

Synthesis and Study Antibacterial Activities of Copper Schiff base Metal Complexes using plant extract as a greener catalyst

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Abstract

Schiff base reaction plays an important role in the formation of C-N bonds in organic synthesis. Schiff bases have a carbon-nitrogen double bond, which is usually unstable, named as imine. The presence of the imine group in these compounds are responsible to show many biological properties. Schiff bases are ligands that typically contain both nitrogen and oxygen donors and are often polydentate in coordinating ability. Schiff base is a kind of organic compound that contains imine (-RC = N-), its own application in the field of chemistry. Environment friendly method for the synthesis of Schiff bases in which we use *Tribulus terrestris* leaves extract as a green catalyst. The antibacterial study was carried out against *Staphylococcus aureus*, *Escheria coli*.

Key words: Schiff base metal Complex, Green synthesis, antibacterial activities.

1. Introduction:

1.1 Schiff base metal Complex: The widespread usage of Schiff base in chemistry, industry, medicine, and pharmacy has Notably enhanced the interest in these compounds (Boulechfar, C and et. al., 2023, Catalano and et. al., 2023). There has been notable attention among academic researchers on Schiff base ligands due to their variety of structural characteristics and wide range of physicochemical properties (Iacopetta, Domenico et. al., 2023, and Venkatesh, Ganesan, et. al., 2023). The interest in Schiff base stems from their structural versatility, which allows them to be

effectively employed as asymmetric and stabilizing agents for various complexes in different oxidation states (Naureen, B. and et.al 2021, Muthukkumar, M.; and et.al 2017). Schiff bases are acquired from the compounds of amino and carbonyl groups with multidentate ligands, which are carried out in generating highly substantial complexes with metal ions. They coordinate with metal ions by Schiff bases nitrogen. Schiff base reaction plays a prime role in the formation of C-N bonds in organic synthesis (Muthukkumar, M. and et.al 2017 and Kargar, H.; and et.al 2022). They show chelation property with O, N, and S electron pair donors with that complexes of metal have a versatile biological activity towards various kinds of pathogens and tumors (Kargar, Hadi, et al., 2022, Ghanghas, Priyanka, et al. ,2021). These ligands are potent for the intriguing novel therapeutic approach to understand diseases and their cure better. The complexes of Schiff base of both transition metal ions comprise N=O or N=O, S donor atoms perform an essential aspect in biological activities was described.

1.2 Green Synthesis of Schiff base metal Complex: The use of toxic chemicals in organic synthesis is considered a very important threat for the health and safety of chemist and environmental pollution (Kargar, Hadi, et al., 2015). Many solvents are organic chemicals with toxic properties. These solvents are very costly, and their by-products are also hazardous for environment and life (Kargar, Hadi, et al., 2022). There are number of organic methods by which Schiff bases can be synthesized but these methods are harmful to environment as many organic reagents are toxic and carcinogenic. Greener methods for the synthesis of Schiff bases are also reported but all these methodologies have some drawbacks like long reaction time, special conditions, special apparatus, cost of dehydrating agent etc. To reduce cost of catalyst herein we are reporting an environment friendly method for the synthesis of Schiff bases in which we use *Tribulus terrestris* leaves extract as a catalyst. This method is economical, short reaction time, free from organic solvent and give high yield of product (Kargar, Pouya Ghamari, et al., 2024). In ancient medicine, extracts of the aerial parts and fruits have been used for its diuretic, tonic, and aphrodisiac properties (Wahab, Aneela, et al., 2014) The preliminary phytochemical study of *Tribulus terrestris* leaves revealed the presence of saponins, flavonoids, glycosides, alkaloids, and tannins (Phillips, and et. al, 2006). The furostanol and spirostanol saponins of tigogenin, ruscogenin, and sarsasapogenin types are frequently found in this plant (Qureshi and et.al, 2014). terretribisamide, 25R-spirost-4-en-3, 12-dione, and tribulusterine, together with 10 known

compounds, N-p-coumaroyltyramine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid from the dried fruits and leaves of *Tribulus terrestris* (Chhatre, Saurabh, et al., 2014) The alkaloids present are harmane and norharmane. The β -carboline alkaloid, tribulusterine, is present in minor quantities in fruits. (Wu TS, et.al, 1999) Gas chromatography-mass spectrometry analysis of methanolic extract of the whole plant of TT revealed the presence of α -Amyrin as the major constituent and seven minor constituents, sterols such as β -sitosterols and stigmasterols were also found to be present (Bremner J et.al, 2005). Phytochemical can be act as antimicrobial and antioxidants (Abirami P et.al, 2011). These Phytoconstituents due their functional groups can show catalytic properties.

1.3 Antimicrobial activity Schiff base metal Complex: The antimicrobial activity of some Schiff base complexes with transition metals is described (Venkatesh, Ganesan, et al., 2024). Generally, the data refer to the lowest concentration of the tested antimicrobial agent (minimal inhibitory concentration, MIC) that can inhibit the visible growth of the bacterium being investigated (Sinha, Dwaipayan et.al., 2022). Schiff bases exhibit higher biological activity compared to free imines, primarily because they serve as crucial intermediates in the synthesis of natural products. Moreover, Schiff bases find versatile applications in everyday life (T.A. Alorini and et.al., 2022).

2.0 Experimental Section

Preparation of plant extract: *Tribulus terrestris* leaves was collected in local area (i.e. easily available in everywhere), the leaves of *Tribulus terrestris* were washed in tap water to remove impurities or dirty particles and cleaned well. *Tribulus terrestris* leaves was dried in 8 days in absence of sunlight. The dried leaves were grind in grinder to obtain *Tribulus terrestris* powder (Mahire, S. P., and S. N. Patel., 2020). 100 gm of *Tribulus terrestris* powder were dissolved in 150 ml of distilled alcohol in stoppered bottle up to 8 days at 60 min. Interval shaking was take place. After 8 days later mixture was filtered, and we were obtained plant extract solution. We were used plant extracted solution as catalyst in reaction (Sinha et.al ,2022).

A) Synthesis of Schiff bases: Generally Schiff base are synthesized by using substituted anilines and substituted aromatic aldehydes in presence of green catalyst (Hosny et.al , 2022),

Extraction was tested for presence of secondary metabolism. The reactions of amine and aldehyde were carried out using *Tribulus terrestris* L. leaves extract. To synthesis Schiff base by using 0.01mol benzaldehyde and 2ml of alcoholic plant extract was added in RBF to this solution 0.01mol aniline added in RBF with constant stirring ,2 ml plant extract is used as a catalyst the reaction mixture was stirred in magnetic stirrer at room temperature, The reaction was completed within 30 mins (Khan et.al, 2022).

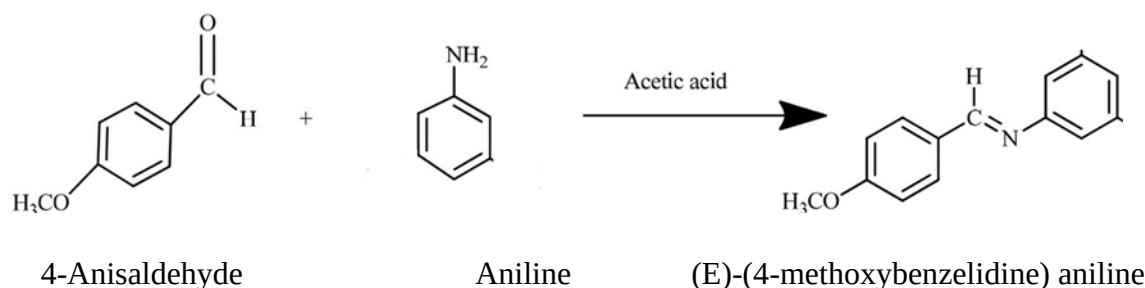


Figure no 1 Schiff base are synthesized by using substituted anilines and substituted aromatic aldehydes in presence of *Tribulus terrestris* L. leaves extract as a green catalyst.

B) Synthesis of the metal complexes by using the synthesized Schiff bases: The aqueous solution of metal acetate/chlorides salt treated with the ethanolic hot solution of ligand to form the metal complexes with the Schiff base ligands (Hosny et.al, 2022). The Schiff base ligand in 20 mL of ethanol, was added in a 1:2 molar ratio dropwise over 10 min at ambient temperature into a 10 mL solution of CuCl_2 stirred for 30 min. The Complex formation Checked by TLC after every 10 min. Crystals of metal complex formed after 30min, Recrystallization of the solid from hot ethanol yielded the compounds (Hosny et.al, 2022).

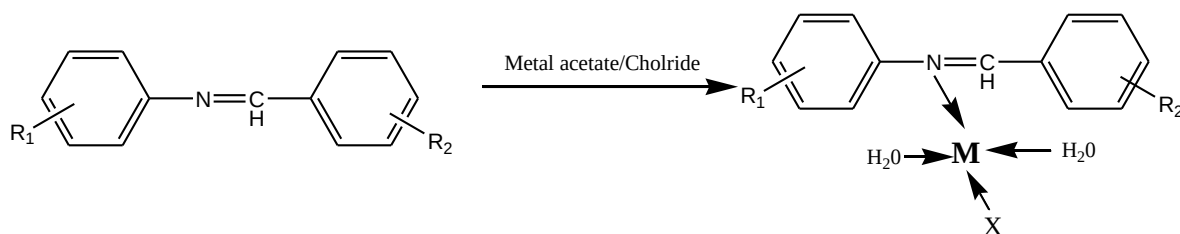
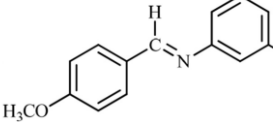
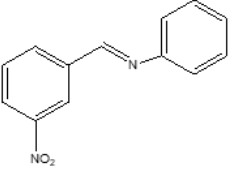
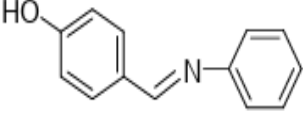
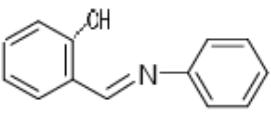
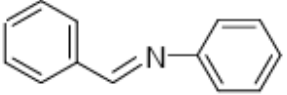


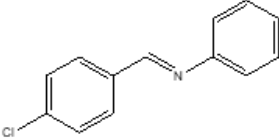
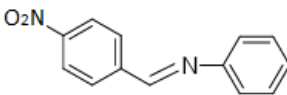
Figure no 2 Reaction for Synthesis of the metal complexes by using the synthesized Schiff bases:

3. Results:

In this synthesis and to obtained high yield of the Schiff base product with *Tribulus terrestris* L. leaves extract green catalyst is developed. This is achieved by adopting the green method for Schiff base reaction between benzaldehyde and aniline with *Tribulus terrestris* L. leaves extract. It is observed that the desired product N-Benzylideneaniline is formed with 85% yield in 30 min (Al Zoubi, Wail. Et.al., 2013).

Table no 1 Synthesis of Schiff base and their Copper Metal Complexes:

Sr. no	Schiff Base structure	Schiff base name	Metal Complex	Sample code
1		(E)-N-(4-methoxybenzylidene)aniline	[Cu((E)-N-(4-methoxybenzylidene)aniline) ₂ .Cl ₂ .2H ₂ O]	C1-Cu
2		(E)-N-(3-nitrobenzylidene)aniline	[Cu((E)-N-(3-nitrobenzylidene)aniline) ₂ .Cl ₂ .2H ₂ O]	C2-Cu
3		(E)-4-((phenylimino)methyl)phenol	[Cu((E)-4-((phenylimino)methyl)phenol) ₂ .Cl ₂ .2H ₂ O]	C3-Cu
4		(E)-2-((phenylimino)methyl)phenol	[Cu((E)-2-((phenylimino)methyl)phenol) ₂ .Cl ₂ .2H ₂ O]	C4-Cu
5		(E)-N-benzylideneaniline	[Cu((E)-N-benzylideneaniline) ₂ .Cl ₂ .2H ₂ O]	C5-Cu

		aniline	aniline) ₂ .Cl ₂ .2H ₂ O]	
6		(E)-N-(4-chlorobenzylidene)-3-phenylamine	[Cu((E)-N-(4-chlorobenzylidene)-3-phenylamine) ₂ .Cl ₂ .2H ₂ O]	C6-Cu
7		(E)-N-(4-Nitrobenzylidene)-3-phenylamine	[Cu((E)-N-(4-Nitrobenzylidene)-3-phenylamine) ₂ .Cl ₂ .2H ₂ O]	C8-Cu

3.1 Antimicrobial testing of synthesized Co Schiffbase metal Complex (T.A. Alorini and et.al., 2022):

B. Cultures Used:		
Microorganism	Strain Name	Strain reference
Gram positive bacteria	<i>Staphylococcus aureus</i>	NCIM 2079
	<i>Bacillus subtilis</i>	NCIM 2250
Gram negative bacteria	<i>Escherichia coli</i>	ATCC 25922
	<i>Klebsiella pneumoniae</i>	NCIM 2719

Table no 2 Antimicrobial Testing of synthesized Copper Schiff base metal Complex

Sample code	Zone of Inhibition in mm	
	<i>S. aureus</i>	<i>E. coli</i>
C1-CU	13	11
C3-CU	0	15
C5-CU	0	14
C6-CU	0	20
C8-CU	0	16
Streptomycin [+ve control]	20	23

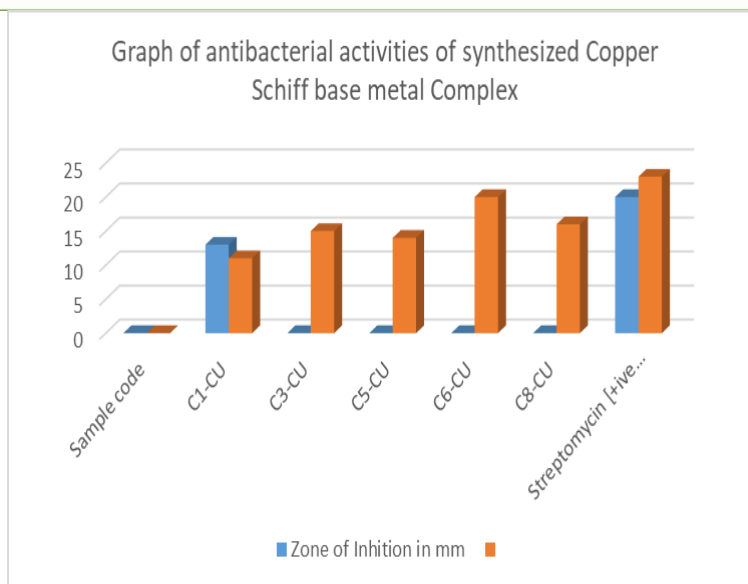
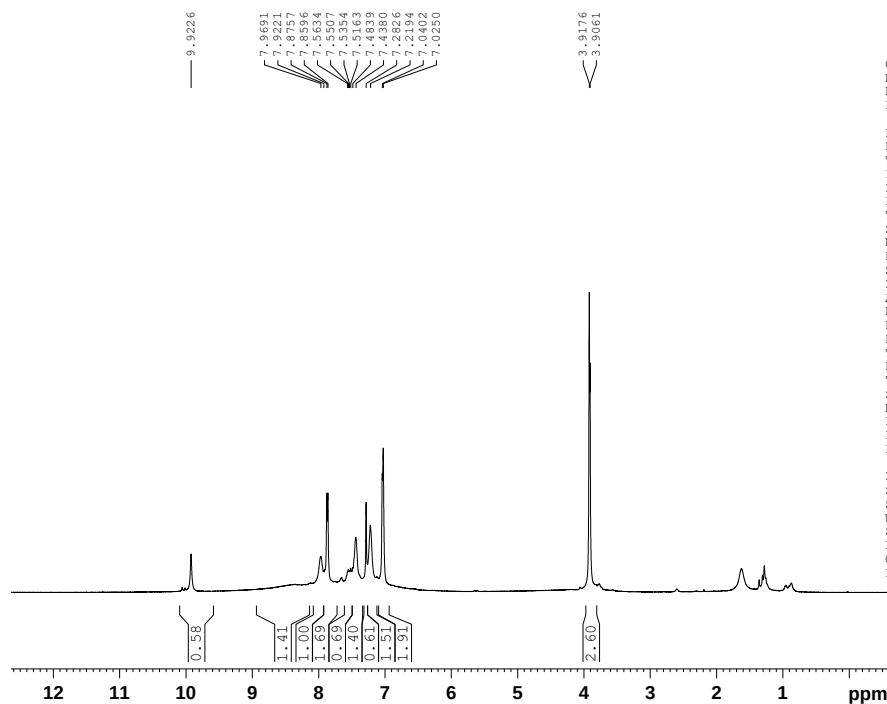


Figure no 3 Graph of antibacterial activities of synthesized copper schiff base metal complex

Characterization spectra:

C1-Cu
1H_8scan CDC13 {D:\Spectra} nmr 7



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters
NAME Feb20-2025
EXPNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 20250220
Time 11.30 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (zg30)
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 64
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 101
DW 34.000 usec
DE 6.79 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Figure no 3: Characterization spectra 01

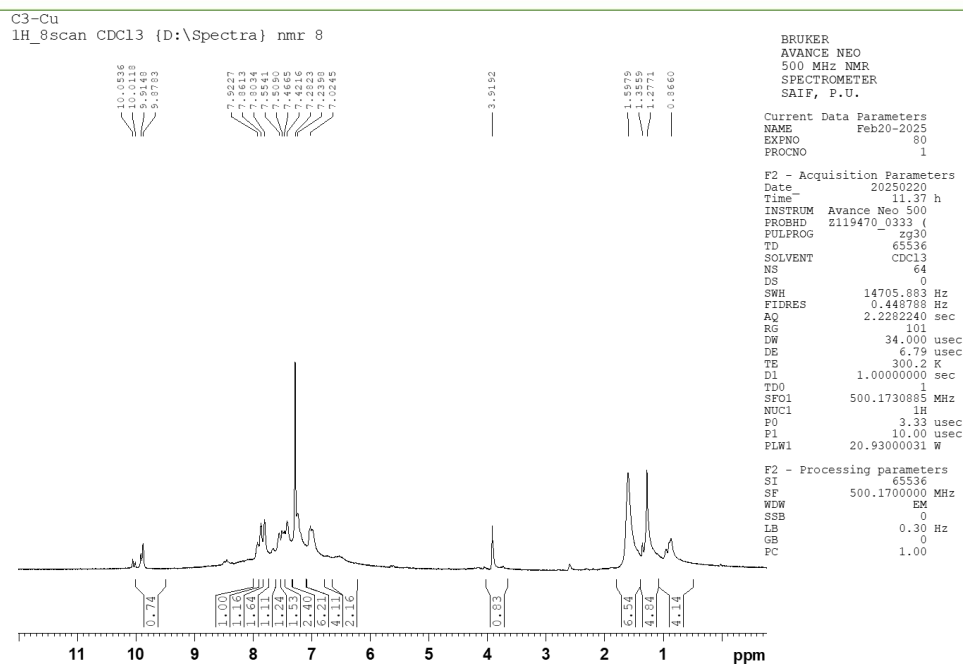


Figure no 4: Characterization spectra 02

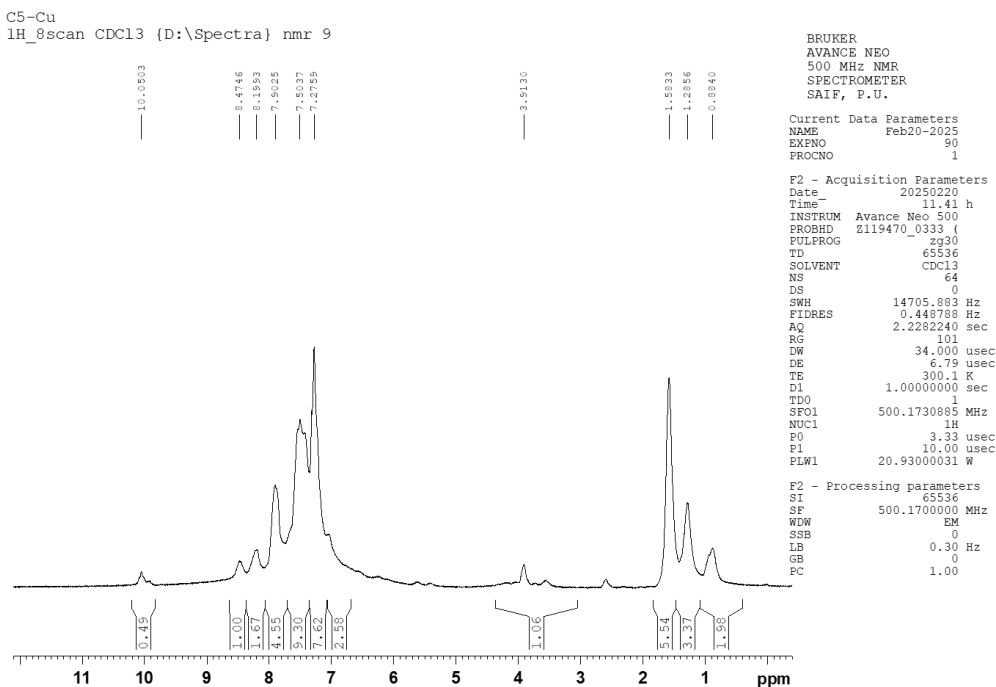
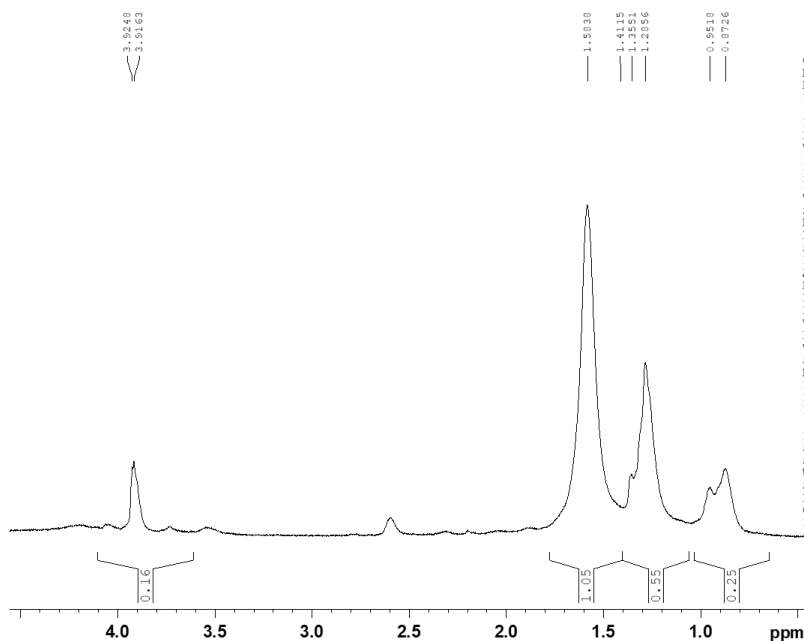


Figure no 5: Characterization spectra 03

C6-Cu
1H_8scan CDC13 {D:\Spectra} nmr 10



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

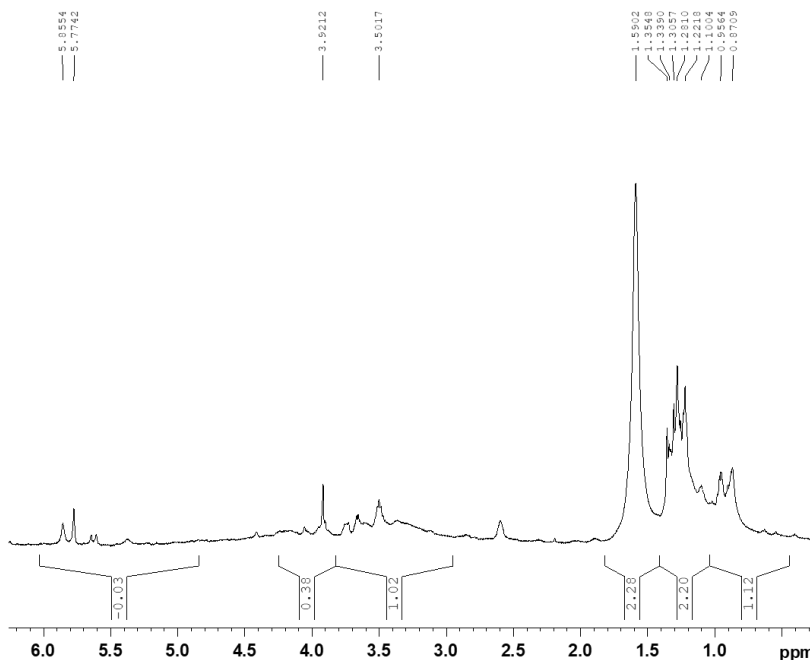
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EXPNO 100
PROCNO 1

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PULPROG zg30
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SOLVENT CDC13
NS 64
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 101
DW 34.000 usec
DE 6.79 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Figure no 5: Characterization spectra 04

C8-Cu
1H_8scan CDC13 {D:\Spectra} nmr 11



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters
NAME Feb20-2025
EXPNO 110
PROCNO 1

F2 - Acquisition Parameters
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NS 64
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FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 101
DW 34.000 usec
DE 6.79 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Figure no 6: Characterization spectra 05

4.0: Discussion

The IR data of the spectra of Schiff base ligands (L1 and HL2) and their complexes are presented in figure 4,5 and 6. The IR spectra of the complexes were compared with those of the free ligands to determine the coordination sites that may be involved in chelation (Al-Hawarin, Jibril I., et al., 2023). There were some guide peaks in the spectra of the ligands, which were helpful in achieving this goal. The position and/or the intensities of these peaks are expected to change upon chelation. New peaks are also guide peaks, as is water, in chelation. These guide peaks are shown (N. Raman, J. Joseph et.al 2006). Upon comparison, it was determined that the $\nu(\text{C}=\text{N})$ stretching vibration is found in the free ligands at 1614 and 1659 cm^{-1} for the L1 and HL2 ligands, respectively. This band was shifted to higher or lower wavenumbers in the complexes, indicating the participation of the azomethine nitrogen in coordination (M—N) 30. The SH stretching vibration, $\nu(\text{SH})$, is not useful, since it displayed very weak bands in both the free HL2 ligand and complexes spectra.

^1H NMR spectra: A review of the literature revealed that NMR spectroscopy has been proven to be useful in establishing the nature and structure of many Schiff bases (R.S. Joseyphus et.al 2006) are shown in figure no 7, 8, 9 and 10, as well as their complexes in solutions. The NMR spectra of Schiff bases were recorded in d_6 -dimethylsulfoxide (DMSO) solution, using tetramethylsilane (TMS) as internal standard.

Microbial Activity: According to the results of the antibacterial activity screening shown in table no 2, the Cu (II) complexes possess effective and selective antibacterial activity against 1 gram-positive bacteria *Staphylo coccus* spore-forming zone of inhibition, 1 gram-negative bacterium *E. Coli* forming zone of inhibition. Cu-Schiff base complex's shown highly potent inhibition activity against gram positive as well as gram negative bacteria is clearly shown in figure no 3 (H.H. Eissa et.al 2013). The Streptomycin [+ive control] was used as standard for antibacterial study. $[\text{Cu}(\text{E})\text{-N-(4-methoxybenzelidine) aniline)}_2 \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}]$ formed 13mm of zone of inhibition against *S. auris* and 11mm against *E. coli*. $[\text{Cu}(\text{E})\text{-4-((phenylimino)methyl) phenol)}_2 \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}]$ formed 15 mm of zone of inhibition against *E. coli*. $[\text{Cu}(\text{E})\text{-N-(benzelidine) aniline)}_2 \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}]$ formed 14 mm of zone of inhibition against *E. coli*. $[\text{Cu}(\text{E})\text{-N-(4-chlorobenzylidene)-3-benzenamine)}_2$

.Cl₂.2H₂O] formed 20mm of zone of inhibition against *E. coli* bacteria. [Cu((E)-N-(4-Nitrobenzylidene)-3-benzenamine)₂ .Cl₂.2H₂O] formed 16mm of zone of inhibition against *E. coli*, bacteria.

5. Conclusion:

Copper-Schiffbase metal complexes formed by using eco-friendly and cheap catalyst. This catalyst is easily available and affordable. The Copper-Schiffbase metal complexes formed were characterized by spectroscopy. On antibacterial testing, these metal complexes showed highly potent antimicrobial activity. In [Cu((E)-N-(4-chlorobenzylidene)-3-benzenamine)₂ .Cl₂.2H₂O] formed 20 mm *E. coli* bacteria. It is highly potent as standard used for testing.

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