (Gentamycin), and for *L. monocytogenes*, it was from 11.32 mm (BA) to 15.66 mm (BN Complex). At a concentration of 500ppm, the zones of inhibition for *E. coli* ranged from 9.32 mm (BA) to 19.53 mm (Gentamycin). For *S. aureus*, the range was from 11.33 mm (BN) to 20.33 mm (Gentamycin), and for *L. monocytogenes*, it was from 11.66 mm (BA) to 15.55 mm [(BN Complex). Table 1].

At a concentration of 250ppm, the zones of inhibition for *E. coli* ranged from 7.0 mm (BA, BN Complex) to 19.53 mm (Gentamycin). For *S. aureus*, the range was from 7.32 mm (BA Complex) to 20.33 mm (Gentamycin), and for *L. monocytogenes*, it was from 7.32 mm (BN, BA Complex) to 13.98 mm (BN Complex). At a concentration of 125ppm, the zones of inhibition for *E. coli* ranged from 7.0 mm (BA, BN Complex) to 18.31 mm (Gentamycin). For *S. aureus*, the range was from 7.0 mm (BA, BN Complex) to 19.64 mm (Gentamycin), and for *L. monocytogenes*, it was from 7.0 mm [(BA, BN Complexes) Table 1].

At a concentration of 250ppm, the zones of inhibition for *E. coli* ranged from 7.0 mm (BA, BN Complex) to 19.53 mm (Gentamycin). For *S. aureus*, the range was from 7.32 mm (BA Complex) to 20.33 mm (Gentamycin), and for *L. monocytogenes*, it was from 7.32 mm (BN, BA Complex) to 13.98 mm (BN Complex). At a concentration of 125ppm, the zones of inhibition for *E. coli* ranged from 7.0 mm (BA, BN Complex) to 18.31 mm (Gentamycin). For *S. aureus*, the range was from 7.0 mm (BA, BN Complex) to 19.64 mm (Gentamycin), and for *L. monocytogenes*, it was from 7.0 mm [(BA, BN Complexes) Table 1].

The study showed statistically significant differences ($p \leq 0.05$) in the antimicrobial activity of different compounds against *S. aureus*. Gentamycin displayed the highest bioactivity, ranging from 19.64 mm to 22.99 mm across all concentrations tested. BN Complex closely followed with a bioactivity range of 11.32 mm to 16.24 mm, while BA Complex demonstrated a range of 8.32 mm to 15.66 mm. regarding the individual compounds, BN and BA exhibited similar bioactivity ranges. BN showed a range of 8.24 mm to 15.30 mm, while BA had a slightly narrower range, ranging from 7.65 mm to 13.33 mm (Figure 5).

Post hoc comparisons revealed that Gentamycin displayed significantly higher bioactivity than all other compounds at all concentrations. BN Complex exhibited significantly higher bioactivity compared to BA Complex, BN and BA at all concentrations. Additionally, BA Complex demonstrated significantly higher bioactivity compared to BN and BA. However, there was no significant difference in bioactivity between BN and BA (Figure 5).

Statistically significant differences ($p \leq 0.05$) in antimicrobial activity were observed among the different compounds against *E. coli*. Gentamycin displayed the highest bioactivity, ranging from 18.31 mm to 21.90 mm across all concentrations (1,000ppm, 500ppm, 250ppm,

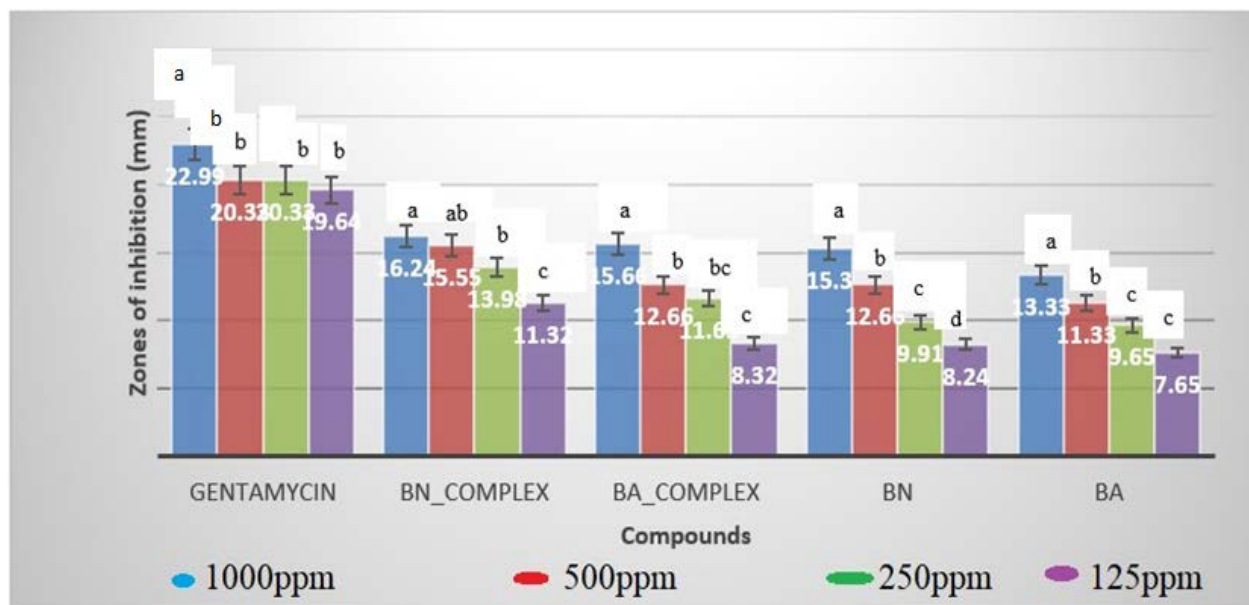
and 125ppm). BN Complex exhibited a range of bioactivity from 7.0 mm to 14.61 mm, while BA Complex demonstrated a range of 7.0 mm to 12.60 mm. The antimicrobial activity of BN ranged from 7.0 mm to 12.27 mm, and BA consistently showed a bioactivity of 7.0 mm across all complex concentrations (Figure 6). Post hoc

analysis indicated that BN Complex had significantly higher bioactivity compared to BA Complex, BN, and BA at all concentrations. Additionally, BA Complex demonstrated significantly higher bioactivity compared to BN and BA. However, there was no significant difference in bioactivity between BN and BA (Figure 6).

Table 1. Antimicrobial activity of Synthesized BA, BN and their complexes against *E. coli*, *S. aureus*, and *L. monocytogenes*

Comp ⁷	Organism	N	Zones of inhibition at different Concentrations				Lsd ⁶	Cv (%)	Mean
			1000ppm ¹	500ppm ¹	250ppm ¹	125ppm ¹			
BA	<i>E. coli</i>	3	11.32 ^a	9.32 ^b	7.0 ^c	7.0 ^c	1.201	12.69	10.45
	LM ²	3	11.32 ^a	11.66 ^a	7.32 ^b	7.0 ^b	1.172	10.96	10.63
	<i>S. aureus</i>	3	13.33 ^a	12.66 ^a	9.65 ^b	8.24 ^b	1.249	12.59	11.74
BAC ³	<i>E. coli</i>	3	12.27 ^a	10.97 ^a	7.65 ^b	7.0 ^b	1.29	14.52	10.69
	LM ²	3	13.3 ^a	12.32 ^a	9.65 ^b	7.0 ^c	1.230	9.78	11.09
	<i>S. aureus</i>	3	15.66 ^a	12.66 ^b	11.63 ^b	8.32 ^c	1.232	11.08	13.19
BN	<i>E. coli</i>	3	12.6 ^a	9.97 ^b	7.32 ^c	7.0 ^c	1.112	12.7	10.53
	LM ²	3	13.66 ^a	11.32 ^b	7.96 ^c	7.0 ^c	1.173	10.95	10.71
	<i>S. aureus</i>	3	15.3 ^a	11.33 ^b	9.91 ^c	7.65 ^d	1.199	11.94	11.93
BNC ⁴	<i>E. coli</i>	3	14.61 ^a	12.66 ^b	7.96 ^c	7.0 ^c	1.286	14.52	10.69
	LM ²	3	15.3 ^a	13.98 ^b	7.96 ^c	7.0 ^c	1.230	11.78	11.09
	<i>S. aureus</i>	3	16.24 ^a	15.55 ^{ab}	13.98 ^{bc}	11.32 ^c	1.232	11.08	13.19
Gent ⁵	<i>E. coli</i>	3	21.90 ^a	19.53 ^b	19.53 ^b	18.31 ^{ab}	1.284	16.045	12.66
	LM ²	3	19.31 ^a	17.98 ^b	17.62 ^b	16.98 ^b	1.26	14.65	13.18
	<i>S. aureus</i>	3	22.9 ^a	20.33 ^b	20.33 ^b	19.64 ^b	1.20	11.20	14.60
Mean (mm)			14.925	13.13	10.28	9.01			
Lsd ($\alpha=0.05$)			1.085	1.111	1.098	1.061			
CV (%)			3.14	4.27	4.19	2.79			
<i>p</i> -value			<.0001	<.0001	<.0001	<.0001			

¹ Compound concentrations, ² *Listeria Monocytogene*, ³ BA Complex, ⁴ BN Complex, ⁵ Gentamycin, ⁶ Least Significant difference, ⁷ compound ^a Means followed with the same letter along the rows are not significantly different.



^a Bars followed with the same letter are not significantly different

Figure 5. Antimicrobial activity of synthesized compounds - BA, BN and their complexes against *Staphylococcus aureus*.

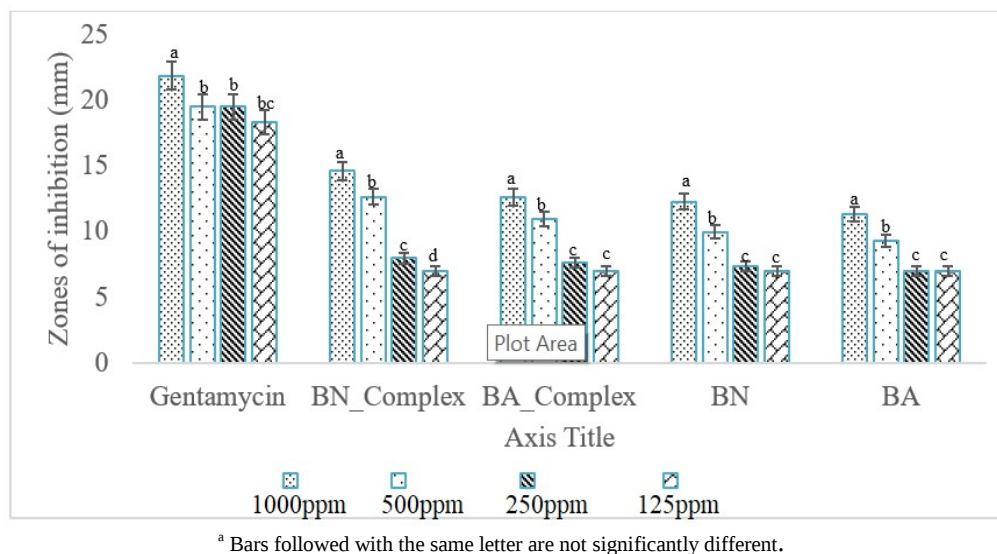


Figure 6. Antimicrobial activity of synthesized compounds - BA, BN and their complexes against *Escherichia coli*

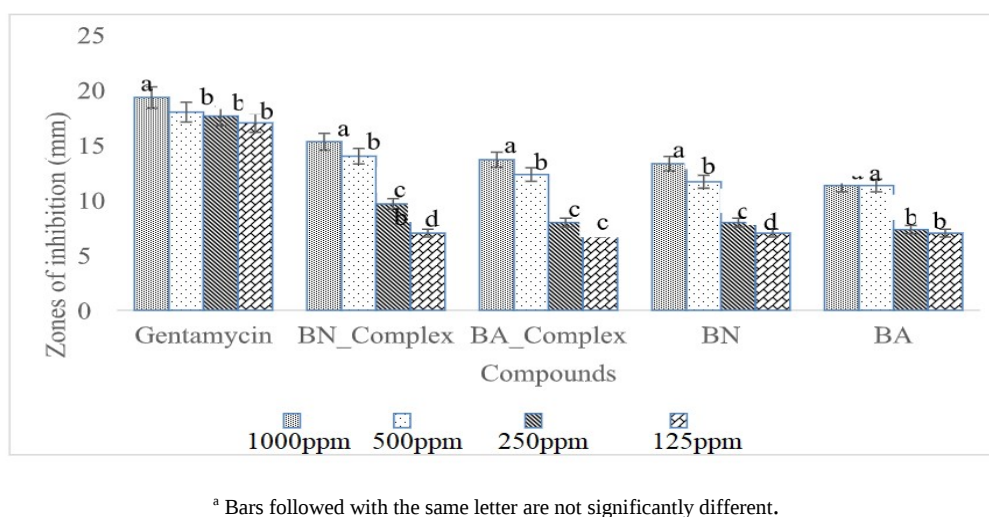


Figure 7. Antimicrobial activity of synthesized compounds - BA, BN and their complexes against *Listeria monocytogenes*

Statistically Significant differences ($p \leq 0.05$ in antimicrobial activity) were observed among the different compounds against *Listeria monocytogenes*. Gentamycin demonstrated the highest bioactivity, ranging from 17.0 mm to 19.31 mm across all concentrations. BN Complex exhibited a bioactivity range of 7.0 mm to 15.30 mm, while BA Complex exhibited a range of 7.0 mm to 13.66 mm. The antimicrobial activity of BN ranged from 7.0 mm to 13.30 mm, and BA consistently showed a bioactivity of 7.0 mm across all complex concentrations.

Post hoc comparisons revealed that Gentamycin had significantly higher bioactivity than all other compounds at all concentrations. Additionally, BN Complex displayed significantly higher bioactivity compared to BA Complex, BN, and BA at all concentrations. Similarly, BA Complex demonstrated significantly higher bioactivity than BN and BA. However, there was no statistically significant difference in bioactivity between BN and BA (Figure 7).

3.2. Discussion of Antibacterial Activities

All the tested compounds demonstrated antibacterial activities on bacteria strains. This in agreement with other

previously reported publication. This could be due to presence of N and O oxygen atom on both Schiff base ligand and Co (II) complexes [7]. In addition the presence of azomethine bond in both Schiff base ligand and the Co (II) complexes was responsible for their antibacterial activity observed as reported by [4]. He reported that azomethine bond may be responsible for formation of the hydrogen bond with chemical structure of bacteria cell hence leading to bacteria cell death.

There bioactivity between BN and BA Schiff was not statistically significant different. However, BN complex showed higher statistically significant difference among all the other test compounds, even higher than BA complexes. This confirmed that coordinating the ligands to metal ion increases their antibacterial as reported by other previous publication in literature [9]. This could be due to changes in polarity of the ligand. Decrease in polarity of ligand and the partial sharing of positive charge of metal and the donor group enhances delocalization of π electrons over the entire complex thereby promoting lipophilicity of the metal ion which is greatly reduced by overlapping of the ligand orbital and the partial sharing of positive charges. This favours the permeability of the

complexes through the lipid layer in the cell membrane. Once the complexes enter the cell, they bind the enzyme of the bacteria thereby disturbing an important cellular process leading to cell death [10].

BN complexed showed better antibacterial agents than another synthesized compound. This showed that nitro group (an electron withdrawing group) increases the bioactivity of the Schiff bases as reported by other previous publication [1]. He reported that introduction of an electron withdrawing group reduces electron density at ortho and para position of the Schiff base moiety making it more favourable for bacterial cell attack.

Lastly, from Figure 5, Figure 6, Figure 7 and Table 1 shows that antibacterial activities increase with increase concentration of the tests compounds. It is possible to have better antibacterial activities than the one explained in Table 1 by increase the concentration even though the toxicity (side effects) of the drugs to human cells increases with increases in concentration of the drug as reported by other previous publication [11].

4. Conclusions

The antibacterial activity of the synthesized compounds was investigated against two gram-positive (*S. aureus* and *L. monocytogene*) bacteria and one gram-negative bacteria (*E. coli*). The inhibition zones were observed on all the test compounds in all concentration. This showed that all the compounds were active against the pathogenic bacteria which were tested but no test compound which displayed better antibacterial activity than Gentamycin (a commercial antibacterial currently in the market).

S aureus showed the highest zones of inhibition among the three organism tested. This implies that *S aureus* is most susceptible to the compound synthesized, followed by *L monocytogenes* and *E. coli* is the least susceptible. This implies that these synthesized compounds can be best antibacterial agent for gram positive organism but not suitable for gram negative organisms.

BN complexes displayed highest zones of inhibition close to that gentamycin. This could be due to presence of nitro group (an electron withdrawing group) in the ligand structure. This suggest that introduction of electron withdrawing group in the structure of Schiff base ligand increases the antibacterial activities of the ligands and their metal complexes. Lastly, it was found that metal complexes has greater antibacterial activity than their respective Schiff base ligand.

ACKNOWLEDGEMENTS

The authors wish to thank Prof Abiy Yenesew university of Nairobi for his assistance in NMR analysis. This work was supported in part by a grant from Chuka university internal grant financial year 2021/2022.

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