

Prospect of Salophene Derivative and its Co(II), Ni(II) and Cu(II) Complexes as

Antimicrobial Agents

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ABSTRACT

Salophene derivative was synthesized by refluxing 4-bromobenzene-1,2-diamine with 5-bromo-2-hydroxybenzaldehyde in 1:2 mole ratio. Complexes of cobalt, nickel and copper with the salophene ligand were also synthesized by refluxing the ligand with corresponding metal ion in 1:1 mole ratio. FTIR, molar conductivity, magnetic moments, and CHN elemental analysis were determined, while antimicrobial potency of the compounds were tested against selected bacteria and some fungal isolates using agar well diffusion method. FTIR spectra of the ligand showed a strong band at 1614 cm^{-1} assignable to C=N stretching vibrations. The band shifted to lower frequencies range of $1603\text{--}1607\text{ cm}^{-1}$ in the spectra of the complexes, indicating coordination between the metals and the nitrogen of the ligand. Molar conductivity values obtained for all the complexes were low ($24.00\text{--}50.20\text{ }\Omega^{-1}\text{cm}^2\text{mol}^{-1}$) indicating non-electrolytic nature. Magnetic moments indicated that all the complexes were paramagnetic with exception of zinc complexes which were diamagnetic. The values (1.99–4.04 BM) suggested octahedral geometry for all the complexes. CHN elemental analysis showed strong agreement with theoretical values for the ligand and the complexes. The results revealed that the complexes showed higher activity (11–24 mm) than the free ligand (08–17 mm) and it was observed that their activity increased with increase in concentration.

Keywords: Salophene, Co(II), Ni(II) and Cu(II) complexes; UV-vis spectroscopy; antimicrobial

INTRODUCTION

The condensation of an aldehyde group with primary amine forms an imine bond (-C=N-), known as a Schiff's base. The Salen ligand system, which is also a Schiff's base, is one of the most studied classes of chelating ligands. The earliest report of a Salen-metal complexes was likely around 1933 by Pfeiffer and his coworkers [1]. The word, "Salen" comes from two abbreviations, sal and en. "Sal" for salicylaldehyde and "en" for ethylenediamine. When two equivalents of salicylaldehyde react with one equivalent of ethylenediamine, potential tetradentate chelating ligand known as 'Salen' is produced. However, when

phenylenediamine (phen) is used instead of ethylenediamine during the reaction, the resulting ligand is called “Salphen” or sometimes “Salophene”. Salophene and its derivatives have extended conjugations with rigid planarity when coordinated with metal ion in square planar, octahedral or square pyramidal geometry, which is an important criterion for material applications. Additionally, the presence of N₂O₂ donor sites in the salophene ligand allows metal ions to adopt various geometries such as square planar, tetrahedral, square pyramidal and octahedral, with additional ligands if needed. To achieve this, a large number of metal ions have been introduced to salophene to produce a variety of complexes [2-4].

A wide range of transition metals, main group metals and inner transition metals have been coordinated with salophene ligand systems. As a multidentate ligand, its complexes have potential applications in several fields such as catalysis, biochemistry, electrochemistry, sensors, molecular magnetism and materials science [5, 6]. Furthermore, the presence of the azomethine group in salophene which is analogous to natural biological systems results in varied biological applications [7]. Salophene has been reported to be used for various biological applications such as antitumor, anti-HIV, antimicrobial, and anticancer [8]. It is widely established that the chemotherapeutic properties of Salophene are enhanced upon complexation. Different complexes of salophene-type ligands have shown effectiveness against selected pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). The potency of these complexes suggests that salophene complexes could be lead structures for the further development of antibacterial metal complexes [9, 10]. Three salophene based complexes were synthesized and screened for their *in-vitro* antibacterial activity against two-gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram- negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The results showed comparable antibacterial activities to commercially available antibiotics such as Ciprofloxacin and Chloramphenicol. Similarly, the *in vitro* cytotoxicity and antimicrobial potency of salophene derivative and its Co(II) complex against human cancer cell lines (Human lung carcinoma cells) and some pathogenic microorganisms respectively showed remarkable potentials as a drug candidate [11]. Also, Cu(II) complexes of some substituted salophene ligands exhibited significant antimicrobial effects against *E. coli* and *S. aureus*. These complexes also showed greater activity against human lung cancer cells, Hela (human cervical cancer cells) and HepG-2 (human liver hepatocellular carcinoma cells) [12]. The continuous increase of resistant bacteria including *Streptococcus pyogenes*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae* as

well as some fungal isolates (*Candida albicans* and *Aspergillus fumigatus*) are currently major challenges in medicine.

Therefore, the discovery of novel lead structures for the design of drugs such as salophene and its various metal(II) complexes to combat infections caused by these bacteria and fungi is urgently needed.

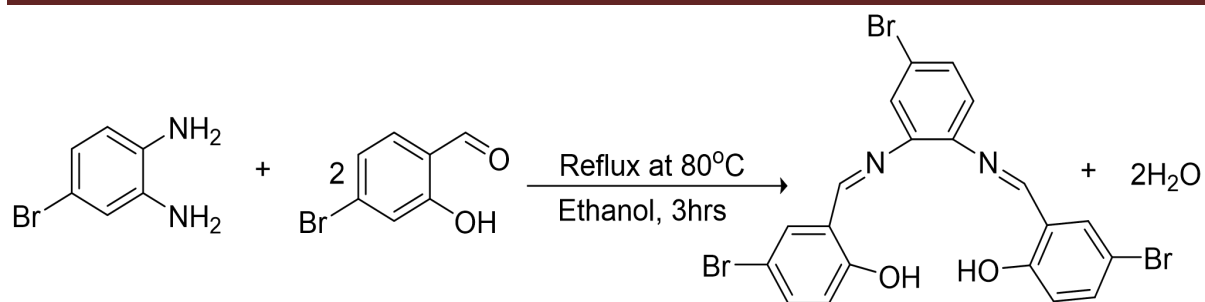
This research focuses on new salophene ligand and its Co(II), Ni(II) and Cu(II) complexes. It is intended to generate cost-effective drug with less or no side effect for treatment of microbial infections with reliable, simple, standard or affordable procedure that could easily be use for the production of effective antimicrobial therapeutic agents. The research can also provide a solution to the problem associated with drug resistance as it may open a new window for more potent antimicrobial drugs. The outcome of this research work would provide researchers that have interest in the same area with helpful scientific information.

MATERIALS AND METHODS

All chemicals used in this work were of analytical grade and were used without further purification. All glass wares were washed with detergent after soaking in conc. HNO₃, rinsed with distilled water and dried in an oven. Weighing was conducted using an electrical Melter balance (model AB54). Infrared spectral were determined using Fourier transform infrared spectrophotometer (FTIR-8400S), range: 4000-400 cm⁻¹. Electrical conductance was measured using Jenway conductivity meter (model 4010), with range 20-200 μS. Melting points and decomposition temperatures were obtained using microprocessor melting point apparatus (WRS-IB). Magnetic susceptibility was determined using magnetic susceptibility balance (MKI Sherwood scientific Ltd). Elemental analyses were recorded using Series II CHNS/O 2400 Perkin Elmer. The UV-Vis spectral information was established using Perkin Elmer UV-Vis Spectrophotometer Lambda-35.

Synthesis of the Salophene ligand (L^a)

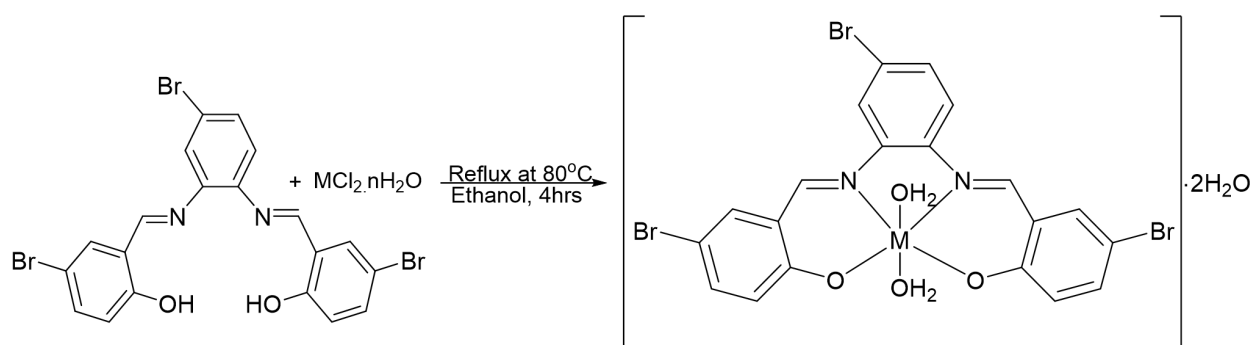
The synthesis of the salophene ligand (L^a) followed a previously reported procedure by Rezakhani et al [13]. Ethanolic solution (25 mL) of 4-Bromobenzene-1,2-diamine (2 mmol) was added to a solution of 5-bromo-2-hydroxybenzaldehyde (4 mmol) in anhydrous ethanol (25 mL) and the yellowish solution stirred and refluxed for 3 h (scheme 1). The precipitated solid compound was filtered, washed with 50% (v/v) ethanol – water several times to remove any traces of the unreacted starting materials and then dried in a desiccator over P₂O₅ for 24 h.



Scheme 1: Synthesis of 4-Bromobenzene-1,2-diamine and 5-bromo-2-hydroxybenzaldehyde (Salophene L^a (C₂₀H₁₃O₂N₂Br₃))

Synthesis of the Salophene (L^a) complexes

The salophene L^a complexes were synthesized in 1:1 molar ratio. The synthesized ligand (L^a) was dissolved in 30 mL of ethanol and the solution was heated to 40°C. Then, a hot ethanolic solution of the corresponding metal salt (MCl₂.nH₂O) was slowly added into a round-bottom flask containing the ligand solution. The resulting mixture was stirred and then refluxed for 4 hours at 80°C (scheme 2). The colored products obtained was cooled to room temperature, filtered off from the reaction mixtures, rinsed with cold ethanol, diethylether and dried under vacuum over P₂O₅ [14].



Where M = Metal (Co(II), Ni(II) and Cu(II))

Scheme 2: Synthesis of 4-Bromobenzene-1,2-diamine and 5-bromo-2-hydroxybenzaldehyde (Salophene L^a) Metal complexes.

Antimicrobial Sensitivity Test

Four bacterial species (*Streptococcus pyogenes*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae*), as well as two fungal species (*Candida albicans* and *Aspergillus fumigatus*), were obtained from Aminu Kano Teaching Hospital and identified at the Microbiology Department, Bayero University, Kano.

The sensitivity test was carried out by preparing sterile nutrient agar and Potato Dextrose Ager (PDA) media and carefully transferred into sterile petri-dishes to an appreciable

amount. It was cooled and solidified at room temperature. The petri-dishes were marked to indicate fungi, bacteria and the positions of the three wells of different test concentrations (1000, 500, 250 $\mu\text{g/ml}$) for both the ligand and the complexes. From the standardized inoculums of each isolate, uniform spreading (using a glass spreader) of 0.1 mL of bacteria and fungi inoculums was done on the surface of dried nutrient agar and PDA respectively. Each fraction of metal(II) complexes and the ligand petri-dishes were placed at the marked positions.

For the fungi, the petri-dishes were kept in a cool dry place for 72 hours while the plates were observed for the presence of zones of inhibition as evidence of antifungal activities.

For bacteria, the petri-dishes were kept in an incubator for 24 hours at 37 °C. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimeters with respect to each isolate and test concentration. The results were recorded as shown in Tables 5 – 8 [15].

RESULTS AND DISCUSSION

Synthesis and physicochemical studies

The Salophene ligand (L^a) was synthesized in the present study by reacting aromatic diamine (*4-bromobenzene-1,2-diamine*) with aromatic aldehyde (*5-bromo-2-hydroxybenzaldehyde*). The synthesized salophene ligand (L^a) was used in synthesizing new complexes of Co(II), Ni(II) and Cu(II). Both the ligand and its respective complexes were characterized using various analytical and spectroscopic techniques.

Table 1: Physical properties of Salophene ligand (L^a) and its metal complexes

Compound	Colour	Melting point Temp. (°C)	Decomposition . Temp. (°C)	%Yield
Ligand (L^a)	Yellow	218	-	84.42
$[\text{Co}L^a(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	Deep maroon	-	280	93.45
$[\text{Ni}L^a(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	Ox-blood	-	295	67.59
$[\text{Cu}L^a(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	Deep Green	-	285	71.59

Salophene ligand (L^a) was successfully synthesized from the condensation of 4-Bromobenzene-1,2-diamine and 5-bromo-2-hydroxybenzaldehyde (Scheme 1). It was found to be very stable in air and moisture at room temperature. It was soluble in DMSO, DMF and in methanol, ethanol, (on heating) while completely insoluble in n-hexane, diethylether and

water (Table 2). The ligand was yellow in colour, amorphous solid having melting point temperature of 218 °C and percentage yield of 84.42% (Table 1).

Table 2: Solubility of Salophene ligand (L^a) and its metal complexes

Compound	n-Hexane	Diethyl Ether	EtOH	MeOH	H ₂ O	DMF	DMSO
Ligand (L^a)	IS	SS	IS	IS	IS	S	S
[CoL ^a (H ₂ O) ₂].2H ₂ O	IS	SS	S	S	IS	S	S
[NiL ^a (H ₂ O) ₂].2H ₂ O	IS	SS	SS	SS	IS	S	S
[CuL ^a (H ₂ O) ₂].2H ₂ O	IS	SS	SS	S	IS	S	S

The metal(II) complexes were prepared by the stoichiometric reaction of Salophene ligand (L^a) with the corresponding metals (cobalt(II), nickel(II) and copper(II)) as chlorides according to the procedure already described. The corresponding reaction was carried out using molar ratio as 1:1 (metal: ligand) in boiling ethanol (Scheme 2).

All the metal complexes of L^a were intensely coloured, with Co(II) complex having deep-maroon while Ni(II) and Cu(II) complexes appeared to be deep-green and yellowish-brown respectively. The colour change may be due to electronic transition within the d-orbitals of the metal ions, charge transfer from metal to ligand and vice versa or nature of the ligand. All were amorphous in nature and decomposed without melting at appreciably higher temperature (280-295 °C) than the corresponding ligand (Table 1). These high temperatures indicated the good stability of the complexes and may be due to coordination between the ligand and the metal ion. The results were in close agreement with what was obtained by Haque et al [16].

The complexes were insoluble in common organic solvents such as chloroform, acetone n-hexane, but slightly soluble in diethylether, methanol, ethanol and completely soluble in DMSO and DMF (Table 2). All complexes were obtained in an appreciable yield (67.59-93.45%).

Table 3: CHN Analysis data of Salophene ligand (L^a) and its metal(II) complexes

Compound	Elemental Analyses Theoretical (Experimental)			
	%C	%H	%N	%Metal
Ligand (L ^a)	43.44 (43.05)	2.37 (2.48)	5.07 (5.09)	--
[CoL ^a (H ₂ O) ₂].2H ₂ O	35.22 (34.96)	2.81 (3.01)	4.11 (4.06)	8.64 (8.55)
[NiL ^a (H ₂ O) ₂].2H ₂ O	35.23 (35.12)	2.81 (2.98)	4.11 (4.31)	8.61 (8.49)
[CuL ^a (H ₂ O) ₂].2H ₂ O	34.98 (34.66)	2.79 (2.83)	4.08 (3.97)	9.25 (8.96)

To accurately establish the formation of the ligand L^a and its corresponding metal(II) complexes, elemental analysis was conducted on the synthesized compounds. The obtained (experimental) C, H, N, and metals (Co, Ni and Cu) percentage data of both the ligand and the metal(II) complexes were compared with the calculated (theoretical) values of their proposed molecular structures. The theoretical and experimental values of the ligand and all the metal(II) complexes are reported in Table 3. The two values were in the acceptable range which provided an evidence for the successful preparation of the proposed ligand L^a and its metal(II) complexes.

Table 4: IR Spectra of the Salophene ligand (L^a) and its metal(II) complexes

Compound	V(C=N) cm ⁻¹	V(M-O) cm ⁻¹	V(M-N) cm ⁻¹	r(M-OH ₂) cm ⁻¹	w(M-OH ₂) cm ⁻¹	V(H ₂ O) cm ⁻¹
Ligand (L ^a)	1614	-	-	-	-	-
[CoL ^a (H ₂ O) ₂].2H ₂ O	1603	478	626	811	592	3424
[NiL ^a (H ₂ O) ₂].2H ₂ O	1603	476	604	803	585	3044
[CuL ^a (H ₂ O) ₂].2H ₂ O	1607	474	626	811	585	3171

The L^a ligand has a number of potential donor atoms that might be involved in coordination with the transition metal ions forming complexes. It is feasible that the coordination of such ligand occurs through hydroxyl oxygen (-OH) and azomethine nitrogen (HC=N) with the metal ions. The infrared spectral bands of the ligand and its respective complexes were presented in Table 4. FTIR spectra of all the complexes were compared with that of the ligand and the conclusions drawn from these observations confirmed the modes of absorption and the complexation of ligand with the metal ions. The band appearing at 1614 cm⁻¹ due to

stretching vibration of azomethine group in the ligand was found to be shifted to lower frequency at 1603-1607 cm^{-1} in the metal(II) complexes, indicating the involvement of the azomethine nitrogen in interaction with the metal ion. The values were consistent with the result obtained by Kuddushi et al [17], 1614 cm^{-1} for the ligand and 1600-1616 cm^{-1} for the respective metal(II) complexes. Similarly, the C-O band was due to bending vibration of the phenolic oxygen (Ph-O) in the ligands, which occurs at 1186 cm^{-1} , and then shifted to 1171-1182 cm^{-1} indicating the deprotonation and coordination of the phenolic oxygen to the metal ion after complexation. This shows that the ligand acted as tetradentate ligand coordinated via the two azomethine N and the two phenolic O.

Further evidence of the coordination of the ligand with the metal ions was shown by the appearance of new bands at 474-478 and 604-626 cm^{-1} which were assigned to the metal-oxygen (M-O) and metal-nitrogen (M-N) stretching vibrations, respectively [18]. These bands were absent in the spectrum of the free ligand, confirming participation of the O and N atoms in coordination with respective metal ions.

The presence of broad diffuse band of strong intensity in the 3044–3424 cm^{-1} region was observed in all the complexes, which may be assigned to the OH stretching vibration for the lattice water. The rocking and wagging vibrations of H_2O can be assigned to the bands at 803–811 and 585–592 cm^{-1} , respectively [19].

Table 5: Conductivity measurement of Salophene ligand (L^a) complexes in DMSO

Compound	Concentration (Mol dm^{-3})	Specific conductance ($\text{Ohm}^{-1} \text{cm}^{-1}$)	Molar conductance ($\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$)
$[\text{CoL}^a(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$	1.0×10^{-3}	50.20×10^{-6}	50.20
$[\text{NiL}^a(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$	1.0×10^{-3}	40.70×10^{-6}	40.70
$[\text{CuL}^a(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$	1.0×10^{-3}	24.00×10^{-6}	24.00

Metal complexes in solution are frequently charged and as such they behave as strong electrolytes, dissociating into their constituent complex ions and counter ions. By using the relation $\Lambda_m = K/C$, the molar conductance of the complexes (Λ_m) can be calculated, where C is the molar concentration of the metal complex solutions. Measurement of Λ_m at a given concentrations would provide a means of determining the charge type of a metal complex, and hence providing its structural information. The molar conductance values of all the prepared metal(II) complexes were obtained in $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ at room temperature using DMSO as a solvent and their results were given in Table 5.

Molar conductance values of the complexes in DMSO (10^{-3}M solution at 25°C) indicated that all the complexes of L^{a} were non-electrolyte in nature having lower values of $24.00\text{-}50.20\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ as shown in Table 5. These readings were found to show strong correlation with work reported by Uddin et al [20].

Table 6: Magnetic susceptibility of Salophene ligand (L^{a}) complexes

Compound	Xg (gmol^{-1})	Xm (gmol^{-1})	μ_{eff} (BM)	n	Magnetic property
$[\text{CoL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	1.12×10^{-5}	6.86×10^{-3}	4.04	3	Paramagnetic
$[\text{NiL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	6.44×10^{-6}	3.93×10^{-3}	3.06	2	Paramagnetic
$[\text{CuL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$	2.70×10^{-6}	1.66×10^{-3}	1.99	1	Paramagnetic

The observed magnetic moment (B.M) values (Table 6) of the L^{a} complexes were determined at room temperature. The magnetic values of Co(II) complex was found to be 4.04 B.M, which was consistent with high spin octahedral geometry showing three unpaired electrons. These values are similar with previous reports [21, 22]. The magnetic value (3.06 B.M) measured for Ni(II) complex indicated the presence of two unpaired electrons there by suggesting that it has an octahedral configuration. Similarly, the magnetic moment value (1.99 B.M) for the Cu(II), also expected for a d^9 system suggesting that it contains one unpaired electron. Hence, the Cu(II) complex appears to be in the octahedral geometry and the results showed strong correlation with values obtained. It can be concluded that all the complexes of L^{a} herein were paramagnetic.

Table 7: Electronic spectral data of Salophene ligand (L^{a}) complexes

Compound	Solvent	$\pi \rightarrow \pi^*$ (nm)	$n \rightarrow \pi^*$ (nm)	LMCT/MLCT (nm)
Ligand (L^{a})	DMSO	239	367	--
$[\text{CoL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$		225	347	486
$[\text{NiL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$		246	350	485
$[\text{CuL}^{\text{a}}(\text{H}_2\text{O})_2].2\text{H}_2\text{O}$		213	380	453

The UV-Vis spectra of ligand L^{a} and its complexes were recorded in DMSO from 200 to 700 nm and the result is presented in Table 7. Two absorption bands at 239 and 367 nm were assigned to intraligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions which were slightly shifted to 213 – 246 nm and 347 – 380 nm upon complexation with metal ions. These results were in strong

agreement with the values reported by Asadi et al [23]. The new bands that were found to appear in all the complexes in the region of 453-486 nm, which were similar to the findings by Alaghaz et al [24], could be assigned to ligand - metal charge transfer (LMCT) transition bands. Therefore, a shift in the λ_{\max} is an indication that coordination has occurred between the metal ion and the ligand.

The proposed structures of the synthesized compounds were presented in Figures 1 and 2.

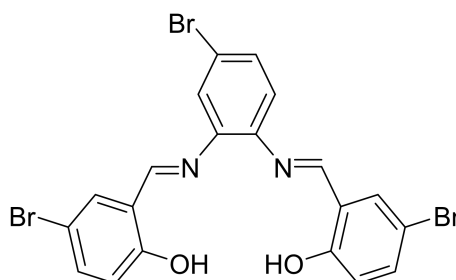
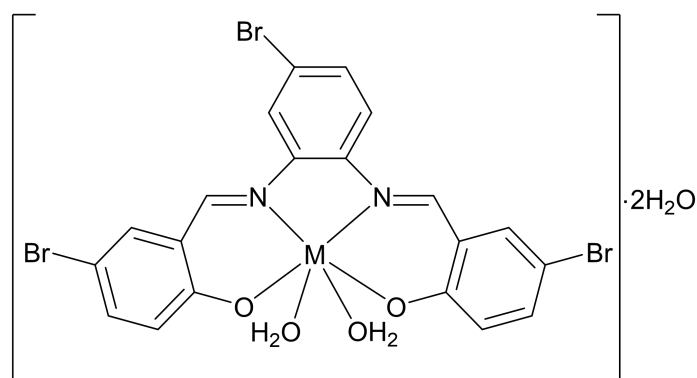


Fig.1: Structural formular of the salophene ligand
($L^a = 2,2'-((4\text{-bromo-1,2-phenylene})\text{bis}(\text{azanylylidene}))\text{bis}(\text{methanylylidene}))\text{bis}(4\text{-bromophenol}))$)



Where M is Co^{2+} , Ni^{2+} or Cu^{2+}

Fig. 2: Structural formular of the synthesized salophene complexes

Antimicrobial activity of Salophene ligand (L^a) and its Metal(II) complexes

The *in-vitro* antimicrobial activity of Salophene (L^a) ligand and its corresponding metal(II) complexes were tested against gram positive (*Streptococcus pyogenes* and *Bacillus subtilis*) and gram negative (*Salmonella typhi* and *Klebsiella pneumoniae*) bacteria as well as some fungal isolates (*Candida albicans* and *Aspergillus fumigatus*). The Salophene and its metal complexes were tested at different concentrations of 1000, 500 and 250 $\mu\text{g/ml}$ using agar well diffusion method. The growth inhibitory zones were measured in diameter (mm) and the

results are summarized and presented in Tables 8a and 8b for both antibacterial and antifungal screening. Ciprofloxacin and Ketoconazole were used as control for bacteria and fungi respectively.

Table 8a: Antibacterial activities of Salophene ligand (L^a) and its metal complexes

Compound	Zone of Inhibition (mm)											
	<i>S. pyogenes</i>			<i>B. subtilis</i>			<i>S. typhi</i>			<i>K. pneumoniae</i>		
	1000	500	250	1000	500	250	1000	500	250	1000	500	250
	(µg/ml)			(µg/ml)			(µg/ml)			(µg/ml)		
Ligand (L ^a)	17	14	09	16	15	11	15	13	08	15	12	09
[CoL ^a (H ₂ O) ₂].2H ₂ O	24	20	15	21	18	15	18	14	13	19	16	13
[NiL ^a (H ₂ O) ₂].2H ₂ O	22	18	16	22	19	14	20	16	13	17	14	11
[CuL ^a (H ₂ O) ₂].2H ₂ O	20	16	14	20	18	14	22	19	14	18	15	11
Ciprofloxacin (Control)	30			28			25			31		

Table 8b: Antifungal activities of Salophene ligand (L^a) and its metal complexes

Compound	Zone of Inhibition (mm)					
	<i>Candida albicans</i>			<i>Aspergillus fumigatus</i>		
	1000	500	250	1000	500	250
	(µg/ml)			(µg/ml)		
Ligand (L ^a)	16	15	10	14	13	09
[CoL ^a (H ₂ O) ₂].2H ₂ O	21	18	13	20	14	13
[NiL ^a (H ₂ O) ₂].2H ₂ O	19	17	14	19	16	12
[CuL ^a (H ₂ O) ₂].2H ₂ O	19	18	14	17	15	11
Ketoconazole (Control)	29			26		

The *in-vitro* antibacterial activity of ligand L^a and its metal(II) complexes were tested against four human pathogenic bacteria; *Streptococcus pyogenes*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae*. Ciprofloxacin was used as a positive control and the agar well diffusion method was used to evaluate the antibacterial activity of the synthesized compounds.

From the antibacterial activity results (Table 8a), ligand L^a showed moderate activity against the tested organisms at 1000 and 500 µg/ml concentrations and low activity at a lower concentration of 250 µg/ml. However, all the complexes exhibited high activity against *Streptococcus pyogenes*, *Bacillus subtilis* and *Salmonella typhi* at the tested concentrations (1000, 500 and 250 µg/ml). However, they only showed moderate activity towards *Klebsiella pneumoniae* at the same concentrations. The activity of these compounds against the tested organisms may be attributed to the presence of imine groups which are involved in transamination reactions in biological systems [25, 26]. A comparative study of the growth inhibitory zone values of the ligand and its metal complexes indicated that the metal complexes exhibited higher antibacterial activity than the free ligands (Table 8a).

On the other hand, the antifungal activity of the synthesized Salophene L^a and its respective metal(II) complexes was measured against two fungal species *Candida albicans* and *Aspergillus fumigatus* and the results were compared with that of ketoconazole as a positive control. The results indicated that the complexes were more active against the fungal isolate used than the free ligand (Table 8b). Comparatively, the cobalt(II) complex showed higher activity against the two organisms while the other complexes exhibited moderate inhibitory action towards the tested fungal isolates.

Generally, metal complexes are more active against micro-organisms than the free ligands, likely due to the greater lipophilic nature of the complexes. This increased activity of the metal complexes can be explained by Overtone's concept and Tweedy's chelation theory [27, 28]. According to Overtone's concept of cell permeability, the lipid membrane surrounding the cell favours the passage of only lipid soluble materials, making liposolubility an important factor in controlling antibacterial activity. Upon chelation or complexation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups [29]. Furthermore, chelation increases the delocalization of π electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity allows the complexes to penetrate the lipid membrane more easily and block the metal binding sites on enzymes of micro-organisms [30].

CONCLUSION

A new Salophene ligand and its metal(II) complexes (Co, Ni and Cu) were successfully synthesized and characterized using various physical, spectroscopic and analytical techniques. The results indicated that the ligand is tetradentate and coordinated to the central metal ion through the azomethine nitrogen and deprotonated phenolic oxygen. Molar conductance values showed the complexes to be non-electrolytic. Elemental and other analytical data showed the stoichiometry of both complexes to be 1: 1 metal to ligand ratio. Magnetic moments and UV-vis spectral studies suggested an octahedral geometry for all complexes. All the metal(II) complexes exhibited superior antimicrobial properties compared to the free ligand under the same experimental conditions. This study also revealed that the antimicrobial growth inhibition ability of the synthesized compounds increased with higher concentrations.

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