

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

Antimicrobial Study of Zn (II) Complexes and Heterocyclic Base Adducts Derived from N (4) Thiosemicarbazone Moiety

Dr.J. R. Gujarathi

Associate Professor, Department of Chemistry, Pratap College, Amalner, Maharashtra, India

ABSTRACT: Zinc (II) complexes and adducts have been synthesized by the reaction of zinc (II) chloride with 3,5-dichloro-2-hydroxy acetophenone N(4) methyl thiosemicarbazone in presence of heterocyclic base like pyridine, 2-chloro pyridine, 3-chloro pyridine, 4-chloro pyridine in the molar ratio 1:1:1. The synthesized thiosemicarbazone has been characterized by $^{13}\text{C-NMR}$, $^1\text{H-NMR}$, ESI-MS as well as IR. Spectral and magnetic measurements indicated octahedral geometry for the six coordinate complexes. The TGA data indicated co-ordinated water molecules in the complexes. The thiosemicarbazone and its Zn (II) complexes have been found antifungal, antibacterial and showed growth inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis* (Gram+ve), *Aspergillus Niger* and *Candida Albicans* fungal species.

KEYWORDS: Metalsalts, Bioactive metal complexes, antimicrobial activity, MIC

I. INTRODUCTION

Thiosemicarbazones are compounds constituting N,S donor atoms. On complexation with metal ion, they show heterodentate nature [1-6]. Thiosemicarbazones are good ligands with high biological activity. Their biological activity is due to ability to coordinate to metal centres. Thiosemicarbazone derivatives also have additional functional groups which can coordinate to metal ion. This suggests the biological activity may also depend on the non coordinating groups. In biochemical systems Zinc (II) sulfur interactions are important, due to the presence of sulfur at the active sites of several enzymes, vitamins and proteins [7]. It is the important transition metal biologically. It plays most vital function which may be concerned with the synthesis of DNA and RNA. The deficiency of Zn (II) has effect on DNA synthesis, delayed wound healing and decrease in collagen synthesis [8]. Thiosemicarbazones react as ligands with metal ions by bonding through the sulfur and the azomethine nitrogen atoms, although in some cases they behave as terdentate ligands bonded through the sulfur and azomethine nitrogen and another donor atom (O or N) [9-14]. Zinc pyrithione drug which is antibacterial and antifungal inhibits the membrane transport processes by blocking the proton pump in fungi. However, at low concentration of the agent fungi are able to inactivate pyrithione [15]. Zinc is the most abundant transition metal in the brain after iron [16]. Zinc(II) carboxylates complexes have good antibacterial activity, inhibitive effect was found towards *E. coli* and *S. aureus* [17]. The interaction between zinc and the gyrase inhibitor ciprofloxacin and various nitrogen-donor ligands have been studied and the complexes were detected more effective than the drugs alone [18]. The complexes of thiosemicarbazones gave promising results. Activity was observed with zinc(II) complexes of picolinaldehyde-S-methyl- and -S-benzylthiosemicarbazones which were found active than metal free ligands [19]. Zinc(II) complexes of salicylaldehyde were found active against *S. aureus* [20]. The antibacterial activity of zinc(II) complex with S-phenylalaninato ligand was higher than the simple metal salt. Good inhibitory activity was also found by the yeast *Candida albicans* [21].

In this research work the synthesis, spectral characterisation and biological studies of six coordinate complexes of zinc (II) with 3,5-dichloro 2-hydroxy acetophenone N (4) methyl thiosemicarbazone have been reported.

II. MATERIALS AND METHODS

All chemicals used are of A.R. grade. Room temperature magnetic susceptibility measurement was carried out by Faraday method. IR spectra were recorded in solid state in the range 4000-200 cm^{-1} range. NMR spectra were recorded

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

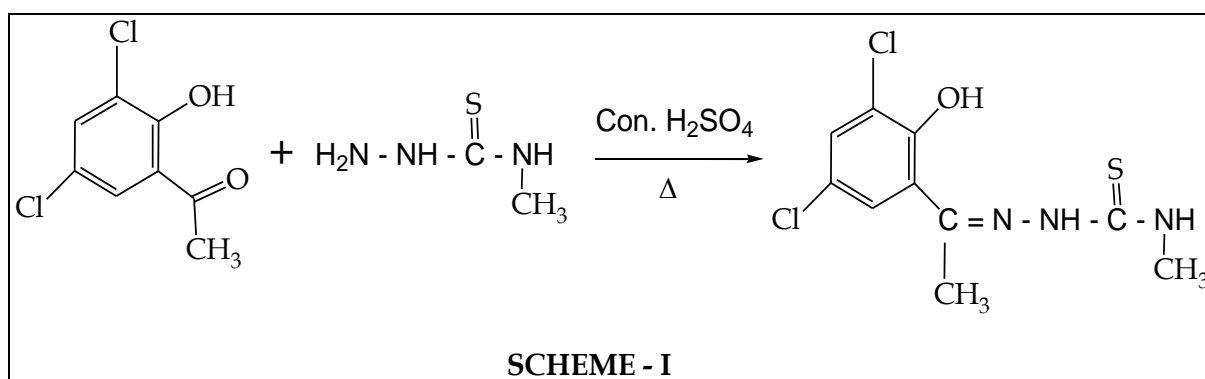
Vol. 4, Issue 12, December 2015

in the mixture of CDCl_3 and DMSO-d_6 (1:1 v/v) with a Bruker AC-300F 300MHz spectrometer. Thermo gravimetric analysis was carried out in the temp rnge 28-800°C. Metal in the complex and adducts was estimated by standerzied E.D.T.A using Erichrome black-T as an indicator and PH-10 buffer solution. Chloride in the complex was determined by Mohr's method.

III. EXPERIMENTAL

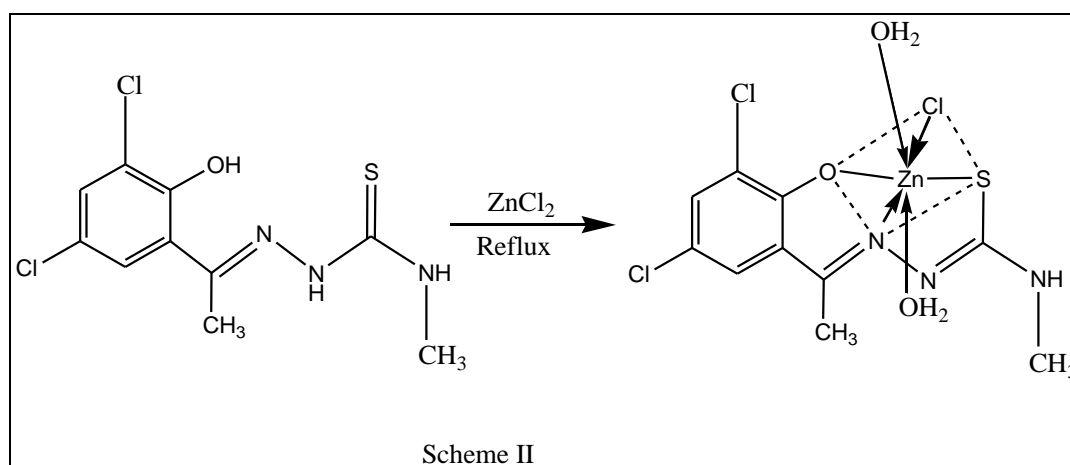
Synthesis of thiosemicarbazone

Ethanolic solution of 5-chloro 2-hydroxy acetophenone (0.01 mole) was added to ethanolic solution of N (4) methyl thiosemicarbazide (0.01 mole) in the mole ratio 1:1. The reaction mixture was refluxed for three hours.. The pale yellow product obtained after cooling was filtered and washed with hot water then cold ethanol and finally with ether. It was recrystalized from hot ethanol and dried in vacuum.



Synthesis of complex

The complex of the type M.L.Cl was synthesized by refluxing ethanolic solutions of thiosemicarbazone (0.01mole), zinc chloride(0.01 mole) in ratio 1:1 for an hour on a hot water bath. The light yellow product obtained was filtered and washed well with hot water, cold absolute ethanol and then ether and dried over in vacuum.



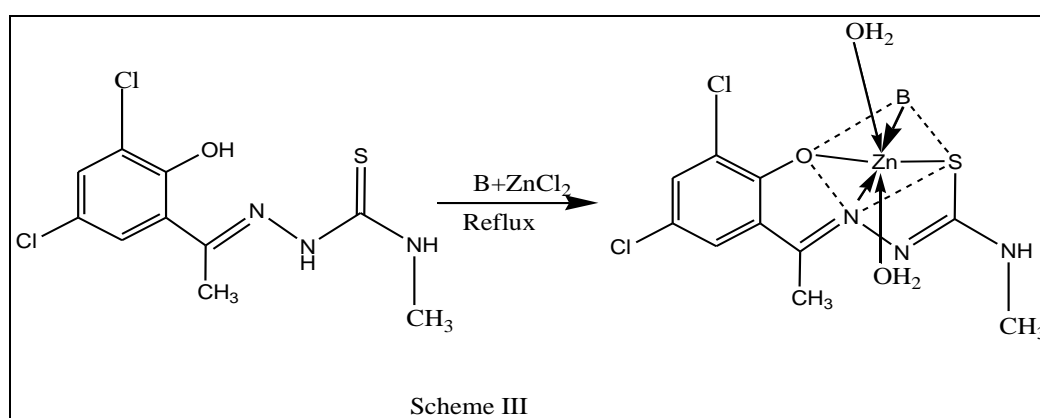
International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

Synthesis of adducts

The adducts of the type Zn.L.B (B is heterocyclic base like pyridine, 2-chloro pyridine, 3-chloro pyridine, 4-chloro pyridine) was synthesized by adding slowly ethanolic solution of $ZnCl_2$ (0.01 mole), heterocyclic base (0.01 mole) to the hot ethanolic solution of thiosemicarbazone (0.01 mole) in the ratio 1:1:1 and refluxing reaction mixture for one hour. The yellow adduct obtained was filtered and washed with hot water, cold ethanol and diethyl ether and dried in vacuum.



(B= pyridine 2-chloro pyridine,3-chloro pyridine,4-chloro pyridine)

TABLE 1 PHYSICAL PROPERTIES

Compounds	Colour	Empirical Formula	Molar conductance $\text{Ohm}^{-1} \text{cm}^2 \text{mole}^{-1}$	Magnetic Moment B.M.
L	Yellow	$C_{10}H_{11} N_3 O S Cl_2$	-	-
Zn-L.Cl.(H_2O) ₂	Yellow	$C_{15}H_{15}N_3O_3SCl_3Zn$	30.4	Diamagnetic
Zn.L.Py.(H_2O) ₂	Yellow	$C_{20}H_{20}N_4O_3SCl_2Zn$	31.7	Diamagnetic
Zn.2-Cl.py.(H_2O) ₂	Yellow	$C_{20}H_{19}N_4O_3SCl_3Zn$	32.6	Diamagnetic
Zn.3-Cl.py.(H_2O) ₂	Yellow	$C_{20}H_{19}N_4O_3SCl_3Zn$	31.8	Diamagnetic
Zn.4-Cl.py.(H_2O) ₂	Yellow	$C_{20}H_{19}N_4O_3SCl_3Zn$	32.7	Diamagnetic

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

Physical data of ligand and its complexes is presented in Table 1. The complex and all adducts are soluble in DMF in which conductivity measurements were made (30⁰C), showing all complexes are non electrolyte [22]. Room temperature magnetic moments measurement at by Faraday method showed diamagnetic behavior expected for d¹⁰ configuration.

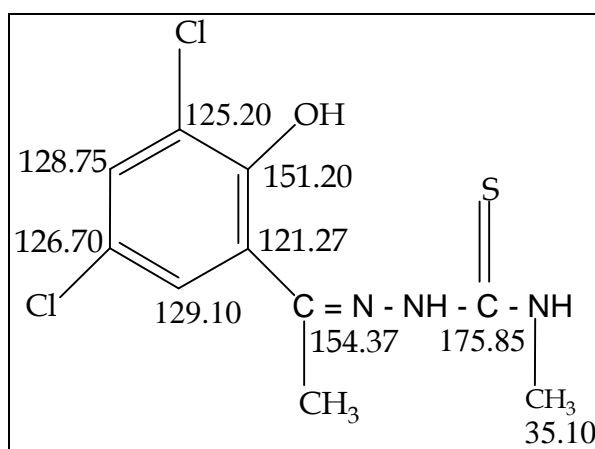
¹H-NMR

Signals at 11.6, 3.30 ppm are assigned to –OH, -CH₃ protons respectively.

¹H-NMR signals at 12.00 and 2.4 ppm are assigned to –OH and –CH₃ protons respectively. The signals at 2.30, 3.01 correspond to ⁴NH and H⁴N-CH₃ respectively. Signal at 10.5 ppm corresponds to ²NH. Aromatic protons show multiplets at 7.0, 7.20, 7.35, ppm.

¹³C-NMR (DMSO-D₆): δppm

125.20 (C = C-Cl), 128.75 (C = C), 127.79 (C = C – Cl), 126.70 (C = C), 129.10 (C = C), 151.20 (C = C – OH), 154.37(C = N), 175.85 (C = S), 35.10 (NH – CH₃).



(Calcd) found ESI-MS m/z, ion M⁺

C₁₀H₁₁N₃O₃SCl₂ (292.17) 292.89, C₁₅H₁₅N₃O₃SCl₃Zn (489.10) 489.73, C₂₀H₂₀N₄O₃SCl₂Zn (532.74) 532.12, C₂₀H₁₉N₄O₃SCl₃Zn (567.19) 567.72, C₂₀H₁₉N₄O₃SCl₃Zn (567.19) 567.89, C₂₀H₁₉N₄O₃SCl₃Zn (567.19) 567.91.

Mass spectral data confirmed the structure of the thiosemicarbazone and complexes as indicated by molecular ion peak (M + 1) corresponding to their molecular weights.

III. ANALYTICAL DATA

1. **L**: % C 41.74 (41.11), % H 3.13 (3.79), % N 14.88 (14.38), % S 10.21 (10.97)

2. **Zn-L.Cl.(H₂O)₂**: % Zn 13.73 (13.37), % Cl 7.84 (7.25), % C 36.05 (36.83), % H 3.73 (3.50), % N 8.24 (8.59), % S 6.81 (6.55).

3. **Zn-L.Py.(H₂O)₂**: % Zn 12.84 (12.28), % C 45.74 (45.09), % H 3.16 (3.78), % N 10.07

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

(10.52), %S 6.64 (6.02).

4. **Zn.2-Cl.py.(H₂O)₂**: % Zn 11.07 (11.53), %C 45.71 (45.09), %H 3.91 (3.38), %N 9.21

(9.88), %S 5.19 (5.65).

5. **Zn.3-Cl.py.(H₂O)₂**: % Zn 11.15 (11.53), %C 45.81 (45.09), %H 3.81 (3.38), %N 9.20

(9.88), %S 5.10 (5.65).

6. **Zn.4-Cl.py.(H₂O)₂**: % Zn 11.18 (11.53), %C 45.88 (45.09), %H 3.90 (3.38), %N 9.25

(9.88), %S 5.09 (5.65).

Elemental analysis data showed that the calculated and experimental percentages are matched and hence confirmed 1:1 mole ratio of metal ion, thiosemicarbazone for complex and 1:1:1 mole ratio for metal, thiosemicarbazone and heterocyclic base for all adducts.

IV. INFRARED SPECTROSCOPIC DATA (cm⁻¹)

1.L.: ν (- OH) 3200; ν (C = N) 1642; ν (- C - S) 790 (s), 1365 (m); ν (N - N) 1060; ν (²N-H) 3250; ν (C - O) 1295.

2 Zn-L.Cl.(H₂O)₂: ν (C = N) 1550; ν (C = N-N=C) 1525, ν (C-S) 705, 1305, ν (N-N) 1150, ν (M - N) 460, ν (M-O) 530, ν (M-S) 330, ν (C - O) 1210, ν (H₂O) 3450,3510.

3 Zn.L.Py.(H₂O)₂: ν (C = N) 1560; ν (C = N-N=C) 1530, ν (C-S) 710, 1310; ν (N-N) 1155, ν (M - N) Base 255, ν (M - N) 465, ν (M - O) 535, ν (M-S) 335, ν (C - O) 1215, Band due to HB 1420, ν (H₂O) 3460,3520.

4. Zn.2-Cl.py.(H₂O)₂: ν (C = N) 1565; ν (C = N-N=C) 1535, ν (C-S) 715, 1315, ν (N-N) 1160, ν (M - N) Base 260, ν (M - N) 470, ν (M - O) 540, ν (M-S) 340, ν (C - O) 1225, Band due to HB 1425, ν (H₂O) 3465,3530.

5. Zn.3-Cl.py.(H₂O)₂: ν (C = N) 1575; ν (C = N-N=C) 1545, ν (C-S) 720, 1325, ν (N-N) 1170, ν (M - N) Base 270, ν (M - N) 475, ν (M - O) 550, ν (M-S) 350, ν (C - O) 1235, Band due to HB 1435, ν (H₂O) 3475,3540.

6. Zn.4-Cl.py.(H₂O)₂: ν (C = N) 1590; ν (C = N-N=C) 1555, ν (C-S) 730, 1335, ν (N-N) 1180, ν (M - N) Base 275, ν (M - N) 480, ν (M - O) 560, ν (M-S) 360, ν (C - O) 1240, Bands due to HB 1440, ν (H₂O) 3485,3550.

The bands in IR spectra due to ν (N—H) appear at 3250 cm⁻¹ for thiosemicarbazone, disappeared in the spectra of all the complexes. The band at 1642 cm⁻¹ due to ν (C = N) of the thiosemicarbazone moiety shifted to 1590 cm⁻¹ upon coordination [23]. This shows the coordination of azomethine nitrogen. The coordination of azomethine nitrogen is confirmed by the presence of a band in the range 460-480cm⁻¹, assignable to ν (Zn-N) for the complexes [24, 25]. A shift in the absorption bands due to the ν (N - N) from 1060 cm⁻¹ to higher region is due to the increase in double bond character off-setting the loss of electron density via donation to the metal. This also confirms the coordination of the thiosemicarbazone through the azomethine nitrogen [26]. The coordination via thiolate sulfur is indicated by decrease ν (C=S) [27,28]. The bands at 330-360 cm⁻¹ assignable to ν (Zn-S) in the complexes confirm sulfur coordination [24]. The new peaks observed in the range 530—560 cm⁻¹ are due to the Zn-O(phenolic) bond [29]. This is indicated by decrease in ν (C-O).

V. TGA ANALYSIS DATA

1. Zn-L.Cl.(H₂O)₂: First step, 114 °C, Mass loss 7.50 % second step, 200 °C, Mass loss, 20.23 % Third Step 350 °C, Mass loss, 48.22 % Fourth Step, 650°C, Mass loss 61.5 %, Residue 780 °C, % of ZnO, 16.10 (16.64).

2. Zn.L.Py.(H₂O)₂: First step, 113 °C, Mass loss 6.10 % second step, 220 °C, Mass loss, 22.32 % Third Step 355 °C, Mass loss, 77.02 % Fourth Step, 669 °C, Mass loss, 62.00 %, Residue, 779 °C, % of ZnO, 15.70 (15.28).

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

3. **Zn.2-Cl.py.(H₂O)₂**: First step, 114 °C, Mass loss 6.50 % second step, 230 °C, Mass loss, 27 % Third Step 365 °C, Mass loss, 48.40 % Fourth Step, 660 °C, Mass loss 62.5 %, Residue 778 °C, % of ZnO, 14.81 (14.35).
- 4 **Zn.3-Cl.py.(H₂O)₂**: First step, 115 °C, Mass loss 6.65 % second step, 235 °C, Mass loss, 28.25 % Third Step 360 °C, Mass loss, 49.02 % Fourth Step, 666 °C, Mass loss, 63.27 %, Residue, 779 °C, % of ZnO, 14.78 (14.35).
- 5 **Zn.4-Cl.py.(H₂O)₂**: First step, 114 °C, Mass loss 6.60 % second step, 240 °C, Mass loss, 28 % Third Step 365 °C, Mass loss, 48.28 % Fourth Step, 669 °C, Mass loss 62.70 %, Residue 800 °C, % of ZnO, 14.88 (14.35).

The hydrated layer was removed below 100⁰C. The two coordinated water molecules were eliminated in the complexes at relatively temperature less than 120⁰C. The two coordinated water molecules in complex and adducts were removed in one step at less than 120 °C The TGA data indicated that the decomposition proceeded in several steps. The compounds are stable up to about 250⁰C. The adducts are more stable than complex. This may be due to heterocyclic bases in coordination. There are three steps after the removal of water molecules in complex and adducts. In the second step the loss is upto 25%. The organic molecule was lost upto 650⁰C. The mass lost corresponding to this step is about 62%. The decomposition was complete and metal oxide was formed at a temperature about 780 °C. The coordination of metal and increase in coordination number in the complexes may be responsible for the thermal stability.

VII. BIOLOGICAL ACTIVITY (AGAR PLATE DIFFUSION METHOD)

Table.4 Minimum Inhibitory concentration L , Zn (II) complexes and standered

Compound	<i>Staphylococcus aureu</i>		<i>Bacillus subtilis</i>		<i>Escherichia Coli</i>		<i>Pseudomonas aeruginosa</i>	
	Gram positive				Gram negative			
	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml
L	0.75	0.42	0.72	0.45	0.77	0.48	0.78	0.47
Zn-L.(H ₂ O) ₃	0.62	0.31	0.65	0.22	0.67	0.23	0.65	0.24
Zn-L.Py.(H ₂ O) ₂	0.63	0.32	0.64	0.24	0.65	0.22	0.62	0.25
Zn-2-Cl.py.(H ₂ O) ₂	0.65	0.33	0.68	0.21	0.66	0.24	0.68	0.26
Zn-3-Cl py.(H ₂ O) ₂	0.67	0.34	0.67	0.23	0.69	0.23	0.67	0.25
Zn-4-Cl py.(H ₂ O) ₂	0.68	0.35	0.66	0.24	0.69	0.22	0.67	0.23
ZnCl ₂ .6H ₂ O	0.20	0.16	0.21	0.16	0.13	0.22	0.11	0.24
Standard	0.15	0.11	0.15	0.14	0.25	0.12	0.15	0.12

(Std-Amphiciline)

The bacterial assay was carried out by the agar plate diffusion method. Activity was measured by measuring the diameter of inhibition zone in mm. The adducts showed good activity against bacterial species than free ligand. The

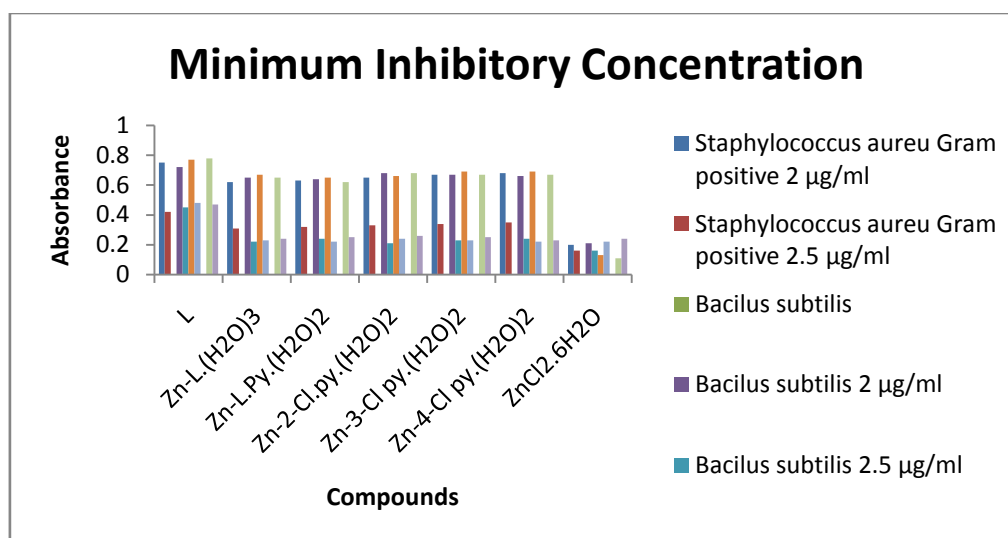
International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

minimum inhibitory concentration was determined by liquid dilution method [30].The solutions of ligand and complexes with 2 µg/ml,2.5 µg/ml and 3 µg/ml contractions were prepared in the solvent DMF.The solution of standard drug ampicilin was also prepared in the same concentration.Inoculums of the overnight culture were prepared.0.2 ml of the inoculums was added to the test tubes containing the solutions of the compounds of different concentrations.After the addition of sterile water to each of the test tubes,these were incubated for 24 hours and observed for turbidity.The absorbance of the turbid solutions was measured at 520 nm.The same method was used for standard [31].It was found that at 2µg/ml the absorbance is more.Less absorbance was observed at 2.5 µg/ml and no absorbance at 3µg/ml.The inhibition is more at 2.5 µg/ml.The metal salt solution showed better inhibition than ligand and complexes. The inhibitory concentration is 2.5 µg/ml. Thus increase in coordination number in complexes and coordination of metal ion to ligand increases microbial activity.

FIG NO.1



VII CONCLUSION

The thiosemicabazone synthesized is tridentate ONS donor.The spectral data indicates octahedral geometry for complexes and adducts.The complex and adducts are diamagnetic.TGA data indicates two water molecules in coordination.The compounds are thermally stable upto200⁰C.The decomposition took place in several steps.The complex and adducts showed growth inhibitory activity against gram +ve bacterial species.The absorbances are more at 2 µg/ml concentration than 2.5µg/ml concentration.The metal salts showed more inhibition.

REFERENCES

- 1.Lobana T.S.,Sharma R.,Bawa G.,Khanna S.*Coord.Chem.Rev.*253 **2009** 933.
2. Garg B.S.,Kurup M.R.P.,Jain S.K.,Bhoon Y.K.*Trans.Metal Chem.*13 **1988** 92.
3. John R.P.,Shreekant A.,kurup M.R.P.,Mobin S.M.*Polyhedron* 21 **2002** 2515.
4. Seena E.B.,Beesy Raj B.N,Kurup M.R.P. Sresh E.*J.Chem.Cryst* 36 **2006** 189.
5. Philip V.,Suni V.,Kurup M.R.P.,Nethaji M.*Polyhedron* 23 **2004** 1225
6. Metasanz A.I.,Perez J.M.,Navarro P.,Moreno J.M.,Colacoi E.,Souza P.*J.Inorga.Biochem* 76 **1999** 29.
7. Prince H, *Adv. Inorg. Radiochem.* 22 **1979**, 349.
- 8.. Sarma L.S,Kumar J.R,Reddy K.J, Thriveni T, Reddy A.V, *J. Braz.Chem. Soc.* 17 **2006**, 463.
- 9.. Souza P, Matesanz A.I.,Fernandez V, *J. Chem. Soc., Dalton Trans.* **1996**,3011..
10. Quoroga A.G, Perez J.M, Lopez-Solera I, Masaguer J.R, Luque A, Roman P, Edwards A. Alonso., C., Navarro -Ranninger C, *J. Med. Chem.* 41,**1998**, 1399.
- 11.Petering D.H., Petering H.G, in: Sartorelli A.C., Johns D.G (Eds.),*Handbook of experimental Pharmacology, Springer, Berlin* ,**1975**, 841.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

12. Petering D.H., Antholine W.E, Saryan L.A., in: Ottenbrite R.M, Butler G.B (Eds.), *Anticancer and Interferon Agents, Synthesis and Properties*, Marcel Dekker, New York, **1984**, 203.
13. Souza P., Sanz L., Fernandez V., Arquero A, Gutierrez E., Monge A., *Naturforsch Z., Teil B* 46, **1991**, 767.
14. Dowling, C, Perkin G. *Polyhedron* 15, **1996**, 2463.
15. Mitic D, Milenkovic M, Milosavljevic S, Godevac D, Miodragovic Z, Anelkovic K, Miodragovic D. *Eur. J. Med. Chem.* 44 **2009** 1537.
16. Chang C J Lippard S J Sigel A, Sigel H, Sigel R.K.O. John Wiley & Sons, Ltd., England. Vol 1 Chapter 12 **2006** 321.
17. Szunyogova E.; Mudronova D.; Gyoryova G.; Nemcova R.; Kovarova J.; Piknova-Findorakova L. *J. Therm. Anal. Calorim.* 88, **2007**, 355.
18. Patel M, Chhasatia M, Parmar P. *Eur. J. Med. Chem.* 45, **2010**, 439.
19. Kizilcikli I, Kurt Y.D, Akkurt B.; Genel A.Y, Birteksoz S, Otuk G.; Ulkuseven B. *Folia Microbiol.* 52, **2007**, 15.
20. Kaczmarek M.T, Jastrzab R, Holderna-Kedzia E, Radecka-Paryzek W.. *Inorg. Chim. Acta* 362, **2009** 3127.
21. Mitic D, Milenkovic M, Milosavljevic S, Godevac D, Miodragovic Z, Anelkovic K, Miodragovic D. *Eur. J. Med Chem.* 44, **2009**, 153.
22. Geary W.J., *Coord. Chem. Rev.* **1971**, 7, 81.
23. John R.P., Sreekanth A., Rajakannan V., Ajith T.A., Kurup M.R.P. *Polyhedron* 23, **2004** 2549.
24. Bermejo E, Castineiras A., Garcia-Santos I., West D.X., *Anorg Z. Allg. Chem.* 630, **2004**, 1097.
25. Nakamoto K, *Infrared and Raman Spectra of Inorganic and Coordination compounds, 5th ed.*, Wiley, New York, **1997**.
26. Bindu P., Kurup M.R.P, Satyakeerthy T.R., *Polyhedron* 1.8, **1999**, 321.
27. West.D.X., Whyte A.C., Sharif F.D., Gabremedhin H., Liberta A.E, *Trans. Met. Chem.* 18, **1993**, 238.
28. Campbell M.J.M, *Coord. Chem. Rev.* 15, 1975, 279.
29. Percy G.C., Thornton D.A, *J. Inorg. Nuclear. Chem.* 34, **1972**, 3351.
30. Salmon S.A, Watts J.L, Cheryal A.J. *Clin. Microbial*, **1995**, 2435.
31. Shankar K., Muralidhar Reddy P, Rohini R, Vadde Ravindra *Der Pharmacia Letter*; 1, **2009**, 97