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SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF IRON(II) COMPLEXES OF ISONICOTINOYLHYDRAZONES

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ABSTRACT

MAINH & HMAINH ligands and their Iron(II) complexes are synthesized and characterized based on physico-chemical, Mass, UV-visible and IR spectral studies. ligands act as neutral tridentate ligands and the general formula for complexes is $[Fe(ML)_2]Cl_2$. Electronic spectral data suggest that the complex has octahedral geometry. Cyclic voltammetric studies reveal that the oxidation/reduction potentials of complexes is shifted towards positive or negative values than its corresponding ligands. Antibacterial activity of ligands and metal complexes are also investigated in the present work.

KEYWORDS: Iron(II) complex, Isonicotinoylhydrazones, cyclic voltammetry, antibacterial activity.

INTRODUCTION

Iron is the most abundant element found in the surrounding environment and plays crucial role for the survival of terrestrial organisms. Iron participates in biochemical processes like ribonucleic reduction, energy production, photosynthesis, nitrogen fixation, oxygen transport, and oxygenation.[1-3] Iron complexes have been known for catalytic, antitumor, and antimicrobial activity.[5–9] Iron and its complexes both are known for its DNA damaging property.[10–12]

Isonicotinoyl hydrazone and its analogues constitute a series of monobasic acids that are capable of binding iron as tridentate (N,N,O) ligands.[13] Hydrazones are efficient chelating agents. The complex formation reaction of iron with isonicotinoyl has been used in the treatment of secondary iron overload.[14-17] Desferrioxamine (DFO), is expensive and cumbersome since the drug requires long subcutaneous infusions and it is not orally active. Isonicotinoylhydrazone deravatives were shown to have high Fe chelation efficacy in vitro.[18, 19]

Studies on iron complexes of isonicotinoyl hydrazones are very limited. Hence it is considered worthwhile to investigate a new series of iron(II) complex of isonicotinoylhydrazones. In the light of the above, isonicotinoylhydrazones and their metal complexes have been prepared and characterized by using physico chemical and spectral techniques. Antibacterial properties iron(II) complexes are described.

EXPERIMENTAL

MATERIALS AND METHODS

Isonicotinic acid hydrazide, 2-Methoxy acetophenone, 4-Hydroxy-2-Methoxy acetophenone agarose were purchased from Sigma-Aldrich. All other provided chemicals were of AR grade and used . The solvents used for the synthesis were distilled before use. Magnetic moment measurements were taken by using lakeshore VSM 7410 instrument at 298K. Molar conductivity measurements were carried out at 298 ± 2K in DMF by using a ELICO CM model 162 conductivity meter. The electronic spectra of copper complexes were recorded in DMF as solvent by using UV lamda50 (Perkin-Elmer) spectrophotometer. IR spectra were recorded for ligands and complexes in the range 4,000–500 cm^{-1} by using Perkin-Elmer spectrum100 spectrometer on KBr discs. Electrochemical studies of complexes were taken on a CH instruments assembly provided with an X–Y recorder. Measurements were taken on degassed (N_2 bubbling for 5 min) solutions (10^{-3} M) containing 0.1 M Bu_4NPF_6 as the supporting electrolyte. The three-electrode system consisted of platinum wire acts as an auxiliary electrode glassy carbon as working electrode and Ag/AgCl as reference electrode.

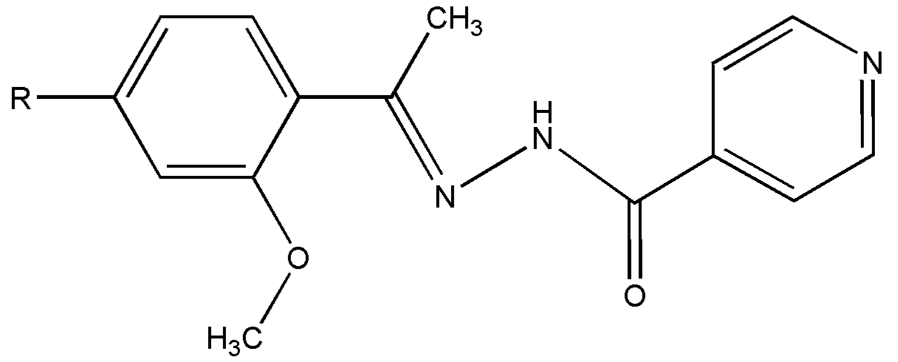
Preparation of Ligands

Ligands were synthesized by reacting isonicotinic acid with different carbonyl compounds. A methanolic solutions of nicotinic acid hydrazide and isonicotinylhydrazide (5mmol), carbonyl compound (5mmol) were mixed in a 100-ml round bottom flask.

Few drops of Hydrochloric acid were added to the above the reaction mixture and heated for 3-6 hours and the reaction mixture is cooled room temperature, yellow or pale yellow coloured crystalline products were obtained. The products which were obtained, washed with methanol and few drops of hexane and dried in vacuum. General structure of ligands is shown in **Figure 1**.

2-Methoxy acetophenone isonicotinoyl hydrazone (MAINH): Yield 71%, M.Pt. 263-265°C, ^{1}H-NMR spectra: δ(8.8-7.4) (doublet 4H), δ(7.1-6.5) (multiplet 4H), δ(3.8)(singlet 1H), δ(2.9) (singlet 3H), δ(2.8) (singlet 3H),assigned to isonicotine ring **H**, phenyl **H**, imine **H**, methyl **H**, methoxy **H** protons respectively. Mass spectra of MAINH shows molecular ion peak at 269.

4-Hydroxy-2-Methoxy acetophenone isonicotinoyl hydrazone(HMAINH): Yield 69%, M.Pt. 270-272°C, ^{1}H-NMR spectra: 13.21δ (singlet 1H), δ(8.6-7.5) (doublet 4H), δ(7.3-7.0) (multiplet 3H), δ(3.6)(singlet 1H), δ(2.7) (singlet 3H), δ(2.5) (singlet 3H),assigned to hydroxy **H** isonicotine ring **H**, phenyl **H**, imine **H**, methyl **H**, methoxy **H** protons respectively. Mass spectra of HMAINH shows molecular ion peak at 285.



R=H, 2-Methoxyacetophenone isonicotinoyl Hydrazone(MAINH)
R=OH, 4-Hydroxy-2-Methoxyacetophenone isonicotinoyl Hydrazone(HMAINH)

Figure 1: Structure of the ligands.

Synthesis of [Fe(MAINH)$_2$]Cl$_2$: In a 250-mL round bottom flask an aqueous solution (10 mL) of metal salt of FeCl$_2$.4H$_2$O (0.99g, 5mmol) were mixed with a hot methanolic solution (20 mL) of the ligand MAINH(1.34g, 5mmol) and the contents were refluxed on water bath under the nitrogen atmosphere for 3hr. On cooling the contents to room temperature, a crystalline brownish black coloured complex was separated out. Then it was collected by filtration, washed with methanol and dried in vaccum. **Yield:** 64%.

Synthesis of [Fe(HMAINH)$_2$]Cl$_2$: In a 250-mL round bottom flask an aqueous solution (10 mL) of metal salt of FeCl$_2$.4H$_2$O (0.99g, 5mmol) were mixed with a hot methanolic solution (20 mL) of the ligand HMAINH(1.42g, 5mmol) and the contents were refluxed on water bath under the nitrogen atmosphere for 4hr. On cooling the contents to room temperature, a crystalline brownish black coloured complex was separated out. Then it was collected by filtration, washed with methanol and dried in vaccum. **Yield:** 64%.

EXPERIMENTS IN ANTIBACTERIAL ACTIVITY

The testing against bacteria and fungi has been carried out by employing the paper disc method. Testing materials apparatus such as test tubes, paper discs, petri dishes, pipettes, conical flasks, and forceps were sterilized before they are used and every precaution was taken to avoid contamination.

Preparation of Sample Solution

The samples were dissolved in DMSO (AR grade) and Whatman No.1 filter paper discs were soaked in different concentrations of the compounds obtained in the range from 100 µg/ml to 25 µg/ml by successive dilutions.

Medium

The medium was prepared by adding 20% agar to the nutrient broth, which was prepared from glucose (15 gm), peptone (12 gm) and salt solution (50 ml) were dissolved in water (1000 ml) and pH was adjusted to 7.2-7.4 and sterilized in an autoclave at 121 °C (at 15 lb pressure) for 15 mins.

Bacteriological testing

The antibacterial activity screening is done by the paper disc method.[20]

Organisms Used

Two gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and two gram-negative (*Salmonella Typhi* and *Escherichia coli*) were selected. The above sterile nutrient agar was melted and taken in labeled containers, cooled to 50°C. Actively growing agar slant culture suspension of bacteria was swab inoculated separately on these solidified agar plates. Sterile filter paper discs (6 mm diameter) prepared from standard Whatman No.1 filter papers were dipped in the test solution of concentration at 100 µg/ml and after drying the discs, they were introduced on to the above inoculated agar plates containing bacterial strains. The plates with test compounds discs were incubated for 24 hr at 35°C. After 24 hr the petri dishes were checked for the growth inhibition zone. The presence of clear zone of growth inhibition around the paper disc indicated the inhibition of growth of organism. The zone of inhibition around the disc in the petri dish was measured in mm.

RESULTS AND DISCUSSION

Iron(II) metal complexes are crystalline, stable and non hygroscopic. They are slightly in organic solvents but soluble in DMSO and DMF. The analytical and physico-chemical data supports the proposed composition of the complexes. Physical properties are given in **Table 1**. ESI mass spectra of $[Fe(MAINH)_2]Cl_2$ is shown in **Figure 2**. Molecular ion peak is observed at m/z value of 664. Thus the proposed molecular formulae of complexes are consistent with molecular weights obtained from mass spectra.

Analytical Studies

The complexes are stable at room temperature, non-hygroscopic, slightly soluble in water, but more soluble in methanol, ethanol and readily soluble in CH_3CN, DMF and DMSO. Analytical data **(Table 1)** are consistent with proposed molecular formulae of complexes. High molar conductivities (45.4 & 57.6) of other two complexes suggest electrolytic in nature.

Magnetic moment studies

The magnetic moment data of Fe(II) complexes are given in **Table 1**. The effective magnetic moment (μ_{eff}) values (5.23 & 5.31) of complexes suggest high spin state iron(II) [20].

Table 1: Physico-chemical properties of metal complexes.

S.No.	Complex	Molecular Formula	Mol.wt (Found)	M.P °C	μ_{eff} (BM)	ΛM^a
1	$[Fe(MAINH)_2]Cl_2$	$FeC_{30}H_{30}N_6O_4Cl_2$	664	>300	5.23	45.4
2	$[Fe(HMAINH)_2]Cl_2$	$FeC_{28}H_{30}N_6O_6Cl_2$	696	>300	5.31	57.6

^{a}Units: Ω^{-1} cm^2 mol^{-1} in DMF

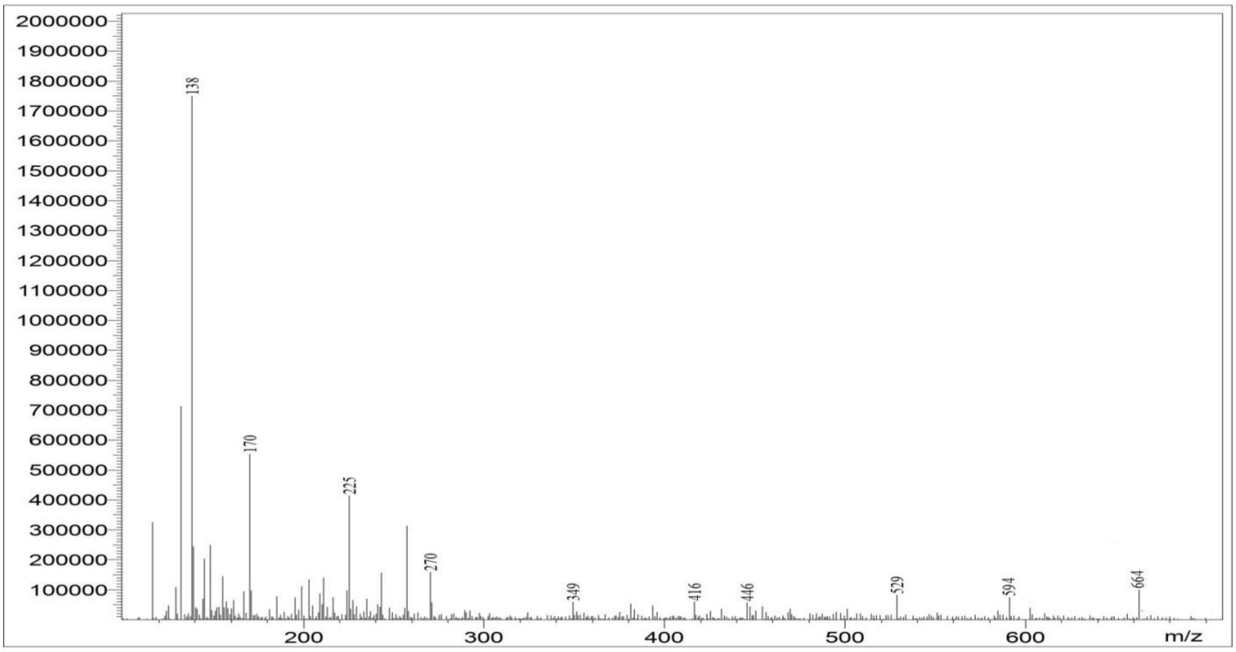


Figure 2: Mass spectrum of $[Fe(HMANH)_2]Cl_2$.

Electronic Spectra

The electronic spectrum of $[Fe(HMANH)_2]Cl_2$ is shown in **Figure 3.** The electronic spectra of iron complexes show a sharp and intense peak in the 33047-32165 cm^{-1} region due to π -π^* transition. One medium intensity band is observed in the range 20958-21364 cm^{-1} due to metal to ligand charge transfer transition (MLCT). A weak peak is observed in the 11053-11341 cm^{-1} region due to the $^5T_{2g} \rightarrow {}^5E_g$ transition suggesting octahedral structure [21] for the complexes.

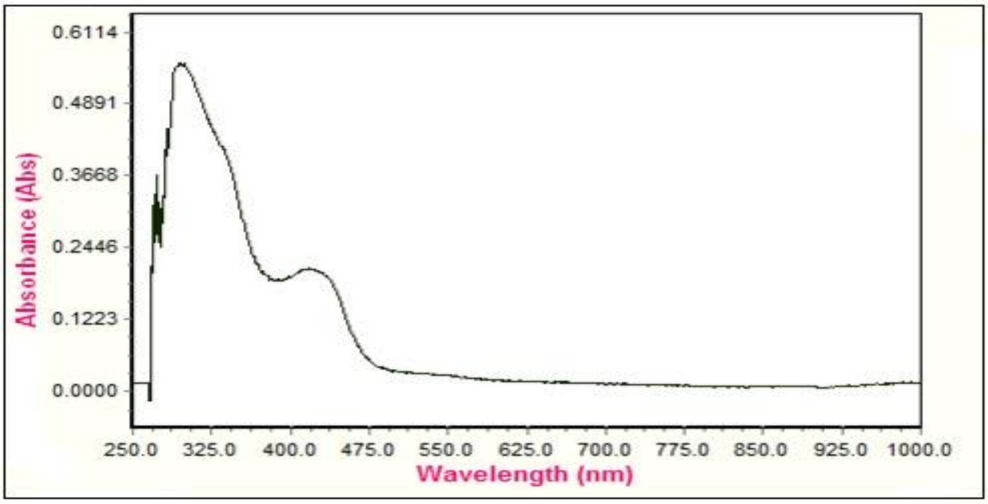


Figure 3: Electronic spectrum of [Fe(HMAINH)$_2$]Cl$_2$.

Table 6. 2: Electronic Spectral data(cm^{-1}) of Fe(II) complexes.

Complex	Bands(cm^{-1})	Assignments
[Fe(MAINH)$_2$]Cl$_2$	33047	π-π^*
	26218	n-π^*
	20958	MLCT
	11053	$^5T_{2g} \rightarrow {}^5E_g$
[Fe(HMAINH)$_2$]Cl$_2$	32165	π-π^*
	27203	n-π^*
	21364	MLCT
	11341	$^5T_{2g} \rightarrow {}^5E_g$

IR Spectral data

[Fe(MAINH)$_2$]Cl$_2$: In the IR spectra of ligands a strong band is observed in the region of 1672 & 1730 cm^{-1} which is assigned to ν(C=O)group. In the IR spectra of complexes this peak is shifted to lower wave numbers suggesting the involvement of >C=O group in chelation. The C=N (imine) vibration is observed in the range of 1612 cm^{-1} in the IR spectra of ligands. This band is shifted to lower or higher wave number in IR spectra of complexes suggesting the participation of azomethine nitrogen atom in complexation with metal ion. Bands are observed in 1291-1232 cm^{-1} & 1052-1022 cm^{-1} in the spectra of ligands and due to symmetric and asymmetric vibrations of C-O-Me group. These bands are shifted to lower wave number in IR spectra of the complexes suggesting the participation of (C-OMe) in chelation with Fe(II) ion.

Table 4: IR spectral data of MAINH & HMAINH ligands and their iron complexes.

MAINH	[Fe(MAINH)$_2$] Cl$_2$	HMAINH	[Fe(HMAINH)$_2$] Cl$_2$	Assignment
-	-	3408	3465	O-H str in
3246	3013	3079	3021	N-H str
2884	-	2961	-	Aliphatic sp^{3}C-H str
1672	1651	1730	1675	C=O str
1612	1602	1612	1603	Azomethine C=N str
1578 1468	1564 1431	1554 1435	1563 1453	Aromatic (C=C) skeleton
1358	1346	1318	1339	Aromatic C-H bending
1291 1022	1257 1038	1232 1052	1219 1039	C-O-Me sym.str. C-O-Me asym.str.
1159 1098	1151 1072	1155 1085	1152 1082	Aromatic C-H bending
853	861	841	856	C-H out of plane bending
756 671	765 668	771 692	772 668	C-C out of plane bending

Cyclic voltammetry

The electrochemical experiments on Fe(II) complexes were performed by using cyclic voltammetry in DMF with TBEP as supporting electrolyte. The electrochemical data of iron(II) complexes are given in **Table 5.** The data revealed that iron(II) complexes has single cathodic wave, corresponding to one electron Fe(II)→Fe(I). The separation(ΔE = 207-267 mV) between cathodic and anodic peaks indicates quasi-reversible character. The potential difference [$\Delta Ep = Ep_c - Ep_a$] for the complexes exceeds the Nerstian requirement of 59/n mV (n = number of electrons involved in oxidation-reduction) which suggests quasi-reversible character of the electron transfer reaction.

Table 5: Cyclic voltammetric data of metal complexes.

Complex	Redox couple	E_{pc}V	E_{pa}V	ΔE (mV)	$E_{1/2}$	logK_c^a	$-\Delta G^{\circ b}$
Fe(HMANH)$_2$	II/I	-0.828	-0.621	207	-0.724	0.187	1073
Fe(MAINH)$_2$	II/I	-0.948	-0.681	267	-0.814	0.125	717

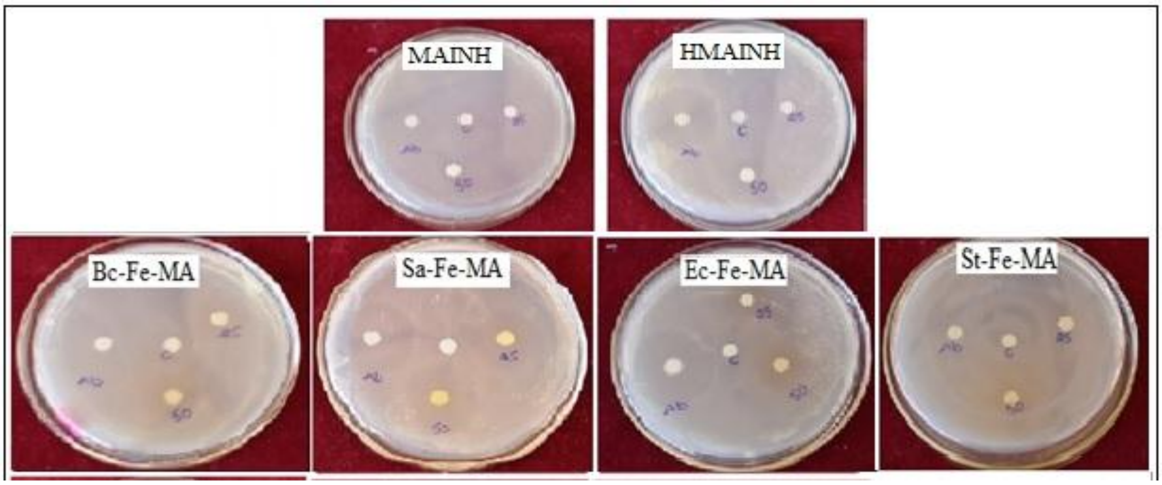
^{a}log $K_c = 0.434ZF/RT\Delta E_p$; $^b\Delta G^o = -2.303RT\log K_c$.

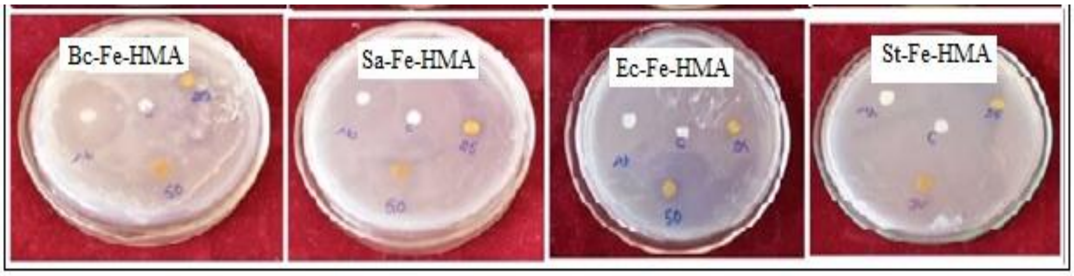
Antibacterial activity

To determine the antibacterial activity of ligands and their Fe(II) complexes, the disc diffusion method was used, with s- Ciprofloxacin as the standard antibiotic. The prepared compounds were tested against *Escherichia coli(-Ve), Salmonella typhi(-Ve), Staphylococcus aureus(+Ve)* and *Bacillus cereus(+Ve)* and *bacteria species* microorganisms. Inhibition zone data are given in **Table 6**. The ligands and their complexes have a less activity against the *E. coli*. The ligands and their metal complexes showed a reasonable result against the growth of S. *Aureus.* A comparative account on the effect of antibacterial activity against the chosen microbes indicates that the ligands and three metal chelates show reasonable activity against *S. Aureus* and *B. Cereus* while the growth of other organisms is moderately inhibited by the ligands and complexes**.** The complexes showed higher antibacterial activity than corresponding ligands**.** Antibacterial activity of standard, ligands and iron complexes are shown in **Figure 4.** The increased activity of metal complexes maybe may be understood on the basis of ***Overtone's concept*** and ***Tweedy's Chelation theory***[22,23]. Graphical representation of Antibacterial activity of the ligands and their complexes is shown in **Figure 5**.

Table 6: Antibacterial activity of ligands and their iron complexes.

Complex	Treatment	Bacterial growth inhibition zone(in mm)			
		B. Cereus	S. Aureus	E.Coli	S.Typhi
s-Ciprofloxacin	5µg/µL	12.56	10.21	10.11	11.63
MAINH	25 µg/µL	0.85	0.72	0.62	0.56
	50 µg/µL	1.39	0.93	0.64	0.79
[Fe(MAINH)$_2$]Cl$_2$	25 µg/µL	2.82	2.65	2.48	2.73
	50 µg/µL	3.70	3.11	2.62	2.98
HMAINH	25 µg/µL	0.73	0.59	0.81	0.79
	50 µg/µL	0.85	0.92	0.90	1.26
[Fe(HMAINH)$_2$]Cl$_2$	25 µg/µL	3.55	2.77	3.18	2.82
	50 µg/µL	4.82	3.55	3.59	3.38





Bc-Bacillus Cereus, Sa-Staphylococcus Aureus, Ec- Escherichia coli, Sa-Salmonella Typhi, Cu-Copper, HMA-HMANH, MA-MAINH,

Figure 4: Photographs of showing the anti-bacterial activity of metal complexes.
(a) B.Cereus (b) S. Aureus (c) E.Coli (d) S. Typhi.

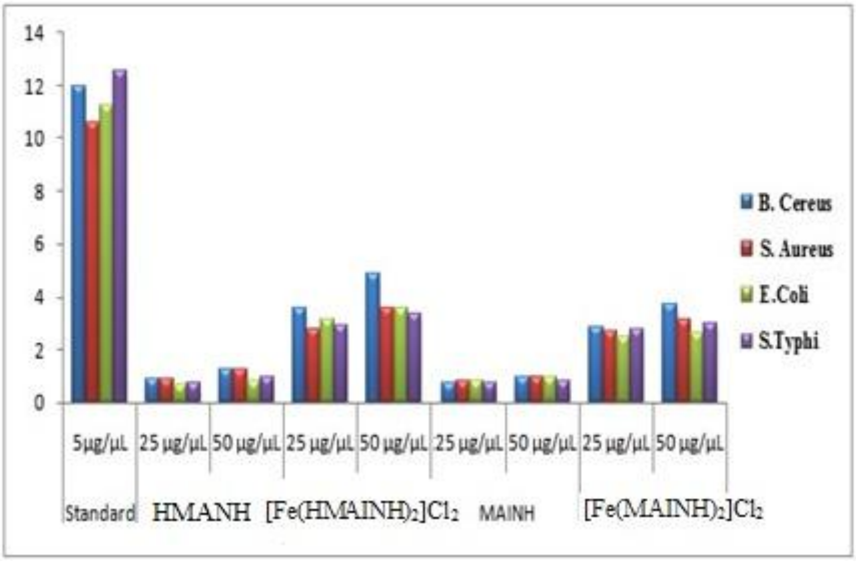


Figure 5: Graphical representation of Antibacterial activity of ligands and their iron(II) complexes.

CONCLUSIONS

Iron(II) complexes of isonicotinoylhydrazones bearing methoxy group have been synthesized based on physico-chemical and spectral studies. Physico-chemical and spectral studies revealed that the complexes have general formula $[FeL_2]Cl_2$ for MAINH & HMAINH ligands(where L=Hydrazone). Ligands act as neutral tridentate ligands. Electronic spectral data suggest that the complexes have octahedral geometry. A comparative account on the effect of antibacterial activity against the microbes indicates that the complexes showed higher antibacterial activity than the ligands. Metal complexes showed considerable activity against *Staphylococcus Aureus* and *Bacillus Cereus*. The highest antibacterial action(4.82mm) has been observed for $[Fe(HMAINH)_2]Cl_2$ complex against Bacillus Cereus.

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