SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF IRON (III) COMPLEX WITH β- SITOSTEROL

YASMEEN BIBI[1], TALAT MAHMOOD[1], IFFAT MAHMOOD[1], NASEEM SHAH[2], FARAH KISHWAR[1], ANEELA WAHAB[1] AND SIKANDAR KHAN SHERWANI[3]

[1]Department of Chemistry, [2]Department of Physics, [3]Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan

Abstract

The complexes of Fe (III) have been synthesized by pH- metric titration using β- sitosterol as a ligand. β-sitosterol reacted with Iron chloride in 1:1, 1:2, 1:3 and 1:4 molar ratio to give metal complexes of the general formula $[ML_3X_3]$ (M = Fe (III) , L = ligand and X = Cl^-). The complexes were characterized by analytical and spectral techniques. The analytical and spectral data suggested octahedral geometry. The data from antimicrobial activity showed that the metal complexes are more effective as equated to the ligand. The complexes showed a wide-range antibacterial activity against both gram-positive and gram-negative bacterial strains as well as significant antifungal activity against the applied fungal strains. Hence the synthesized complexes displayed remarkable antimicrobial activity as compared to ligand.

Introduction

Current research exposed significant development in the usage of transition metal complexes as drugs for cure of numerous human diseases such as inflammation, cancer, diabetes, lymphomas, and neurological disorders (Shazia, *et al*., 2010; Sanjay *et al.,* 2009]. A recent study showed that pepto-bismol (bismuth subsalicylate) has been found active for the cure of diarrhea, heart burn, nausea and stomach upset (Revanasiddapa *et al.,* 2010). In another study cisplatin [cisdiammine-dichloroplatinum (II)] has been used for the control of tumors (Shazia, *et al*., 2010). Metal complexes like platinum (II) porphirins, titanocenedichloride, ruthenium (III) imidazole, ferrocenes and many organometallic analogues have shown anti-cancer activity (Shazia *et al*., 2010; Oguniran *et al*., 2008; Alina and Anacona, 1998; James and Price, 2004). Silver (I) sulfadiazine, an anti-bacterial metal complex is found to be active for the cure of burns and for the control of malaria. Iron (III) complexes of quinoline have been used for the treatment of malaria (Shalita, 1989; Pathel, 2006). The complexes of gold like aurothioglucose, aurothiomalate and aurothiopropanol have been used as anti-inflammatory and anti-arthritic agents. Copper and iron complexes are found effectual for catalyzing dismutation of the superoxide anion (Shazia, *et al*., 2010). Manganese (II) complex of biscyclohexylpyridine substituted macrocyclic ligand was effective to cure cell and tissue oxidative wounds by acting as superoxide anion scavenger (Shazia, *et al*., 2010; Patel and Petal, 2010). The complexes of vanadium and chromium significantly relieve glycaemia in patients with diabetes (Anyakulu and Prabhakara, 1986).

In this study we are reporting the synthesis and characterization of Fe (III) complexes with β-sitosterol (ligand).The intense interest in synthesis of metal complexes depends on the fact that β-sitosterol is a naturally occurring plant sterol (Budavari, 1996; Drumm *et al.,* 1990; Huang *et al.,* 1991; Oka *et al.,* 1973; Pyle *et al.,* 1976; Thorpe, 1972; Turchetto *et al.,* 1993; Morton *et al.,* 1995) and has rich biological activities (Beckstrom and Duke, 1997; Chandler *et al.,* 1979; Hac-Wydro, 2013; Kim *et al.,* 2014; Tao *et al.,* 2013). It is a fact that the activity is usually enhanced upon metal complexation (Shazia, *et al*., 2010; Perez *et al.,* 2009). The structures of synthesized complex was confirmed by elemental analysis and spectral studies and the complexes were screened for their antimicrobial activity.

Materials and Methods

Reagent and glassware: All the reagents were used of analytical grade, purchased from Bio Basic Inc and Merck. All glassware used was of standard quality. For pH-metric study Iron (III) chloride was used.

Instrumentation: Denver Instrument, TP- 214 was used for weighing. Jenway, model 3510 was used for pH metric titration. For stirring the solution hot plate stirrer (lab Tech) with bead was used. For the separation of solvent from complexes, Rota Vapor R-210, BUCHI (Switzerland) was used. Melting points were determined on Gallen Kamp apparatus and were uncorrected. Perkin Elmer (2400 CHN) elemental analyzer was used for elemental analysis. UV/Visible spectra were recorded on Shimadazu (UV/Visible 1800) spectrophotometer.

Experimental

pH metric titration: pH metric titration was done at 27 ± 5^0C. Before titration of sample solution sodium hydroxide (NaOH) solution was standardized using oxalic acid.

pH metric titration of ligand (β-sitosterol): For this purpose 40 mL of βs solution (10^{-2}M) in chloroform and 10mL of chloroform were taken and then purified nitrogen gas was purged in this solution. After that βs solution was titrated against 0.1 M standard NaOH solution. pH values were plotted against the added volume of standard NaOH.

pH metric titration of Fe (III) with β-sitosterol: In order to get metal-ligand complex pH metric titration of Fe with β-sitosterol was done. To obtain 1:1 metal ligand solution, 10mL of metal solution (10^{-2}M) and 10mL of β-sitosterol solution (10^{-2}M) were mixed. The mixture obtained was subjected to titration by using (0.1M) standard NaOH solution. The change in color at different pH confirmed the formation of complex. The above method is likewise repeated for 1:2, 1:3 and 1:4 metal ligand ratios.

Biological Assay

The biological activities of βs and its complexes have been studied for their antibacterial and antifungal activities using agar-well method (Perez, *et al.,* 2009; Wuthi-udomlert *et al.,* 2011). For screening of antibacterial and antifungal activity 10mg/mL of sample was used. All plates were incubated at 28 ± 2^oC for 24-48 h and after incubation diameter of the zone of inhibition was noted by vernier caliper. For antibacterial activity Gentamicin was used as a standard and for antifungal activity Gresiofulvin was used as a standard.

Determination of Minimum inhibitory concentration (MIC):
Minimum inhibitory Concentration (MIC) was determined by Micro broth dilution method using 96-well microlitre plate (Smyth *et al.,* 2008; Vaghasiya *et al.,* 2000). Two fold serial dilutions of extracts was made in 100 µl broth and subsequently 10 µl of two hour refreshed culture matched with 0.5 Mac Farland index was added to each well. One well served as antifungal agent control while the other are served as culture control. Microtitre plate was incubated for 24 hours at 37 ºC. The MIC was read as the well showing no visible growth.

Results and Discussion

pH metric titration: As a reference titration of β- sitosterol (βs) with standard NaOH was performed (Fig. 1). In the plot of pH against volume of NaOH added, near pH 12.2 only one curve was observed for ligand (βs) and notable changes in titration curves of βs and its complexes were observed (Fig.1), which specifies complexation among metal and βs. Complexation of βs with Fe was confirmed by a change in color from yellow to brown. In case of βs-Fe (1:1) at pH 10.08, βs-Fe (1:2) at pH 11.22, βs-Fe (1:3) at pH 11.31 and in βs- Fe (1:4) at pH 11.42 (Fig. 1) change in color was observed. With the change of ratio there is no change in the pattern of pH graph. Physical parameter of the complexes are given in Table 1.

Species distribution graph: With the help of pH-metric data Species distribution curve was also plotted to find out best pH values for M, ML and ML_2 complexes (Bates, 1964). In βs –Fe complex no change in color takes place before pH 3.5 indicating presence of free metal or ligand but at pH 4.5 maximum percentage of ML seemed to be formed whereas maximum percentage of ML_2 species appeared at pH 10 (Fig. 2). Species distribution graph is helpful for the determination of favorable condition for the formation of sterol-metal complex.

Mole- Ratio method: Stoichiometry of metal-sterol complexes was studied by Mole-ratio method. This method is usually useful to study the number of ligands attached to metal in a given metal/ ligand environment. For this study stock solution of metal and ligand having 10^{-2} M concentration were used. To study mole ratio, solution of metal and ligand were prepared having metal to ligand ratios of 1:1,1:2,1:3,1:4,1:5,1:6,1:7,1:8,1:9, and 1:10. The absorbance of the complex is recorded at a particular wavelength (λ_{max}). In βs- Fe complexes maximum absorption takes place at 246 nm. The curve was plotted between mole ratios of metal to ligand verses absorbance. The straight line portions of obtained curve are extrapolated and the point of cross describe the metal to ligand ratio in the complex (Sawyer *et al.,* 1984).These mole-ratio were plotted against absorbance that showed ML_3 complexation between βs and Fe (Fig .3).

Uv Visible Spectroscopy: The UV data of ligand (βs) and its Fe complex is presented in Table 2. The ligand (βs) showed two major absorption peaks in the UV spectrum at 210 and 280nm.These band represent

$\pi \longrightarrow \pi^*$(47619.05 cm^{-1}) and n $\longrightarrow \pi^*$(35714.29cm^{-1}) transitions .These bands shifted to higher wavelength in βs-Fe complexes of each mole ratio. The complex βs – Fe (1:3) displayed characteristics absorption peaks at 246 nm (40650.41 cm^{-1}), 292 nm (34246.58 cm^{-1}), 344 nm (29069.77 cm^{-1}) and 580 nm (17241.38 cm^{-1}) (Fig. 4) and exhibited octahedral geometry (Fig. 5)

Table 1. Physical parameters of βs-Fe complexes.

Sample code	Color	Melting point ^oC	Yield (%)	pH	Elemental analysis (%) (Calcd)		Proposed formula
βs-Fe (1:1)	Brown	243	82	10.08	60.87 (60.83)	9.02 (9.05)	$[FeL_3](Cl_3)$
βs-Fe (1:2)	Brown	246	80	11.22	69.69 (69.61)	11.01 (11.03)	
βs-Fe (1:3)	Brown	242	87	11.31	74.69 (74.61)	10.95 (10.86)	
βs-Fe (1:4)	Brown	240	85	11.42	76.70 (76.68)	10.95 (10.90)	

Table 2. UV/Visible data of the complex.

S. No	Sample code	λ (nm)	ν (cm^{-1})	Band assignment	Geometry
1	βs	210	47619.05	$\pi \longrightarrow \pi^*$	Octahedral
		280	35714.29	$n \longrightarrow \pi^*$	
2	$\beta s - Fe$ (1:3)	246	40650.41	$\pi \longrightarrow \pi^*$	
		292	34246.58	$n \longrightarrow \pi^*$	
		344	29069.77	$^6A_{1g} \longrightarrow {}^4T_{2g}(G)$	
		580	17241.38	$^6A_{1g} \longrightarrow {}^4T_1(G)$	

Table 3. Antibacterial potential of βs, βs -Fe (1:1, 1:2, 1:3 and 1:4) against gram positive bacteria.

Gram positive bacteria	Zone of inhibition in mm (mean± S.D)					
	βs	βs-Fe(1:1)	βs-Fe(1:2)	βs-Fe(1:3)	βs-Fe(1:4)	Standard Genta
Bacillus cereus	-	-	-	-	-	15
Bacillus subtilis	-	-	-	-	-	15
Bacillus thruingiensis	-	-	-	-	-	15
Corynebacterium diptheriae	14±2	22±2	20±0	26±0	-	28
Corynebacterium hofmanii	12±2	25±0	25±1	25±0	-	28
Corynebacterium xerosis	11±1	20±1	22±0	27±2	-	30
Staphylococcus epidermidis	16±1	-	-	19±0	-	25
Streptococcus saprophyticus	18±1	-	-	29±1	-	30
Staphylococcus aureus	-	-	-	-	-	15
Staphylococcus aureus AB 188	-	-	-	-	-	15
M. smegmatis	-	-	-	-	-	15
Streptococcus fecalis	-	-	-	21±1	-	25
Streptococcus pyogenes	17±0	-	-	-	-	15

Table 4. Antibacterial potential of βs, βs -Fe (1:1, 1:2, 1:3 and 1:4) against gram negative bacteria.

Gram negative bacteria	Zone of inhibition in mm (mean± S.D)					
	βs	βs-Fe(1:1)	βs-Fe(1:2)	βs-Fe(1:3)	βs-Fe(1:4)	Standard Gentamicin
Enterobacter aerogenes	12±0	-	-	28±1	-	30
Escherichia coli ATCC 8739	20±1	-	-	29±1	-	30
Escherichia coli	19±1	-	-	19±1	-	25
E. coli multi drug resistance	12± 0	-	-	17±0	-	20
Klebsiella pneumoniae	11±2	15±1	14±1	18±2	-	20
Salmonella typhi	10±2	19±0	12±1	22±0	-	25
Salmonella paratyphi A	-	-	-	-	-	15
Salmonella paratyphi B	-	-	-	-	-	15
Shigella dysenteriae	-	-	-	-	-	15
Serratia marcesens	12±1	20±2	17±1	24±2	-	28
Acinetobacter baumanii	14±0	16±2	19±2	17±2	-	20
Campylobacter jejuni	12±1	-	-	-	-	15
Campylobacter coli	16±1	-	-	-	-	15
Helicobacter pylori	-	-	-	-	-	15
Hemophilus influenzae	-	-	-	-	-	15
Vibrio cholerae	14±2	-	-	-	-	15
Aeromonas hydrophila	-	-	-	-	-	15
Proteus mirabilis	-	-	-	-	19±1	25
Pseudomonas aeroginosa	12±2	-	-	-	12±2	20
Pseudomonas aeruginosa ATCC	-	-	-	-	15±1	20

Table 5. Minimum Inhibitory Concentration (MIC) of βs and sterol-metal complexes of gram positive bacteria and gram negative bacteria.

Extract / *Bacteria*	*MIC (mg/mL)*				
	βs	βs-Fe (1:1)	βs-Fe (1:2)	βs-Fe (1:3)	βs-Fe (1:4)
Gram positive bacteria					
Corynebacterium diptheriae	95	78	88	68	-
Corynebacterium hofmanii	92	10	42	22	-
Corynebacterium xerosis	100	24	44	18	-
Staphylococcus epidermidis	95	-	-	78	-
Streptococcus saprophyticus	92	-	-	10	-
Streptococcus fecalis	-	-	-	72	-
Gram negative bacteria					
Klebsiella pneumoniae	-	15	66	112	-
Acinetobacter baumanii	-	36	110	114	-
Serratia marcesens	-	94	86	52	-
Salmonella typhi	100	46	58	72	-
Proteus mirabilis	-	-	-	-	72
Pseudomonas aeroginosa	-	-	-	-	64
Pseudomonas aeruginosa ATCC	-	-	-	-	85
Enterobacter aerogenes	95	-	-	84	-
Escherichia coli ATCC 8739	90	-	-	70	-
Escherichia coli	100	-	-	76	-
E. coli multi drug resistance	95	-	-	92	-

Table 6. Anti –Fungal activity of βs, βs -Fe (1:1, 1:2, 1:3 and 1:4).

Yeasts	Zone of inhibition(mm)					
	βs	βs-Fe(1:1)	βs-Fe(1:2)	βs-Fe(1:3)	βs-Fe(1:4)	Standard Gresiofulvin
Candida albicans	-	20±3	27±1	24±2	-	30
Candida albicans ATCC 0383	-	24±1	24±0	26±1	-	30
Saccharomyces cerevisiae	-	-	-	-	-	12
Candida galbrata	8±0	21±1	23±1	20±1	-	25
Candida tropicalis	-	23±2	21±3	27±0	-	30
Candida kruzei	-	18±2	22±1	19±1	-	25
Dermatophytes						
Microsporum canis	7±1	12±2	12±2	12±2	-	15
Microsporum gypseum	-	-	-	-	-	12
Trichophyton rubrum	-	-	-	-	14±1	15
Trichophyton mentagrophytes	-	-	-	-	13±1	15
Trichophyton tonsurans	-	-	-	-	16±0	20
Saprophytes						
Aspergillus flavus	-	20±2	22±2	26±3	-	30
Aspergillus niger	-	16±2	15±1	16±0	-	20
Fusarium specie	-	10±2	12±1	13±1	-	15
Penicillium sp	8±1	16±2	15±2	18±3	-	20
Rhizopus	-	-	-	-	-	12
Helminthosporum	-	-	-	-	-	12

Table 7. Minimum Inhibitory Concentration (MIC) of βs and sterol-metal complexes against Yeast.

Yeasts	MIC (mg/ml)				
	βs	βs-Fe (1:1)	βs-Fe (1:2)	βs-Fe (1:3)	βs-Fe (1:4)
Candida albicans	-	92	90	72	-
Candida albicans ATCC 0383	-	22	94	62	-
Candida galbrata	100	88	90	80	-
Candida tropicalis	-	82	92	72	-
Candida kruzei	-	78	86	58	-
Dermatophytes					
Microsporum canis	100	120	120	120	-
Trichophyton rubrum	-	-	-	-	98
Trichophyton mentagrophytes	-	-	-	-	90
Trichophyton tonsurans	-	-	-	-	95
Saprophytes					
Aspergillus flavus	-	12	66	64	52
Aspergillus niger	-	60	-	32	64
Fusarium specie	-	70	-	48	-
Penicillium sp	100	76	-	86	-

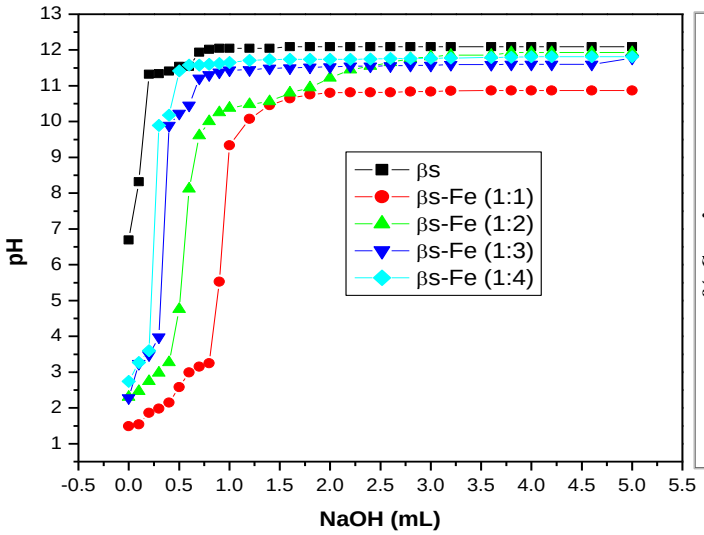


Fig.1. pH- metric plots of βs=ligand and βs-Fe (1:1, 1:2, 1:3 and 1:4) =Complex of βs with Iron.

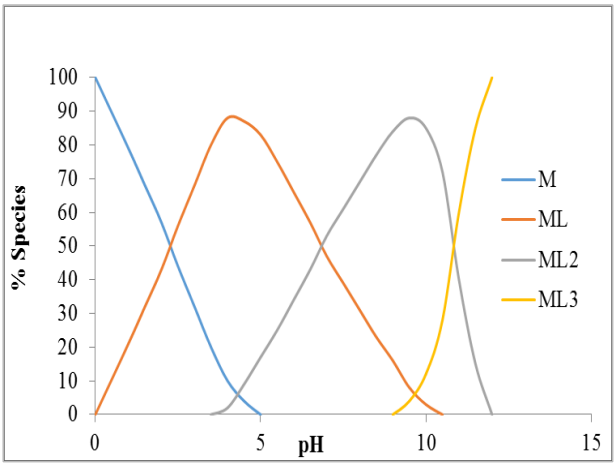


Fig.2. Species distribution graph of βs – Fe complex.

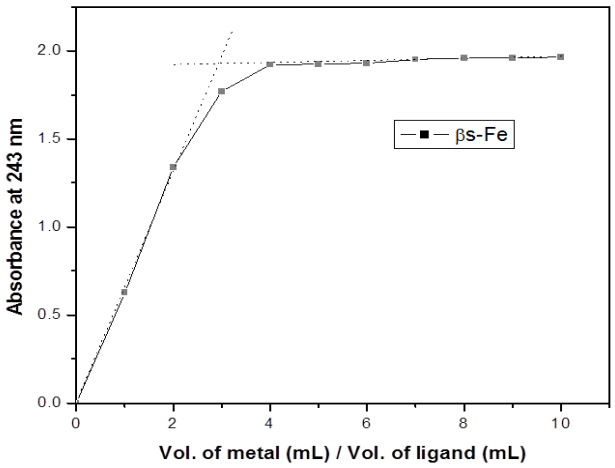


Fig.3. Mole ratio plot of βs – Fe complex.

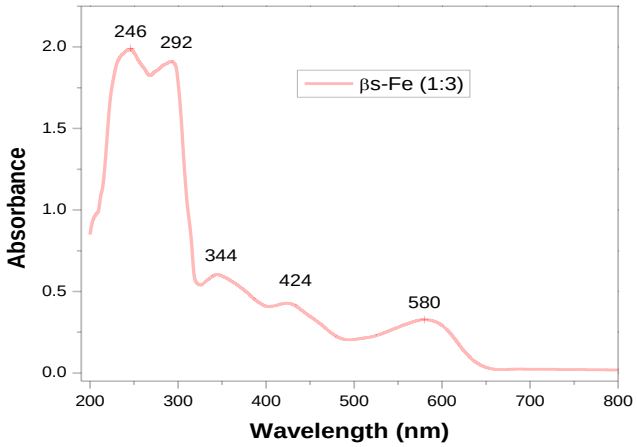
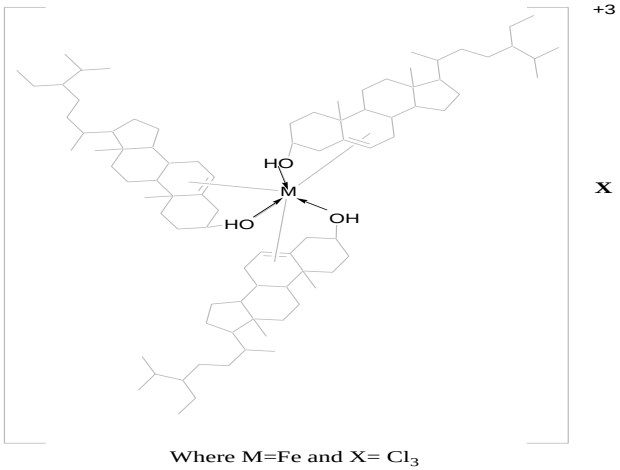


Fig. 4. UV/Visible spectra of βs-Fe (III) =Complex of βs with Iron.



Where M=Fe and X= Cl_3

Fig. 5. Proposed structure of βs – Fe complex.

Biological activity

Anti-bacterial activity: The ligand βs is found to be effective against three fungal strains *C. galbrata, M. canis, P. sp* with zone of inhibition 8, 7and 8 mm respectively (Table 3). The complex βs–Fe (1:1, 1:2 and 1:3) was observed active against *C. diptheriae, C. hofmanii, and C. xerosis* with zone of inhibition ranging between 19-29mm in gram positive bacteria. The complex βs-Fe (1:4) was the only one that was found inactive against gram negative bacteria. Whereas the complex βs-Fe (1:1, 1:2 and 1:3) were found to be most effective against *K. pueumoniae, S. typhi, S. marcesens* and *A. baumanii* with zone of inhibition range between 12-24mm while the complex βs-Fe (1:2) showed significant activity against *E. aerogenes , E. coli ATCC 8739 , E. coli, E. coli multi drug resistance* with zone of inhibition ranging between 17-29 more ever the complex βs-Fe (1:4) was found effective against *P. mirabilis, P. aeroginosa* and *P. aeroginosa* ATCC with zone of inhibition 19,12 and 15 respectively in gram negative bacteria (Table 4). It has been observed that in βs–Fe (1:2) highest MIC 88mg/mL exist against *C. diptheriae* whereas βs–Fe (1:1) showed lowest MIC 10mg/mL against *C.hofmanii* in gram positive bacteria. The complex βs–Fe (1:3) showed highest MIC 76 mg/mL against *S. typhi* in gram negative bacteria while βs–Fe (1:1) showed lowest MIC 15 mg/mL against *K. pneumonia* (Table 5).

Anti-fungal activity: The complex βs-Fe (1:1, 1:2 and 1:3) displayed antifungal activity against *C. albicans, C. albicans ATCC 0383, C. galbrata, C. tropicalis* and *C. kruzei* with zone of inhibition range between 18- 27 in yeast whereas in dermatophytes βs-Fe (1:1, 1:2 and 1:3) complexes showed moderate activity against *M. canis* with zone of inhibition 12mm and βs- Fe (1:1, 1:2 and 1:3) complexes showed excellent activity against *A. flavus , A. niger, F. specie* and *P. sp* with zone of inhibition ranging between 10 -26mm in saprophytes (Table 6). In yeast the highest MIC 94mg/mL was found in βs-Fe (1:2) against *C. albicans ATCC 0383*, whereas βs-Fe (1:1) showed lowest MIC 22mg/mL against *C. albicans ATCC 0383.* The complex βs-Fe (1:1, 1:2 and 1:3) showed MIC 20m g/mL against *M.canis* in dermatophytes. The complexes βs-Fe (1:3) have highest MIC value 86mg/ml against *P. sp* while βs-Fe (1:1) showed lowest MIC 12mg/ml against *A. flavus* in saprophytes (Table 7).

Conclusion

Iron (III) complexes were synthesized using β- sitosterol as a ligand and their anti-microbial activities were also assessed. The pH-metric titration showed the best pH for the formation of complex, mole ratio method justified that the complexation occurs in 1:3 mole ratio. UV /Visible data helped to predict the proposed structure of the complex. Antimicrobial activity of the complexes showed that the complex in 1:3 mole ratio had remarkable antibacterial and antifungal activities against the tested organisms.

The results of antimicrobial activity showed that all the tested organisms were much more inhibited by the complexes as compared to ligand giving the opportunity for their use in medical practice. Our work contributes to develop these metal complexes as potent antibacterial and anti-fungal agent against a wide range of bacterial and fungal strains. It can be further used as an antibacterial agent and antifungal agent in pharmaceutical industry for mankind, after testing their toxicity on human beings. However, still more scientific evaluation and clinical trials are required to establish its therapeutic efficacy.

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