Synthesis, Characterization, and Biological Activity of Cu (II) and Ag(I) Hetero-bimetallic Complex with [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)] and 1-Nitroso-2-Naphthol

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Abstract: **In the present research work, the reagent *N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde) is synthesized by Schiff base reaction. The reagent is characterized by FT-IR and NMR. This reagent is coupled with Cu (II) to get a monometallic complex. Another monometallic complex has been prepared by using 1-Nitroso-2-Naphthol as a reagent with Ag (I). The combination of these two will give a Hetero-bimetallic complex. This prepared complex is characterized by using FT-IR, powder XRD, and Elemental analysis. The said hetero-bimetallic complex shows better antibacterial, antifungal & anti-inflammatory activity than the mono-metallic complex.**

Keywords: **Schiff base, Hetero-bimetallic, Powder XRD, Cu (II)**

INTRODUCTION

The chemistry of coordination compounds comprises an area that spans the entire spectrum, from theoretical work on bonding to the synthesis of organometallic compounds.[1]

Bimetallic complexes are key targets for many applications because of their desirable functional properties. For instance, two metal centres can facilitate cooperative multi-electron processes with transition metal ions. The potential of bimetallic complexes in enhancing reactivity is a source of optimism and hope for researchers in the field. [2,3]

Preparing a new Schiff base mixed ligand was perhaps the most crucial step in developing metal complexes that exhibit unique properties and novel reactivity. The electron donor and electron acceptor properties of the ligand, structural-functional groups, and the position of the ligand in the coordination sphere, together with the reactivity of coordination compounds, may be factors for different studies.[4]
The ligands in the bimetallic complexes are multi-dentate and can coordinate with at least two metals. Compared to mono-metallic complexes, bimetallic complexes provide a faster reaction rate and better selectivity. In short, bimetallic complexes are a faster-growing field because of their promising bioactivities. The bimetallic complexes can be homobimetallic with two similar metal centers or heterobimetallic with dissimilar metal centers. Bimetallic complexes bring a combined set of properties that may be attributed to the two identical or different metals. The two different central metals in heterobimetallic complexes may have a combined property contribution attributed to both metals, which enhances the properties of the complex as compared to the homobimetallic complex.[5] The metal centers of the bimetallic complexes tend to cooperate by changing the reactivity and or physical properties of the complexes therein. Each metal may contribute individually or collectively to the overall properties of the complexes.[6] Heterobimetallic complexes can be prepared via the stepwise addition of different metal ions. [7-9]

The pharmacological properties of metal complexes depend on the properties of both the metal and the ligand. In many cases, the addition of metal ions to an organic ligand can lead to an increase in its biological activity.[10] A literature survey reveals that many bimetallic complexes have been designed, and their applications in several fields, such as agriculture [11], industry [12], medicine [13], etc., have been studied. In recent years, more research papers were found on the synthesis, characterization, and study of the antimicrobial activities of homometallic complexes with transition metals like Cu, Co, Mn, and Zn [14], the bimetallic complexes of Ru(II)-Fe(III)bimetallic complexes have been used for detecting signal amplifying and degrading oxalate which is an industrial pollutant. [15] In some hetero-bimetallic complexes, the study of the electronic effect of aluminium-containing ligands in Ir-Al and Rh-Al was also reported.[16]

Complexes of copper in oxidation state +2 were found to show significant antioxidant and anti-free radical activity. [17, 18] Bimetallic copper complexes are potential models for several important biological systems containing a couple of sites [19] and have been studied extensively. [20–25] The synthesis of a new copper (II) complex with a multidentate Schiff base ligand also contributes to the development of coordination chemistry. [26–28]

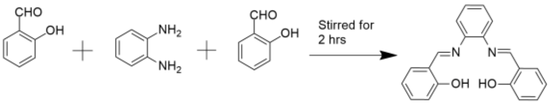
In the present work, the reagent (*N*, *N*-1,2-phenylenbis (2-hydroxybenzaldehyde)) is selected as a primary ligand obtained by the Schiff base reaction. This reagent forms a complex with Cu (II), which gives a monometallic complex. It is coupled with another (1-nitroso-2-naphthol) Ag (I) monometallic complex, which offers the heterobimetallic complex. The heterobimetallic complex shows more promising biological activities than the monometallic complex. To the level of our knowledge, Cu (II)-Ag(I) bimetallic complex with reagents (*N*, *N*-1,2-phenylenbis (2-hydroxybenzaldehyde)) and (1-nitroso-2-naphthol) has not been reported yet.

EXPERIMENTAL

All chemicals are purchased from commercial sources and used without further purification. O-phenylenediamine, Salicylaldehyde, copper chloride, silver chloride, and 1-nitroso-2-naphthol were purchased from Sigma Aldrich. Infrared spectral studies were recorded in the KBr phase on a Shimadzu 8201 FTIR spectrophotometer. Elemental analyses for C, H, and N were performed on a Perkin Elmer 240 C elemental analyser.

A) Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)]

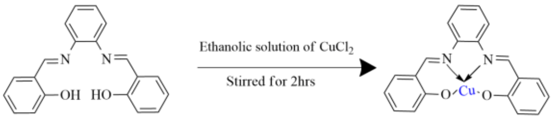
An ethanolic solution of O-phenylenediamine was stirred with Salicylaldehyde to obtain the final product [R_1]. R_1 is recrystallized with the help of ethanol.



Scheme I : Synthesis of [N, N-1,2-Phenylenebis (2-Hydroxybenzaldehyde)]

B) Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)] Cu (II) Complex

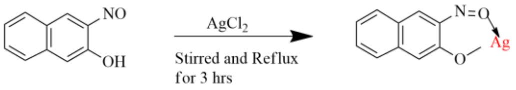
An ethanolic solution of copper chloride was added to an ethanolic solution of (R1) while stirring. A green precipitate formed upon stirring at room temperature. Stirring continued for one hour to ensure the reaction was complete. The product was filtered, washed with hot ethanol, then cold water, and dried under a vacuum.



Sheme II : Synthesis of [*N*, *N*-1,2-Phenylenebis (2-Hydroxybenzaldehyde)] Cu (II) Complex

C) Synthesis of 1-Nitroso-2-Naphthol Ag (I) complex

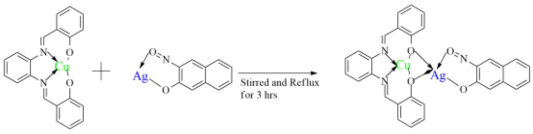
The hot ethanolic solution of (R_2) was added to the ethanolic solution of silver nitrate with stirring. The black precipitate (R2M2) was formed upon stirring at room temperature. Stirring was continued for one hour to ensure the reaction was completed. The product was filtered off, washed with hot ethanol, followed by cold water, and then dried under a vacuum.



Scheme III : (Synthesis of 1-Nitroso-2-Naphthol) Ag(II) Complex

D) Synthesis of Heterobimetallic Cu(II)-Ag(I) Complex

The ethanolic solution of (R1M1) was added to an ethanolic solution of (R_2M_2) with stirring. This mixture was refluxed for 3 hours. The product was filtered off, washed with hot ethanol, followed by water, and dried under vacuum



Scheme IV: Synthesis of $C_{30}H_{18}AgCuN_3O_4$ Complex

RESULT AND DISCUSSION

IR Spectrum:

The infrared spectra for the metal complex ligand $C_{20}H_{12}CuN_2O_2$ were recorded. A spectral band at

1274.23 cm^{-1} originated, which is attributed to phenolic C–O str. Band. When the ligand $C_{20}H_{12}CuN_2O_2$ formed heterobimetallic complexes with the Cu (II) metal chelates of 1-Nitroso-2-naphthol, shifting and splitting of the phenolic C–O str. band was observed. Therefore, in $C_{30}H_{18}AgCuN_3O_4$, spectral bands emerge at 1274 and 1230 cm^{-1}. The consequent shifting and splitting of phenolic C–O str. bands in the heterobimetallic complexes justifies the coordination through phenolic oxygen.

The FT-IR spectra for the metal complex ligands $C_{20}H_{12}CuN_2O_2$ and $C_{10}H_6NO_2Ag$ arise at 435.11 and 440.21 cm^{-1} due to M─O(str) modes, respectively. There is a shift in the position of these bands towards the higher energy side after the formation of heterobimetallic complexes, which is observed at 570.45 cm^{-1}

	$C_{20}H_{12}CuN_2O_2$	$C_{10}H_6NO_2Ag$	$C_{30}H_{18}AgCuN_3O_4$
υ (ArC=C)	1434.42	1405.47	1445.89
υ (ArC-H)	908.12	971.74	3053.10
υ (CH=N)	1608.53	-------------	1605.93
υ (N=O)	---------	1342.96	1304.12
υ (C-O)	1274.23	1230.12	1025.02
υ (M-O)	435.11	440.21	570.45
υ (M-N)	525.14	---------	542.31
υ (M-Cl)	482.14	465.31	-----------

NMR Spectroscopy:

The ^{1}H NMR spectra of the Schiff base ligands demonstrated characteristic azomethine (CH=N) protons at 4.89 ppm as a singlet, respectively. The (CH_2) protons present in all the ligands were observed at 3.05–3.98 ppm as a singlet.

Physical parameters of the Metal complexes

Complex		$C_{20}H_{12}CuN_2O_2$	$C_{10}H_6NO_2Ag$	$C_{30}H_{18}AgCuN_3O_4$
Molecular Weight		375.54	279.86	655.40
Colour		Dark green	Maroon	Brown
Analysis (%), Found (Calculated)	C	61.80 (63.90)	40.23 (43.01)	52.31 (54.92)
	H	2.20 (3.19)	1.35 (2.14)	1.70 (2.74)
	N	5.80 (7.45)	3.50 (5.00)	4.42 (6.40)
	Cu (II)	8.24 (16.91)	--------	4.20 (9.69)
	Ag (I)	--------	20.23 (38.54)	5.32 (16.45)
Yield (%)		78.04	65.31	67.95

Powder XRD:

The powder XRD pattern of the Ni-Ag bimetallic complex recorded in the range (2θ = 0-80°) was shown in Fig. XRD pattern of the bimetallic complex shows the sharp crystalline peaks indicating its crystalline phase. The average crystallite size (dXRD) of the complex was calculated using Scherer's formula. The bimetallic complex has an average crystallite size of 70 nm.

Biological activity:

Procedure: Antibacterial activity against *E. coli*, *Bacillus subtilis*, *S aureus*, and *P. aeruginosa* bacteria by the well diffusion method. The inoculums of the microorganism were prepared from the bacterial cultures. 15 ml of nutrient agar (Hi Media) medium was poured into clean, sterilized Petri plates and allowed to cool and solidify. 100 µl of broth of the bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Once the agar was hardened, the Sample Slides were placed on the plate, and the plates were incubated at 370°C for 24 hours. Antibacterial activity was evaluated by measuring the diameters of the zones of inhibition (ZI).[29]

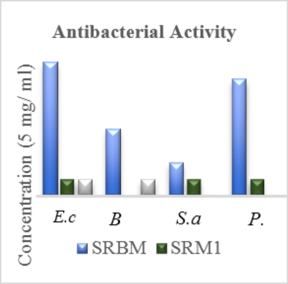
Antibacterial Activity of samples against *E. coli*, *Bacillus subtilis*, *S Aureus*, *P. aeruginosa*,

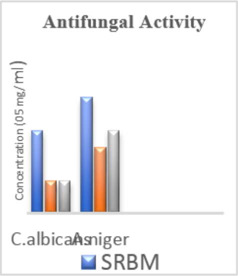
SAMPLES	$C_{30}H_{18}AgCuN_3O_4$ Complex (SRBM)	$C_{20}H_{12}CuN_2O_2$ (SRM1)	$C_{10}H_6NO_2Ag$ (SRM2)
CONCENTRATION (mg/ml)	5 mg	5 mg	5 mg
ZONE IN DIAMETER (mm) *E. coli*	08	02	02
ZONE IN DIAMETER (mm)	07	00	02

Bacillus subtilis			
ZONE IN DIAMETER (mm) *Staphylococcus Aureus*	05	01	00
ZONE IN DIAMETER (mm) *Pseudomonas*	08	01	00

Procedure: Antifungal activity against Candida Albicans A. Niger & A.Barsiliniase (Agar well plate diffusion Method) WELL DIFUUSION METHOD FOR THE DETERMINATION OF ZONE OF INHIBITION Antifungal activity Stock solution for antifungal activity: For antifungal study sample concentration of 5mg and 10 mg stored in a refrigerator till further used. Antifungal activities of the sample were evaluated using agar well diffusion assay. The assay was carried out according to the method of (Hufford et al., 1975). Sabouraud dextrose agar (Hi Media) was used for the growth of the fungus. Media with acidic pH (pH 5.5 to 5.6) containing a relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121ºC for 15 minutes. Twenty-five ml of molten (45°C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish. For counting of spores (fungi) were counted by suspending them in normal saline to make the volume up to 1ml and then counted with the help of a hemocytometer (Neubauer chamber). Once the agar was hardened, 6mm wells were bored using a sterile cork borer. Then 0.1 mL (100μl) from each stock solution of the sample, having a final concentration of 5 mg and 10mg, was placed in each well, and the plates were incubated for 72 hours at 29 °C. The antifungal activity was measured as the diameter (mm) of the clear zone of growth inhibition.[30,31,32]

SAMPLES	Concentration	ZONE IN DIAMETER (mm) against *Candida albicans*	ZONE IN DIAMETER (mm) against *A. Niger*
$C_{30}H_{18}AgCuN_3O_4$Complex	5 mg	05	08
$C_{20}H_{12}CuN_2O_2$ (SRM1)	5 mg	01	04
$C_{10}H_6NO_2Ag$ (SRM2)	5 mg	01	05





CONCLUSION

The synthesized Schiff's base was characterized by TLC, FT-IR. The downward shift of the band in complexes indicates that the ν (CH=N) and ν (N=O) groups of the Ligand are coordinated to the metal. Biological studies of these complexes reveal that these complexes show antibacterial activity against *E. coli*, *Bacillus subtilis, Staphylococcus Aureus* and *Pseudomonas* as well as antifungal activity against *Candida albicans*, *A. Niger*. As compared to monometallic complexes, a bimetallic complex shows promising biological activities. All metal complexes are coloured and thermally stable solids.

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