Mixed Ligand Complexes Containing 2, 2'-Bipyridine with Acetaldehyde Thiosemicarbazone: Synthesis, Characterization and Antifungal Activity

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Abstract

Mixed ligand complexes with chlorides of copper(II) and iron(III) of general composition $[M(L1)(L2)Cl_2]$ were synthesized using acetaldehyde thiosemicarbazone, a derivative of thiourea as primary ligand and 2, 2'- bipyridine as secondary ligand. The compounds were structurally characterized by Elemental Analysis (EA), Fourier Transform Infrared (FT-IR), Nuclear Magnetic Resonance (^{1}HNMR) and Electronic spectroscopies. The infrared spectra data showed that the chelating behaviour of the secondary ligand towards the transition metal ion is through the two pyrindyl nitrogen atoms, whereas the primary ligand indicated the involvement of thiolato sulphur and azomethine nitrogen atom. The electronic spectra results displayed the existence of d-d electronic only in the copper complex, other bands seen correspond to n→π* transition and intra-ligand charge transfer and confirmed the proposed structures. The results of the elemental analysis for the compounds were in good agreement with the theoretical values. The complexes are of 1:1:1 stoichiometry and are non-electrolytes in solution. The antifungal activities evaluated by the diameter of zone of inhibition in (mm) against *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Candida albicans* revealed that the copper complex has more activity than that of iron. The study however showed that complexation of the ligand with metal ion resulted in increased antifungal activity.

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Introduction

The good complexing ability of thiosemicarbazones to both transition and main group metallic cations is attributed to the extended delocalization of electron density over the thioamide sulphur and azomethine nitrogen bond system (–NH–C(S)–NH–N=) which is further enhanced by substitution at the N(4) position. The importance of thiosemicarbazone metal chelates in medicinal chemistry is increasing because they have been used as drugs and are reported to possess a wide variety of biological activities against pathogenic organisms. The antitumour activity of such thio compounds was revealed in their ability to inhibit ribonucleotide reductase (RR), a necessary enzyme for deoxyribonucleic acid (DNA) synthesis (Sandercock *et al.,* 2007), other pharmacological activities include: antiamoebic, antimalarial, antiviral (Kolocouris *et al.,* 2002) antibacterial, antifungal, antioxidants and anticancer agents (Liu *et al.,* 2009; Sampath *et al.,* 2003; Aly *et al.,* 2010; Vinuelas-Zahinos *et al.*, 2011; Halder *et al.*, 2012; Kumar *et al.*, 2013; Kpomah and Kpomah, 2018; Ammar and Mohammed 2019). Metal complexes of biologically important ligands are sometimes more effective than thier corresponding free ligands, this may be due to modified pharmacological and toxicological properties when administered in the form of metallic complexes.

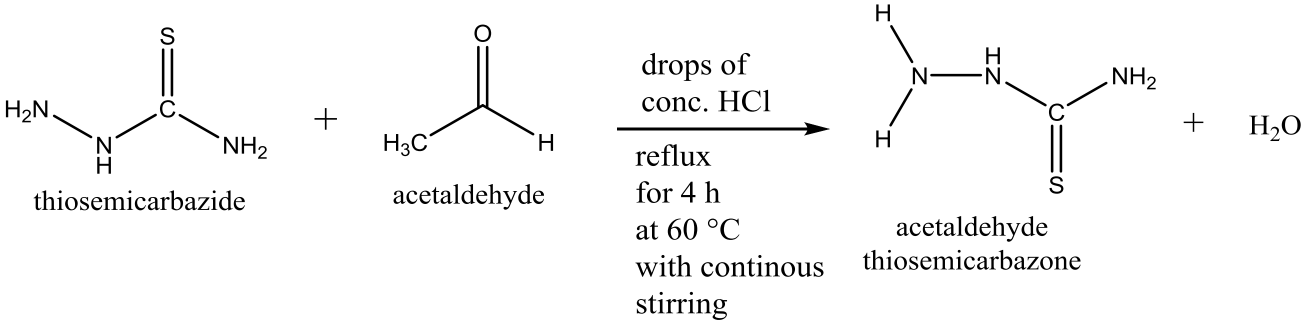
Mixed ligand complexes with metal ion bound to two different biochemically important ligands have aroused interest as model for metallo-enzymes. They have a key role in biological chemistry because the mixed chelation occurs commonly in biological fluids as millions of potential ligands are likely to compete for metal ions *in -vivo*. These create specific structures and have been implicated in the storage and transport of active substances through membranes (Ahmed *et al.*, 2012). This present work describes the synthesis of copper and iron mixed ligand complexes of acetaldehyde thiosemicarbazone a derivative of thiourea, synthesized by simple condensation reaction of thiosemicarbazide and acetaldehyde with 2, 2′ –bipyridine (bipy) to achieve the desire mixed ligand complexes

Materials and Methods

Analytical grade chemicals and solvent were used in this study. Thiosemicarbazide and 2, 2′ - bipyridine are both products of Sigma Aldrich. The metal salts used for the synthesis were obtained from British Drug House (BDH). Other reagents and solvents like methanol, ethanol, acetone, chloroform, dichloromethane, dimethylsulphoxide (DMSO) and concentrated hydrochloric acid were all products of BDH and were used without further purification. The mixed ligand complexes were synthesized using standard procedure. Melting points of the ligand and metal complexes were determined using Optimelt Automated melting point System. The conductivity measurements were taken using Jenway 4510 Conductivity Meter. The CHN Elemental Analysis was done using Thermo Flash 1112 CHNSO Elemental Analyzer. Electronic spectra of the ligand and the complexes were recorded in Dimethylsuphoxide (DMSO) solution on Shimadzu 10UV scanning UV-Visible spectrophotometer in the range 200–800 nm. The infrared (IR) spectra were recorded on Shimadzu 8400S FTIR spectrophotometer as KBr pellets in the range 4000 – 400 cm^{-1}.

Synthesis of Acetaldehyde Thiosemicarbazone (ADTSC)

10 mmol, (1.92g) thiosemicarbazide was dissolved in methanol (60 mL) by refluxing at 60 °C. In the refluxing solution, acetaldehyde 10 mmol, (1.56 mL) solution in methanol (30 mL) was added; this was then followed by the addition of few drops of concentrated HCl. The reaction mixture was continuously stirred and refluxed for 4 hrs at 60 °C. The volume of reaction mixture was reduced and kept in the refrigerator overnight. White crystals of ADTSC precipitated out, the crystals was washed with methanol and dried in the desiccator over silica gel (Mahto, 1981).



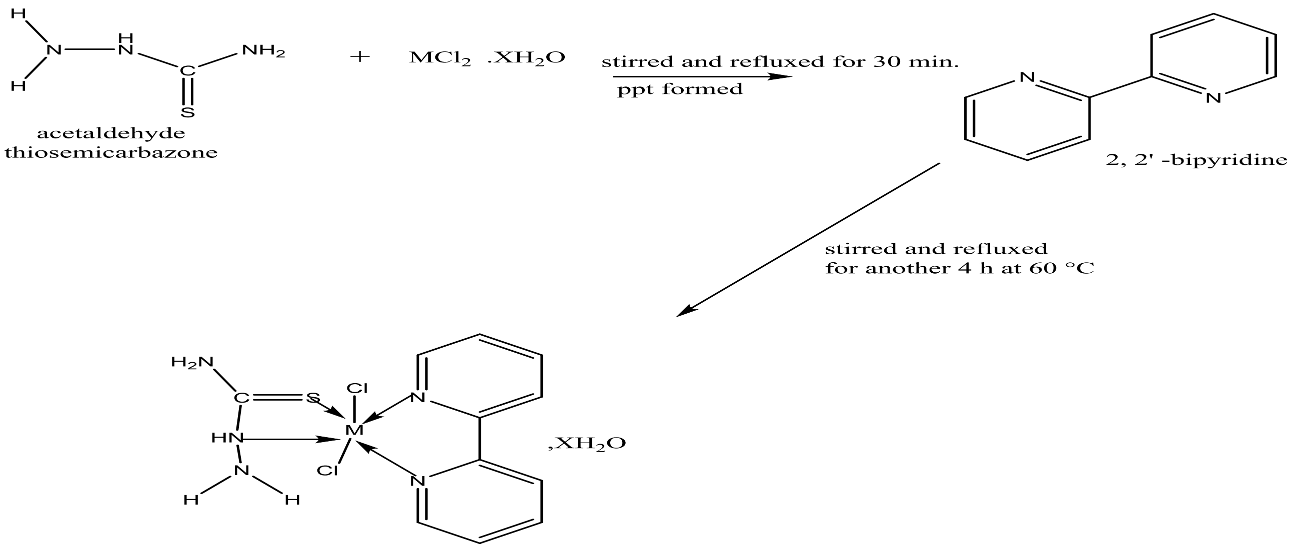
Scheme 1 : Synthetic Preparation of acetaldehyde thiosemicarbazone (ADHT)

Synthesis of Mixed-Ligand Copper Complex of 2, 2′ - bipyridine and Acetaldehyde Thiosemicarbazone

To refluxing 1 mmol (0.135 g) in 15 mL methanolic solution of the ligand, acetaldehyde thiosemicarbazone was added slowly to a 15 mL hot methanolic solution of the metal salts $CuCl_2.2H_2O$ (1mmol 0.170 g). The reacting mixture was constantly stirred and refluxed for 30 min. 1 mmol (0.156 g) 15 mL methanolic solution of 2, 2′ -bipyridine was subsequently added slowly to the refluxing mixture. The entire mixture was refluxed under continuous stirring for 4h at 60°C. The resulting mixture was kept at room temperature for slow evaporation. The green crystals so formed was filtered off after 96 h. The crystal was washed with distilled water and small quantity of cold ethanol and dried over silica gel in a desiccator (Agarwal *et al.,* 2009).

Synthesis of Mixed-Ligand Iron Complex of 2, 2′ - bipyridine and Acetaldehyde Thiosemicarbazone

To refluxing 1 mmol (0.135g) 15 mL methanolic solution, acetaldehyde thiosemicarbazone was added slowly to a hot 15 mL methanolic solution of 1 mmol $FeCl_3$ (0.162 g). The reacting mixture was constantly stirred and refluxed for 30 min. Subsequently, 1 mmol (0.156 g) methanolic solution of 2, 2′ bipyridine was added slowly to the refluxing mixture. The entire mixture was thererafter refluxed under continuous stirring for 4 h at 70 °C. The resulting mixture was concentrated and kept at room temperature for slow evaporation. The brown crystals so formed were filtered off after 96 h. The compound was washed with distilled water and small quantity of cold ethanol and dried over silica gel in a desiccator (Agarwal *et al.,* 2009).



Scheme 2 : Synthetic Preparation of the mixed ligand Complexes [M(ADHT)(2, $2^{'}$-bipy)Cl_2]

Antifungal Activity Test

The antimicrobial activities of the compounds were screened by adapted qualitative diffuse metric methods (i.e. distribution of the tested solutions on filter paper discs, or in spots on solid media that have been inoculated with test microbial strains). Media plates of sensitivity test agar (STA) were prepared and inoculated from overnight slant cultures of the test organisms and spread as uniformly as possible throughout the entire media. Discs impregnated with 60 µg/ml solution of the antimicrobial sample were then placed on the inoculums media. Blank paper discs of dimethylsulphoxide were used as control. The plates were filed with the SDA agar (two-thirds) and the fungi specie inoculated into it and the sample solutions added as in the antibacterial sensitivity test above except that the inoculated plates were incubated at 37 ^{o}C for 72 hours. The activities of the compounds were represented by size of the diameter in mm, this size also known as inhibition zones were measured using the zone reader. In all experiments, results were recorded in triplicate (Cheesbrough, 2009).

Statistical Analysis

Data are expressed as the mean of five (5) replicates ± standard deviation, means were analysed using One Way Analysis of Variance (ANOVA) Posthoc (Turkey), $p<0.05$ were considered as statistically significant. Descriptive statistics (Frequency count, simple percentage) was also used. All statistical analysis was done using Statistical Package for Social Science (SPSS) version 16.

Results and Discussion

Physical Properties of the Compounds

The physical properties of the complexes are presented in Table 1. They are in good agreement with the suggested formula of copper and iron complexes. The molar conductance measurements of the complexes in DMSO indicate that they are both nonelectrolytes. The higher melting point of the complexes observed when compared with the free ligand could be attributed to the increased molecular mass, enhanced stronger lattice structure and stronger interaction which accompanied the coordination of the ligand to the central metal ions in the complexes. The results of partial elemental analysis are in good agreement with assigned formulations (Zeinab, 2006; Oladipo *et al.*, 2005).

Electronic Spectra

Electronic spectra data of mixed ligand complexes are listed in Table 2. Acetaldehyde thiosemicarbazones showed five absorption bands in the region 198 nm (50505 cm-^{1}), 208 nm (48076 cm-^{1}), 276 nm (36231 cm-^{1}) corresponding to $n\rightarrow\pi^*$ transition, while the bands assigned to $\pi\rightarrow\pi^*$ transition are 301 nm (33222 cm-^{1}) and 311 nm (32154 cm-^{1}). Upon complexation a blue shift was observed due to the polarization of the C=N bond caused by the metal ligand electron interaction during the chelation. This also clearly indicates the coordination of azomethine nitrogen to the metal atom.

Table 1: Analytical data of the Compounds

					Elemental Analysis Found/(Calcd) (%)			
Formulation and Empirical Formula	M/ Wt. (g/mol)	Colour	Yield (%)	M.p. (^oC)	C	H	N	EC 10^{-3} M (ohm^{-1} cm^2 mol^{-1})
AHDT $C_3H_7N_3S$	117	Milky white crystals	84	189.9	29.87 (30.75)	5.98 (6.02)	35.22 (35.03)	
Cu(AHDT)(bipy) (Cl_2) $C_{13}H_{15}Cl_2CuN_5S$	405.97	Green crystals	60	245	38.41 (38.29)	3.54 (3.71)	16.96 (17.17;)	18.23
Fe(AHDT)(bipy) (Cl_2).3H_2O $C_{13}H_{21}Cl_2FeN_5S$	398.98	Green crystals	60	245	39.46 (39.03)	3.54 (3.78)	17.46 (17.50)	18.23

EC = Electrical Conductance 10^{-3} *M solution in DMSO,* Ohm^{-1} cm^2 mol^{-1}.

Table 2: Electronic Spectra of the Compounds (nm and cm^{-1})

Compound	d^n Configuration	Wavelength nm(cm^{-1})	Assignments/ Transitions	Stereochemistry
AHDT $C_3H_7N_3S$		198 (50505) 208 (48076) 276 (36231) 301 (33222) 311 (32154)	$n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $\pi\rightarrow\pi^*$ $\pi\rightarrow\pi^*$	-
Cu(AHDT)(bipy)(Cl_2) $C_{13}H_{15}Cl_2CuN_5S$	d^9	199 (50251) 208 (48076) 224 (44642) 229 (33594) 358 (27932) 683 (14640)	$n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $n\rightarrow\pi^*$ Charge Transfer d–d ($^2B_{1g}\rightarrow^2A_{1g}$)	Octahedral
Fe(AHDT)(bipy) (Cl_2).3H_2O $C_{13}H_{21}Cl_2FeN_5S$	d^5	198(50505) 208(48076) 215 (46511) 273 (36630) 371 (26954)	$n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $n\rightarrow\pi^*$ $n\rightarrow\pi^*$ Charge Transfer	Octahedral

In the electronic absorption spectra of Cu(AHDT)(bipy)(Cl_2) there are about four detected absorption bands around 199-299 nm assigned to $n\rightarrow\pi^*$. The band assinged to charge transfer for Cu(AHDT)(bipy)(Cl_2) is found at 358 nm. This band resulted from the small hyperchromic effect of the bands found in the uncomplexed ligand upon complexation, this finding is in accordance with the studies of previous electronic spectra of copper(II) thiosemicarbazone complexes (Chandra and Kumar, 2007). The d-d transition of the complex was also recorded in visible region by concentrating the solution. The broad band at ca 683 nm (14640 cm-^{1}) is assigned to $^{2}B_{1g} \rightarrow {}^{2}A_{1g}$ transition in square planer geometry (Chandra and Kumar, 2007; Chaudhary and Shelly, 2012).The broad bands shown in $Fe(AHDT)(bipy)(Cl_2).3H_2O$ between 198-289 nm (50505-36630 cm^{-1}) correspond to $n \rightarrow \pi^*$. The iron complex is coloured inspite of the fact that it has d^5 electronic configuration, the colour may be attributrd to charge transfer band at 371 nm (26954 cm^{-1}) (Soumitra *et al.*, 2002; Sreekanth and Kurup, 2003; Subba *et al.*, 2010)

IR Spectra

The IR spectra is presented in Table 3. The primary ligand, acetaldehyde thiosemicarbazone showed strong bands observed at 3373 and 3262 cm^{-1}; these bands are assigned to ν(NH) vibrations. The most notable change in the ligand spectral upon coordinated to metal ion is the observed hypsochromic shift in C=N at ca 50-04 cm^{-1} in the spectra of the complexes (Wilkinson *et al.*, 1987; Abou-Melha and Faruk, 2008). This finding may be taken as an evidence for the participation of ν(C=N) azine group in coordination to the metal ion (Abou-Melha and Faruk, 2008; Hitesh and Saavani, 2012). The band at 999 cm^{-1} in the free ligand due to ν(C=S) and δ(C=S) stretching vibrations are shifted to lower frequencies at 759 – 900 cm^{-1} in the spectra of the complexes, suggesting coordination through the thioketo sulphur with the metal atom (Mendes *et al.*, 2007; Hitesh and Saavani, 2012). The complexes exhibit strong and sharp bands at 2042 and 2108 cm^{-1} and weak bands at 759 and 769 cm^{-1} which can be attributed to Arν(C=N) and ν(C=S) respectively. (Abu El-Reash *et al.*, 1994; Gupta *et al.*, 2002; Bermejo *et al.*, 2005; Aguirre *et al.*, 2006; Kumar and Kumar, 2013). Strong band found at 1095 cm^{-1} in the ligand assigned to ν(N–N) vibration, is found at higher frequencies of 1116-1145 cm^{-1} in the spectra of the complexes, this increase is due to the increase in the bond strength. The absorption frequency of all characteristic bands of ligand decreases upon complexation except the hydrazinic ν(N-N) band (Garg *et al.*, 1988). The principle behind this phenomenon is due to the donation of the unpaired electrons from one of the nitrogen atom to the metal ion, incidentally deflating the repulsion force between the two adjacent nitrogen electrons. This decreases the distance between the two nitrogen atoms, subsequently, shifting the absorption frequency to a higher value (Abu-Affan *et al.*, 2009). This again confirms the coordination via the azomethine nitrogen (Rapheal *et al.*, 2007). Conclusive evidence of bonding of the ligand to the central metal ion is provided by the appearance of new bands observed at 407-476 cm^{-1} which are tentatively assigned to ν(M-N) and ν(M-S) (metal-ligand) stretching bands supporting the coordination of the ligand as bidentate N-S chelating agent (Sengupta *et al.*, 1998; Abou-Melha and Faruk, 2008).

The coordination of the secondary ligand 2, 2$'$–bipyridine is indicated by the positive shift of ν(C=C), ν(C=N) ring stretching frequencies and the presence of the deformation modes at around 1556 cm^{-1} respectively. The position of the bands found in the spectrum of 2, 2$'$–bipyridine has been completely changed in the spectra of $Cu(AHDT)(bipy)(Cl_2)$ and $Fe(AHDT)(bipy)(Cl_2).3H_2O$ where it is used as co-ligand, and new bands appeared at 1492 and 2140-2193 cm^{-1} in $Cu(AHDT)(bipy)(Cl_2)$ and $Fe(AHDT)(bipy)(Cl_2).3H_2O$ respectively, confirming the coordination nature of 2, 2$'$- bipyridine. An additional band indicating ν(M-N$_{Azo}$) coordination with the metal ion at ~534 and 617 cm^{-1} has also been observed in $Cu(AHDT)(bipy)(Cl_2)$ and $Fe(AHDT)(bipy)(Cl_2).3H_2O$ respectively (Chaudhary and Shelly, 2012). Based on the above spectral evidences, it is confirmed that the ligands are biidentate, coordinating via the azomethine nitrogen and thiolate Sulphur in the primary ligand while, the secondary ligand cooordination was through the two pyridyl nitrogen atom.

^{1}H-NMR Spectra

The ^{1}H-NMR recorded in DMSO of spectra of the complexes in (ppm) are collected in Table 4. The spectra of acetaldehyde thiosemicarbazone showed -CH_3 (methyl proton) signals at 0.89 ppm, this signal shifted slightly downfield in the spectrum of the mixed ligand complexes. The -CH (aldimine proton) signals in the uncoordinated acetaldehyde thiosemicarbazone was observed at 6,65 ppm, this signal in the complexes resonates at 7.92- 7.94 ppm. The spectra showed no peak at 4.00 ppm an attribute of SH protons. Meanwhile peaks were seen at 11.33 ppm and 7.68 ppm which was attributed to N-H group (azomethine proton) N_2H (imino proton) respectively in acetaldehyde thiosemicarbazone. This is an indication that the ligand is in thione form, which is in conformity with IR spectrum. The downfield shifts in the signals of the N–H and N_2H are attributed to the coordination through the azomethine nitrogen and the thiocabonyl surphur atom, which are in agreement with the findings of previous of work of Gangadharan *et al.* (2010). The spectrum also displayed the characteristics signals of aromatic proton due to the pyridine fragment in the mixed ligand complexes, this was seen between 8.88-8.95 ppm.

Table 3: IR Spectra Assignments of the Compounds in (cm^{-1})

IR Band Assignment (KBr, cm^{-1})	AHDT $C_3H_7N_3S$	bipy	Cu(AHDT)(bipy)(Cl_2) $C_{13}H_{15}Cl_2CuN_5S$	Fe(AHDT)(bipy)(Cl_2).3H_2O $C_{13}H_{21}Cl_2FeN_5S$
ν(OH), H_2O				3781
ν(N-H)	3373 br 3263 br		3435	3394
Arν(C-H)		3055	3053	3080
ν(C=N)	1645 s		1600	1608
[ν(CS) + ν(CN)]	1286 m		1249	1293 1240
Arν(C=C)		1556	1492	1437
Arν(C=N)		2291	2108	2042
Ar(C-H) Bending			727	
Arν(C-C) Bending		742 758	650	
ν(N–N)	1095 m		1130	1132
ν(C=S)	999 s		769	759
M-N_{Azo}			617	534
M-N_{Aro}			476	474
M-S			416	407

Table 4: The ^{1}H-NMR Recorded in DMSO of Spectra of the Compounds in (δ, ppm)

COMPOUND	-CH_3 (METHYL PROTON)	CH_{Aro} Methylene Proton	CH (aldimine)	-$^4NH_2$ (IMINO PROTON)	-^{2}NH (AZOMETINE PROTON)	-OH (ALCOHOL)	CHEMICAL FORMULA
(AHDT)	3H, 3(s)1 0.89		1H, 1 (s)2 6.65	2H, 2(s)3 7.68	1H, 1(s)4 11.33		$C_3H_7N_3S$
Cu(AHDT)(bipy)(Cl_2)	3H, 1, 3 0.87 1.95, (d) 2.36, (d)	8H, (m), 8.93	1H, (m), 7.91	2H, 8(s), 7.21	1H, 5 (s), 9.15- 10.94 11.29		$C_{13}H_{15}Cl_2CuN_5S$
Fe(AHDT)(bipy)(Cl_2).3H_2O	3H, 1, 3 1.90, (d) 2.22, (d)	8H, (m), 8.91	1H, (m), 7.94	2H, 8(s), 7.25	1H, 5 (s), 9.15- 10.94	6H, (d), 11.01	$C_{13}H_{21}Cl_2FeN_5S$

Antifungal Activity

The result of fungicidal screening in Table 5 shows that the complexes were more active than the free ligand against pathogenic fungi. The mode of action may involve the formation of a hydrogen bond through the azomethane nitrogen atom with the active centers of the cell constituents, resulting in interference with the normal cell process (Gupta *et al.*, 2002; Abd El-Wahab *et al.*, 2004). The activity of the complexes was found to be greater than those of the primary ligands. These derivatives could act through a dual mechanism of action combining the pharmacological properties of both ligands and the metal salt (Navarro *et al.*, 2001; Sanchez-Delgado *et al.*, 2004). This increase in the inhibitory activity of the mixed ligand complexes as compared to the other drugs is an indication that they are very much effective against the various pathogenic microbial species tested. The biological behavior revealed that copper complex has higher activity than that of iron.

Table 5: Antifungal activity Data of the Compounds after 72 Hours Using Sensitivity Disc (60 µg/mL). Zone of Inhibition in (mm).

Test Samples	*Aspergillus Niger*	*Penicillium chrysogenum*	*Rizopus stolonifer*	*Candida albicans*
(AHDT)	10.00 ±1.00**	6.80 ± 0.58**	11.00 ± 1.00**	10.00 ± 0.00**
bipy $C_{10}H_8N_2$	6.00 ±0.20**	5.20 ± 0.48**	09.00 ± 2.00**	08.00 ± 1.00**
Cu(AHDT) (bipy)(Cl_2)	32.67 ± 2.52**	30.70 ± 2.15**	31.00 ± 0.00**	32.40 ± 0.00**
Fe(AHDT) (bipy) (Cl_2).3H_2O	31.33 ± 1.53**	27.00 ±0.00**	28.00 ± 2.00**	29.00 ± 3.00**
CuCl.2H_2O	0.00±0.00*	0.33 ± 0.58*	0.67 ± 0.58*	0.00±0.00*
$FeCl_2$.6H_2O	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*

All values are mean of replicate determinations ± standard deviation, values in the same column with different superscript symbol (**) are significantly different from the control (*) (P< 0.05), one-way analysis of variance (ANOVA) followed by post hoc LSD.

Conclusion

The structural analysis indicates that coordination of the anionic thiosemicarbazone ligand is via the imine nitrogen and thiolato sulphur atom and the two pyridyl nitrogen atoms in the secondary ligand. Other coordination site being occupied by chloride ions in an octahedral geometry for both complexes. The electronic spectra results displayed the existence of d-d electronic only in the copper complex, other bands seen correspond to n→π* transition and intra-ligand charge transfer. The iron complex is coloured inspite of the fact that it has d^5 electronic configuration, the colour may be attributrd to charge transfer band at 371 nm (26954 cm^{-1}). The results of the elemental analyses for the compounds were in good agreement with the theoretical values. The complexes are of 1:1:1 stoichiometry and are non-electrolytes in solution. The antifungal activities were evaluated by the diameter of zone of inhibition in (mm) against *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Candida albicans.* The copper complex has more antifungal activity than that of iron. The study however showed that complexation of the ligand with metal ion resulted in increased antimicrobial activity. Finally, coordination process induces changes in the properties of the mixed ligand complexes, these physicochemical transformations due to structural modifications accomplished enhanced the antifungal activity.

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