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Conventional and Green Synthesis, Characterization and Antimicrobial potency of Complex of Cu (II) with [2-((Z) (4-hydroxy-3-methoxy-5-((E)-thiazol-5-yldiazenyl)benzenylidene)amino)benzoic acid] (MThBABA)

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Abstract: **Schiff base ligand and its complex with Cu (II) are made by conventional and microwave methods. Both the ligand and Cu(II) complex were characterized by elemental analysis, electronic spectra, IR and ESR spectroscopy, molar conductance and magnetic susceptibility. The Cu (II) complex is solid, coloured & non - hygroscopic in nature. The ligand is co-ordinated to metal ion via azomethine (N), N=N and phenolic, carboxylic anionic (O) thus behaves as tetradentate ligand & it is bi negatively charged. Octahedral geometry has been suggested by considering magnetic susceptibility values and electronic spectral analysis. It is non - electrolytic nature based on it's the molar conductivity data. In order to understand their microbiological activity both the ligand and metal complexes have been screened.**

Index Terms: **Microbiological activity, Octahedral geometry, Schiff base (MThBABA), Spectra, Tetradentate.**

I. INTRODUCTION

In the field of Coordination Chemistry (Shirodkar et al., 2010) Schiff base and its metal complex hold an essential part. Metal complexes of Schiff's base include donor atoms such as Nitrogen, oxygen (Bhattacharya et al., 1998; Djebbar et al., 1997; Liu et al., 1996; Wu et al., 1996).

Biological and catalytic activities (Wahhenri et al., 2001) are shown by Chelating ligands containing donor atoms like N, S and O. Schiff base is used for finding metal cations at the micro level.

One of the branches of green chemistry is Microwave-assisted synthesis. It is a high rate of development in organic, organometallic and coordination chemistry. are the advantages of Microwave-assisted synthesis has benefits like reduced pollution, low cost, shorter reaction time and simplicity in processing and handling. There are some research papers on the synthesis of metal complexes by microwave synthesis. (Mahajan et al., 2009; Mishra et al.,2012; Mohanan et al., 2010; Sharma et al, 2010).In this paper, binuclear metal complex of Cu (II), with Schiff base :2-(((Z)-4-hydroxy-3-methoxy-5-((E)-thiazol-5-yldiazenyl)benzylidene)amino)benzoic acid have been synthesized by methods like conventional & micro wave & both process are compared

II. MATERIALS AND METHODS

All chemicals employed in the present study were of analytical grade and were used without further purification and purchased from Loba Chemical. All glasswares used for experimental purpose were made up of borosil glass. An

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analytical balance of 1 mg sensitivity was used for sample weighing. The melting point or the decomposition temperature of all the prepared ligand and metal complexes were observed by EQ 730 equiptronics .A digital pH meter ELICO LI- 127 was used for pH measurement of buffer solutions.

Element analyzer CHNO model Flash EA 1112 series was used to find the percentage compositions of the elements (CHNO) of the ligand and its Cu(II) complex.

ICPAES (Inductive coupled plasma atomic emission spectroscopy) in an Iris Intrepid II XSP model instrument was used to analyze the metal contents of the complex. Vibration spectra (IR) of schiffs base and Copper complexes (KBr discs) was taken by Perkin Elmer Spectrophotometer 8400 FTIRUV-Vis Spectrophotometer. ELICO SL -159 was used to record the electronic Spectrum of complexes. E - 112 ESR Spectrometer at I.I.T. Mumbai was used to record the Electron spin resonance spectra (ESR). ELICO SL - 303 model conductometer was used for the molar conductivity measurements of freshly prepared solution (10-3M) in DMF

Gouy's method was used to find magnetic susceptibility using mercury tetraisothiocyanatocobaltate as the callibrant.

A. *Conventional Method Of Synthesis Of Ligand [2-((Z)-(4-Hydroxy-3-Methoxy-5-((E)-Thiazol-5-Yldiazenyl) Benzenylidene) amino)Benzoicacid*

Aminothiazol (0.005 moles) solution was diazotised by adding sodium nitrite solution (0.005moles). The cold diazonium salt was poured into this vanilline solution (25cm^3 of 10% sodium hydroxide) (0.005moles) stirring it continuously till red crystals separates out. After an hour the product was filtered& washed with saturated solution of sodium chloride.0.01 moles of this azo compound was taken along with 0.01 moles of 2-aminobenzoic acid & 50 ml of alcohol. It was attached to water condenser and refluxed for 3 hours. After that mixture was poured to a beaker and kept in the fridge overnight. The product was filtered dried & crystallised (Vogel A 1089). Brown crystals of schiffs base, 2-(((Z)-4-hydroxy-3-methoxy-5-((E)-thiazol-5-yldiazenyl) benzylidene)amino)benzoic acid. In the entire paper it is represented as L (Fig. 1).

B. *Green Synthesis: Preparation Of Schiffs Base By Microwave Method*

0.005 moles of azo compound and 0.005 moles of 2-aminobenzoic acid along with few drops of pure alcohol were taken in a beaker which was then irradiated in the microwave oven at 180 0 for 2 minutes . The reaction was completed in a short time (2 min) with higher yields. Brown crystals of schiffs base, 2-(((Z)-4-hydroxy-3-methoxy-5-((E)-thiazol-5-yldiazenyl)

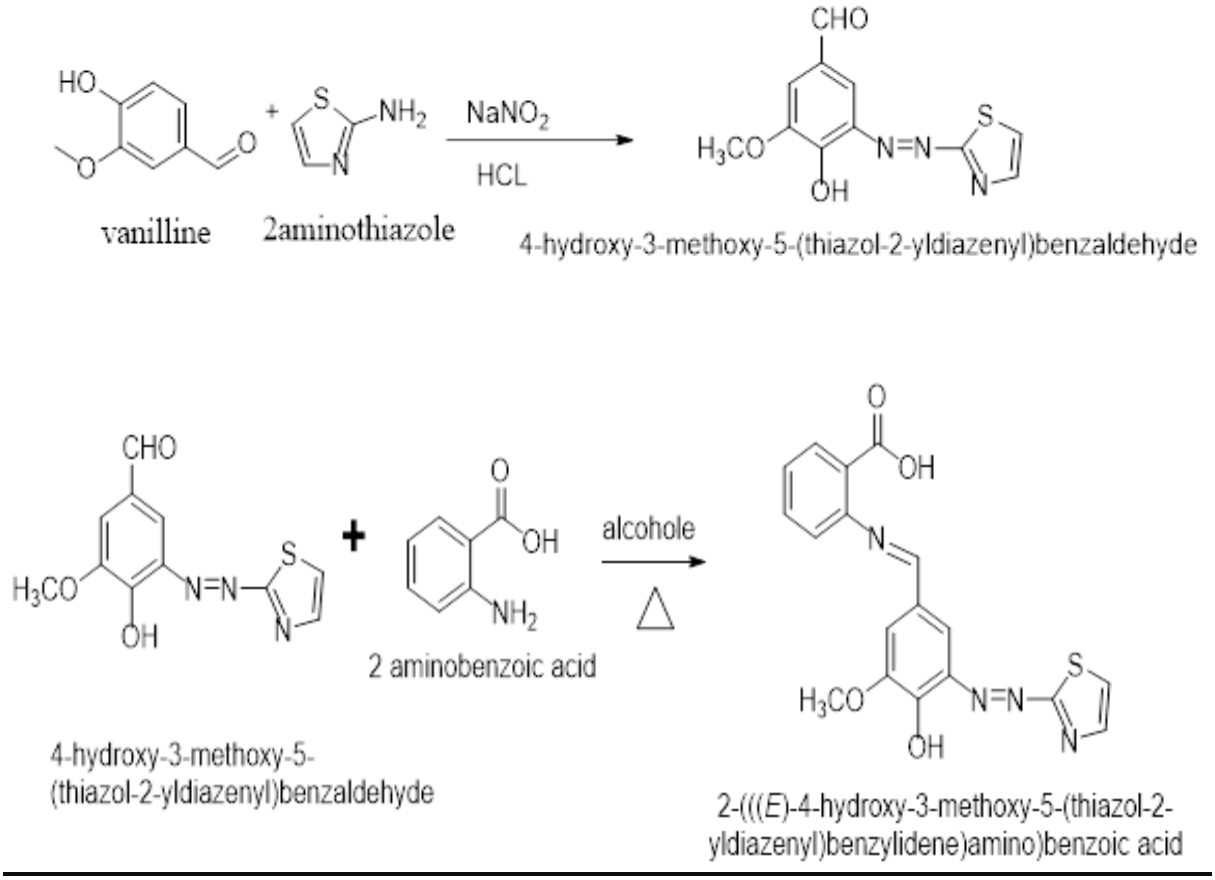

Fig. 1. Preparation of schiffs base

benzylidene)amino)benzoic acid were obtained.

C. Preparation Of Copper Complex By Conventional Method

At pH 5.6 - 6.4, hydrated copper sulphate and ligand (dissolved in ethanol) in molar ratio of 1:1was refluxed on water bath for 2.-3 hours. When cooled at room temperature dark brown colored copper complex separated which was filtered, washed with water and then with ethanol & then recrystallized (Vogel A,1989), It was dried in vacuum desiccator with the help of anhydrous $CaCl_2$ (yield 60 - 70%).

D. Green Synthesis: Preparation Of Copper Complex By Microwave Method

The reaction mixture containing hydrated copper sulphate and ligand in 1:1 ratio in 50 ml borosil beaker along with 3–4 mL of dry ethanol was irradiated by the microwave oven at 700 Watts for 2 minutes. In a short time (1–2 min) the reaction was completed with higher yields. The resulting product was then recrystallized with ethanol and ether and finally dried under reduced pressure over anhydrous $CaCl_2$ in a desiccator. The progress of the reaction and purity of the product was monitored by TLC using silica gel G (yield: 91–92%).

E. Biological Studies

Antibacterial screening aims to determine the susceptibility of the pathogenic microorganism to test whether the complex can be used as a therapeutic agent.

The stock solution of 2000 ppm of ligand and Cu(II) complex was prepared on active ingredient basis. It was kept at room temperature till it was used. Antimicrobial potency of ligand and its Cu(II) complex was investigated by Cup plate method . Sterile Sabourauds agar plates for fungus Candida albican and Sterile Mueller Hinton agar plates for bacterial test cultures were seeded with 1ml of 24 hour old, 0.1 O.D. cultures. Wells were punched in the above media and compounds 50 µl were added. Depending on their culture plates are incubated for 48 hours at 37°C / R.T. Inhibited area around the wells were measured in millimeters.

Table I. The Analytical and physical data of ligand and Metal complexes

Compounds (Colour)	Molecular Weight	Reaction period Conventional methods	Reaction period Micro synthesis	M.P.	% Elemental Analysis Found (Calculated)						^m Ohm^{-1} cm^2mol^{-1}
					C	H	O	S	N	M	
MThBABA (greenish brown)	382.39	4 hours 79%	0.4 Minutes 92%	244	56.51 (56.54)	3.68 (3.69)	16.80 (16.79)	8.38 (8.39)	14.66 (14.65)	- -------	
$[MThBABA]_2$ $Cu_2.4H_2O$ (dark brown)	954.16	3.2 hours 81%	1.2 Minutes 94%	278	44.84 (44.85)	3.76 (3.76)	19.90 (19.91)	6.66 (6.65)	11.63 (11.62)	13.19 (13.18)	26.2

III. RESULTS AND DISCUSSION

Copper complex is colored, solid, non- hygroscopic and stable at room temperature. The Analytical and physical data of ligand and their metal complexes are recorded in Table I.

Table II. The Important IR bands of Ligand and Their Metal Complexes

Compound	**υ phenolic (-OH) cm^{-1}**	**υ(C-O) stretching cm^{-1}**	**υ(C=O) stretching cm^{-1}**	**υ(C=N) cm^{-1}**	**υ(M-N) cm^{-1}**	**υ(M-O) cm^{-1}**
MThBABA (greenish brown)	**3376**	**1272**	**1683**	**1629**	**----**	**-------**
$[MThBABA]_2Cu_2$ 4 H_2O	**----**	**1368**	**1677**	**1624**	**499**	**461**

A. Infrared Spectral Analysis

IR was recorded with a Perkin Elmer FTIR -8400S Spectrometer (4000-400 cm^{-1}) using KBr pellets.

The most important conclusions based on infrared spectra bands of the Ligand MThBABA and complexes $[MThBABA]_2 Cu_2 .4H_2O$ are tabulated in Table II, (figure 2 & figure 3).

A broad band at 1630 cm^{-1}in the IR spectrum due to C=N stretching of azomethine group (Lever, 1973) ligand has shifted to lower regions in IR of Cu (II) complexes at 1627 cm^{-1} reveals the coordination of azomethine nitrogen to metal atom in complexes (Anitha et al., 2013). The shifting was due to the donation of electron density from Nitrogen to metal.

IR of ligand depicts a weak band around 3561 cm^{-1} due to intra molecular hydrogen bonded -OH group (Anitha et al., 2013; Bellamy, 1954; Hasan et al., 2016) which is missing in the case of Cu complexes proposing the dissociation of the phenolic proton on complexation and involvement of phenolic anionic oxygen in coordination. It is further supported by the fact that the strong band at 1275 cm^{-1} due to C - O (phenolic and carboxylic) in the ligand has been shifted to the 1316 cm^{-1} in the spectra of complexes (Anitha et al., 2013).

IR of schiff's base shows a band due to C = O stretching frequency (Hasan, 2016; Omar, 2012) at 1672 cm^{-1} which has displaced by 20—60 cm^{-1} in the bi nuclear copper complex. It is shown at1652 cm^{-1}, The shifting of this band indicates the involvement of oxygen atoms from the hydroxyl group of COOH which bonds with the metal ions (Hasan, 2016).

A vibration spectrum of ligand does not depict any peak at region 400-500 cm^{-1}. But in binuclear complex new peak arises at 400-500 cm^{-1}. The band at 473 cm^{-1} is due to stretching frequencies of υ(M-O) (Hasan, 2016). Likewise band at 4447 cm^{-1} are of stretching frequencies of υ(M-N) (Hasan, 2016; Omar, 2012) which infers that Oxygen and Nitrogen atom are involved in coordination. A broad band around 3449 cm^{-1} in the spectra of copper complex is due to the symmetric and asymmetric stretching modes of coordinated water molecule

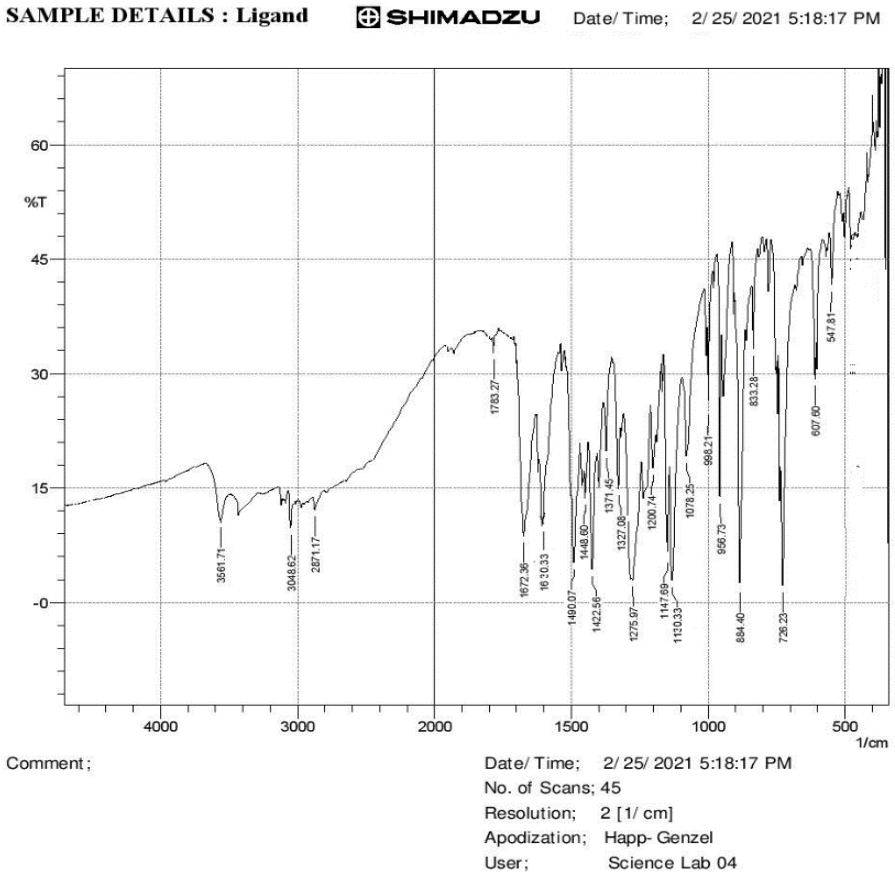

Fig. 2. IR Spectra of ligand

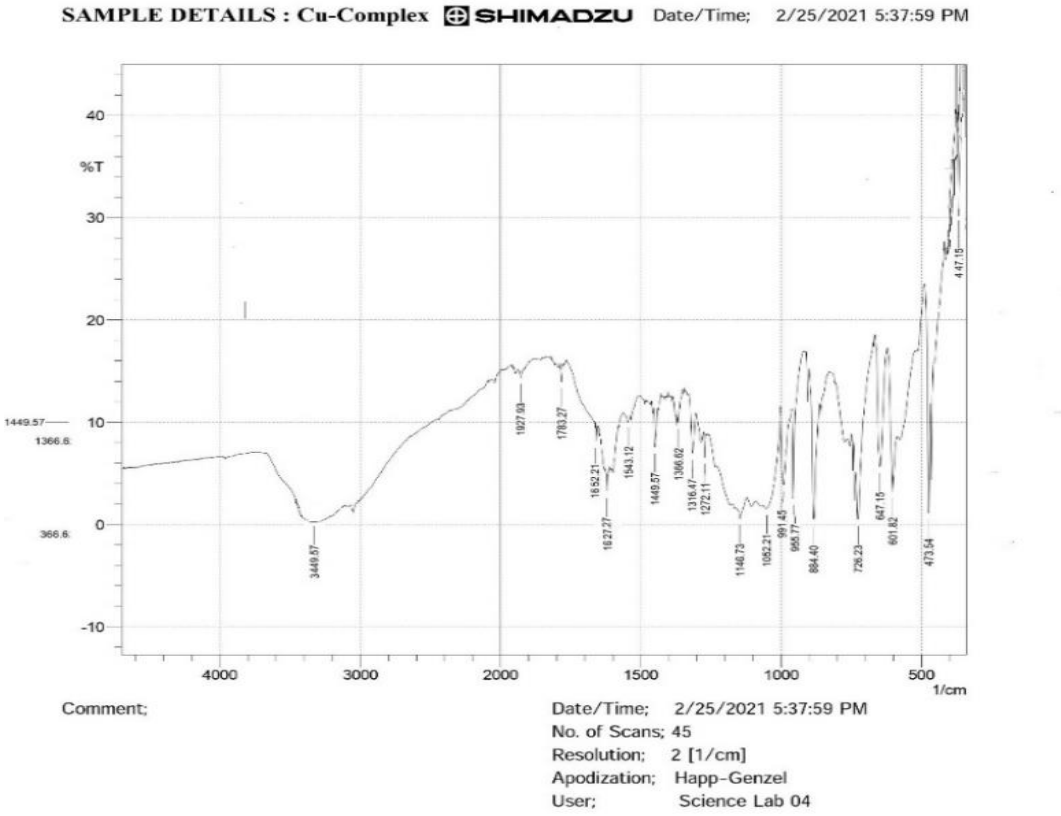

Fig. 3. IR Spectra of Cu complex

(Abdul & Ahmed, 2018). An additional band at 1144 cm^{-1} suggests that water molecules are coordinated to metal ion (Omar, 2012).

Hence from IR spectra it is inferred that ligand behaves as {bi negatively} tetradentate ligand co-ordinated to metal ion via azomethine (N), N=N and phenolic, carboxylic anionic (O).

B. Molar Conductance

Copper complex (1x10^{-3} M) in DMF Molar conductance of (Λm) is 26 $Ohm^{-1}cm^2mol^{-1}$ at room temperature. Using the known molar conductivities non - electrolytic nature (Madan et al., 2018; Omar, 2012; Umendra & Sulekh, 2011) of the complex was inferred.

C. Electronic Spectra and Magnetic Susceptibility

Table III. Electronic Spectra and Magnetic Moment of the Cu (II) Complex

Compound	Band, λ max (nm)	Band, λ max (cm^{-1})	Assignments	μ_{eff} (B.M.)
MThBABA (greenish brown)	360	**27777 cm^{-1}**	$^2B_{1g} \rightarrow {}^2Eg$ ($dx2 - y2 \rightarrow dxy,dyz$),	**1.59**
	600	**16166 cm^{-1}**	$^2B_{1g} \rightarrow {}^2B_{2g}$ ($dx2 - y2 \rightarrow dzy$),	
	780	**12820 cm^{-1}**	$^2B_{1g} \rightarrow {}^2A_{1g}$ ($dx2 - y2 \rightarrow dz2$),	

Measurements of Complexes

LABMAN-Visible Spectrophotometer is used to record electronic spectra. Data is shown in Table III.

The electronic transition takes place in UV-Visible electromagnetic region.

ESR parameters for Cu (II) complex are shown in Table IV.

Two g values ($g\parallel$ and $g^{\perp}$). of ESR spectra of bi nuclear complex ($g\parallel > g^{\perp}$) shows that the unpaired electron is delocalized in dx^2- y^2 orbital in the ground state of metal and spectra are characteristics of axial symmetry. The g_{avg} was

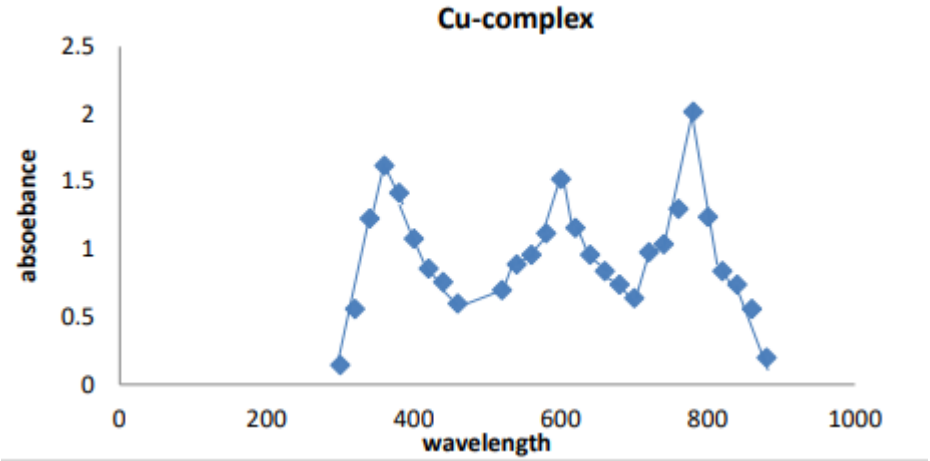

Fig. 4. Electronic Spectra of the Cu (II) Complex Figure

The electronic spectra of the copper complexes recorded in DMF supported a near octahedral geometry and also propose that water groups are coordinated axially to Cu (II) ions (Omar, 2012). The spectrum of the Cu(II) complex exhibits absorption bands at 780nm,600nm, 360nm. These bands may be considered to the following three spin allowed transitions (Omar, 2012): $^2B_1g \rightarrow {}^2A_1g$ ($dx^2 - y^2 \rightarrow dz^2$), $^2B_1g \rightarrow {}^2B_2g$ ($dx^2 - y^2 \rightarrow dzy$), and $^2B_1g \rightarrow {}^2Eg$ ($dx^2 - y^2$) suggesting octahedral geometry .The magnetic moment value 1.59 B.M which is abnormally small consistent with the octahedral Cu (II) complex (Lever, 1984; Omar, 2012) (Fig. 4).

D. Electronic Spin Resonance (ESR) Spectra

Knowledge of unpaired electron and extent of delocalization is given by ESR spectrum

By using X - band at frequency 9.5 GHZ under the magnetic field strength 3400 gauss in DMF the ESR spectra of copper complexes have been taken at room temperature. The values of calculated by equation [$(g_{avg}) = 1/3\ (g\parallel + 2g^{\perp})$].

The $g\parallel > 2.3$ reveals an ionic environment and $g\parallel < 2.3$ indicates a covalent character in metal ligand bonding this is as per Kvelson & Neiman (Maki, 1958). Using this concept, in this Cu compound $g\parallel$ shows the prevalence of covalent character in metal - ligand bond (Maki, 1958). Hathaway expression gives the exchange coupling interaction $G = (g\parallel - 2) / (g^{\perp} - 2)$.

If G value is more than four then exchange interaction not possible, Cu (II) complex have G value less than four indicating considerable exchange interaction the complexes (Abragam & Bleaney,1970; Mishra et al., 2002; Maki,1958); Umendra & Sulekh, 2011.

E. Determination of Composition of the Extracted Species

Composition of complex was found to be 1:1 (Cu: MThBABA) was verified by using Mole Ratio Method and Job's Continuous Variation Method (Makhijani & Barhate, 2013)

Table IV. Electronic Spin Resonance Parameters of The Complexes

Metal Complexes	$g\parallel$	$g^{\perp}$	g_{avg}	Δg	G
$[MThBABA]_2\ Cu_2$	2.28	2.081	2.147	0.179	3.456

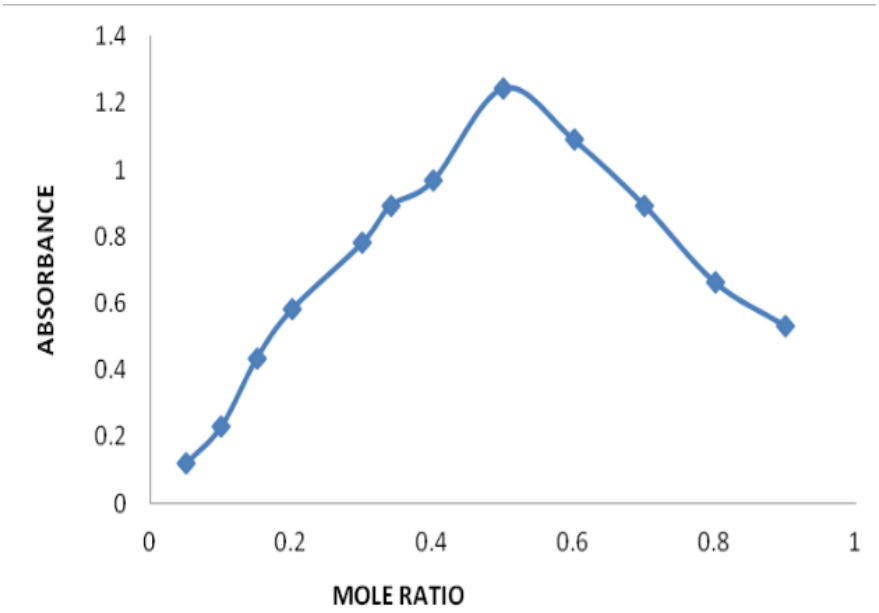

Fig.5. Cu:MTHBABA Job's Continuous Variation method

Graph of Cu:MTHBABA Job's Continuous Variation method is shown in Fig. 5.

F. Proposed Structure of Complex

Analytical, magnetic, spectroscopic data and microbiology data suggests that the chelating nature of ligand forms stable complexes with Cu (II). Based on experimental evidence the probable structures of the complexes can be shown as (Fig.6).

G. Biological Activity

Schiff base ligand MThBABA and its Cu complex were assessed for antibacterial potency by cup plate method (Bsasubramanyam et al, 2005; Sandhar et al., 2006) against "gram positive bacteria (staphylococcus aureus and corynebacterium diphtheria), gram negative bacteria (Escherichia coli and Klebsiella pneumonia)"and "antifungal

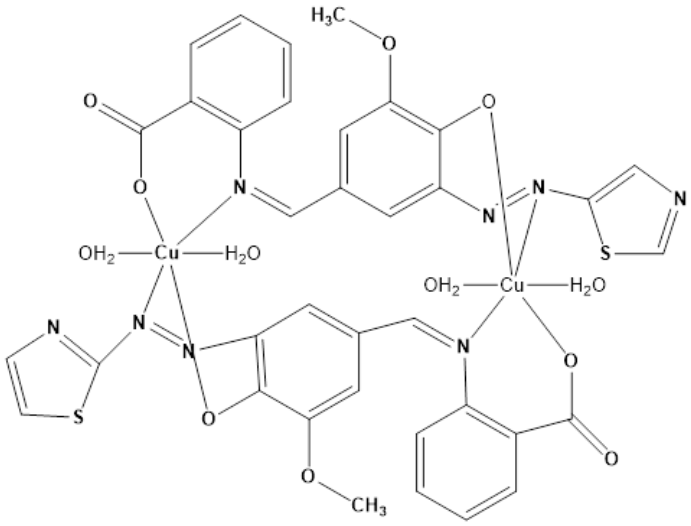

Fig.6. Proposed Structure of Complexes

Table V. Inhibition zone of growth in millimeters

Complexes	Cultures					
	S.a	C.d.	E.c	K.p	C.a	Asp.
MThBABA	22	9	07	06	02	10
$MThBABA]_2$ Cu $_2 4H_2O$	33	12	11	11	04	14

activity against Candila albicans and aspergillus species" (Omar,

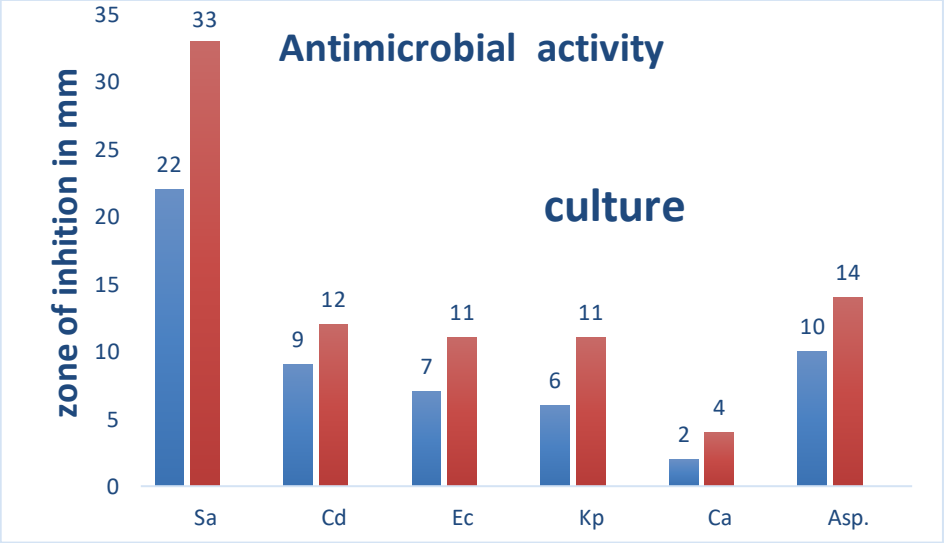

Fig.7. Graph showing zone of inhibition of growth in millimeters

2012)

Data from Table -V (figure 7) reveals that metal chelates of Cu(II) complex show higher antibacterial and antifungal activity as compared to free ligands. Metal chelates show enhanced biological activity as compare to ligand. It can be explained by chelation theory and overtones concept.

There is overlap of the ligand orbital and partial sharing of positive charges of metal ion with donor groups. This chelation reduces the polarity of the metal ion. The delocalization of electrons over the whole chelate ring enhanced the lipophillicity of the complex with this higher lipophillicity (Bsasubramanyam et al, 2005; Sandhar et al.,2006) the Cu complex penetrates to greater extent into lipid membrane and hampers the enzyme activity of microorganism.

The respiration process of the cell is also hampered as metal complexes block the synthesis of proteins, which restricts further growth of the organism. The variation in the activity of different complexes against different organisms depends either on the impermeability of the microbe's cells or difference in the microbe's ribosomes (Bsasubramanyam et al, 2005).

CONCLUSION

The data obtained from above study infers that Schiff base MThBABA [2-((Z)-(4-hydroxy-3-methoxy-5-((E)-thiazol-5-yldiazenyl)benzenylidene)aminao)benzoicacid] shows tetradentate complexing agent towards metal Cu(II) ions. Analytical data of complexes matches well with their molecular formula. IR spectra proves that ligand behaves as bi negatively, tetradentate ligand co-ordinated to metal ion via azomethine (N) , N=N and phenolic, carboxylic anionic(O). The ratio of metal to ligand is 1:1whch is obtained by elemental analysis and proved by job method and mole ratio method which further supports binuclear complex.

The electronic spectral and magnetic susceptibility measurements concludes that binuclear Cu(II) complex with MThBABA is octahedral in nature. ESR spectra of complexes gives knowledge of the extent of the delocalization of unpaired electron which inferes the covalent bonding between metal and ligand Considering the above data the structure for the binuclear has been proposed which fully agrees with theoretical

consideration (Fig.6). The metal complexes shows enhanced microbiological activities as compared to the ligand.

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