Antimicrobial Activity of Synthesized Ferrocyanides from Transition Metals

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Abstract

Transition metal complexes of V(II) Cr (II), Zr (II), Ag (II) and Cd (II) were synthesized and characterized by X-ray diffraction analysis. Antimicrobial potential of these complexes with specified fungus and bacteria have been evaluated. Antibacterial screening of these complexes has been carried out against Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Shigella flexneri, Pseudomonas aeruginosa and antifungal screening of these complexes have been carried out against Byssochlmys fulva, Aspergillus Niger, Aspergillus fumigatus. Silver ferrocyanide and Cadmium ferrocyanide have shown higher inhibitory effect against Staphylococcus aureus with 32mm and 30mm inhibition zone respectively. In antifungal activity also Silver ferrocyanide and Cadmium Ferrocyanide has shown maximum inhibition zone with 29mm and 25mm diameter respectively against Aspergillus Niger.

***Keywords**: Transition metal complexes, antibacterial and anti-fungal activity*

1. Introduction

Now a days the antimicrobial activity of the established drugs is constantly reduced due to the resistance that bacteria develop throughout the years is the greater threat to public health worldwide. Few transition metal complexes of d- block element show higher antimicrobial activity against various bacteria and fungi compared to those of clinically used antibiotics. Several transition metal ions in the living organism work as enzymes or carriers in macrocyclic ligand field environment (Norman, 2011). Among all the hexacyanoferrates, of transition metals, nanoparticles of few metal transition series such as zinc, cobalt, chromium, iron, nickel and copper have significant attention of scientific fraternity due to their peculiar solid state represent in SEM analysis and structural attributes including chemical, photomagnetic, electro chromical, electro catalytical, ion exchanging and ion-sensing properties. Transition metals present in different oxidation states and can react with a various

number of negatively charged ions (Costamagna et al., 2000). The activity of transition metals has started the development of novel metal-based drugs in medical system having various pharmacological applications and may introduced in unique therapeutic opportunities in medical field (Tao et al., 2010). Therefore, vary meaningful research in this field might generate simple models for biologically occurring metallo-enzymes and thus the research in this field will help in developing our understanding of biological systems of various living organism (Sekhon, 2011). There is little citation on antifungal and antibacterial activity of transition metal ferrocyanides are reported in literature (Warra, 2011; Bharti, 2012).

Transition metals have an important place in the field of medicinal and biochemistry. The exploration of research has shown significant progress in use of transition metal complexes as drugs in medical field to treat various human and animal diseases (Kostova, 2006; Alessio, 2011). These complexes act as in various therapeutic agents in pharma industry and as antimicrobial agents (Sharma, 2020; Arora, 2012; Arora, 2012) Scientist shown significant progress report in utilization of metal complexes as drugs to treat various human diseases like cancer, tumor treatment, several neurological disorders, lymphomas and diabetes. In addition, properties hexacyanoferrates exhibit both ionic conductivity as well as redox properties which make them relevant in nano science and technology (Rafique et al., 2010, Alessio, 2011). Most of the transition metal complexes have a zeolitic structure which works as the diffusion of ions for maintenance of charge neutrality. Because of this quality, hexacyanoferrate do not undergo dissolution upon oxidation or reduction, thereby, making these compounds very useful for diverse applications in various field of science and technology (Thompson, 2011). The combination of more than one metal complexes leads to important compounds which have been found to have applications in various fields like sensor fabrication display technologies solid state batteries chemical precipitation of radioactive caesium from waste solutions and hydrogen storage. Transition metals are also capable of catalyzing the reduction of hydrogen peroxide, which is generated by redox enzymes during the chemical reaction (Hubin, 2003; Majed, 2009). Due to this unique property, they are widely used in the construction of amperometric biosensors. The various scientists worldwide have been focused on the unique properties of hexacyanometallates such as simplicity of manipulation of structures, occurrence of multi-redox centres the possibility of forming solid films, etc. (Malik, 1976; Tewari, 1996). Reduction of H_2O_2 is facilitated by the use of transition metal hexacyanoferrates because they decrease the kinetic barrier leading to lower operation potentials (Kamaluddin, 1994; Viladkar, 1996).

To find the possible role of transitional metal ferrocyanides as antimicrobial agent in medical and pharmacy industries, the present investigation has been undertaken to screen the complexes for their antibacterial and fungal activity.

2. Experimental

2.1 Synthesis of Metal Ferrocyanides

Five transition metal ferrocyanides namely Silver ferrocyanide, Chromium ferrocyanide, Cadmium ferrocyanide Zirconium ferrocyanide, Vanadium ferrocyanides were synthesized by the Kourim's procedure (Kourim, 1964). In this procedure a solution of potassium ferrocyanides (167ml, 0.1M) was added to solution of Vanadium pentaoxide, chromium

oxide, zirconium oxychloride, silver nitrate and cadmium chloride metal salt (500ml, 0.1M) with constant stirring. A slight excess of metal salt solution markedly improves the coagulation of the precipitate after that the reaction mixture was heated on a water bath at 80^{o}C for 3-4 hrs. The precipitate was filtered under vacuum and washed thoroughly with distilled water. It was dried in an oven at 60^{o}C. Colored powders thus obtained were quite stable in water. All the synthesized complexes were found to be insoluble in water. These were also been characterized on the basis of X-ray diffraction analysis.

2.2 X-ray diffraction analysis of metal complexes

X Ray Diffractometer was used for recording XR Diffraction patterns of transition metal complexes. Characterizations of all synthesized metal complexes were done by X ray diffraction studies. d values of the observed peaks of metal complexes are in good agreement with the published data (PC-PDF file no 46-0910, 23-0188,14-0291, 01-0244, 24-0164, and 01-0433).

2.3 Collection of Bacterial and Fungal Strains

Six bacterial strain Escherichia *coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Shigella flexneri, Pseudomonas aeruginosa* and three fungal strain *Byssochlmys fulva, Aspergillus Niger, Aspergillus fumigates u*sed in the present investigation were collected from Himachal Pradesh University Shimla.

2.4 Screening of metal complexes for antibacterial activity

Antimicrobial activity was tested by using paper disc method. Metal complexes formed and medicinal plant extracts chosen for present investigations (Perez, 1990)**.** Six bacteria for antibacterial activities with their respective antibiotics have been used for present investigation. A solution of Muller Hinton Agar for the growth of bacteria in Erlenmeyer flask and covered the flasks with cotton plug and wrapped them with aluminum foil. This medium was prepared by dissolving 38g of Muller Hinton Agar/1000ml of distilled water. The medium was autoclaved and poured into petri dishes. Then these flasks are autoclaved for 15-20 min. in an autoclaving machine. In the mean time we cleaned the petridishes and wrapped them in an autoclave bags. These petridishes were also autoclaved for 15-20 minutes. After 15-20min. when media for the growth of bacteria and petridishes have been autoclaved, we kept them in a cabinet, Laminar air flow, to avoid contamination. Firstly dried the petridishes in a cabinet then pour the solution of Muller Hinton Agar with the depth of 1.5mm in each petriplate. Let the solution to get solidify. Then these petridishes having Muller Hinton Agar were punchered with 6mm diameter vial. In each petriplate made five well, one in the centre and four in its surroundings with appropriate gaps between all of them. Then we spread the bacteria by J shape spreader in the entire petridishes one by one, having Muller Hinton Agar media. In each petridish we spread different bacteria. Then in each vials we pour 100µl solution of different ferrocyanides of metals except in the centre vials. Centre vial were dispensed with 100 µl of antibiotics with their respective bacteria as positive control (*Ampicillin, Gentamycin, Tetracycline, Ciprofloxacin*).

Plates were then incubated at 37^{o}C for 16 hours. The antibacterial activity was assayed by checking the diameter of the inhibition zone formed around the well.

Following antibiotics were used as positive control

Bacteria	Antibiotics used
Escherichia coli	*Ampicillin*
Salmonella typhi	*Ampicillin*
Staphylococcus aureus	*Gentamycin*
Bacillus cereus	*Tetracycline*
Pseudomonas aeruginosa	*Gentamycin*
Shigella flexneri	*Ciprofloxacin*

2.5 Screening of metal complexes for antifungal activity

Procedure for antifungal activity

Three funguses for antifungal activities with their respective antibiotics have been used for present investigation. A solution of Sabouraud agar for the growth of fungus in Erlenmeyer flask and covered the flasks with cotton plug and wrapped them with aluminum foil. Suspend 65 grams of Sabouraud Dextrose Agar in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes. Then these flasks are autoclaved for 15-20 min. in an autoclaving machine. In the meantime, cleaned the Petri dishes and wrapped them in an autoclave bag. These petridishes were also autoclaved for 15-20 minutes. After 15-20min. when media for the growth of fungus and Petri dishes have been autoclaved, kept them in a cabinet, laminar air flow, to avoid contamination. Firstly, dried the petridishes in a cabinet then pour the solution of Sabouraud Agar with the depth of 1.5mm in each Petri plate. Let the solution to get solidify. Then these petridishes having Sabouraud Agar were punchered with 6mm diameter vial. In each petriplate we made five well, one in the centre and four in its surroundings with appropriate gaps between all of them. Then we spread the fungus by J shape spreader in the entire petridishes one by one, having Sabouraud Agar media. In each petridish spread different bacteria. Then in each vials pour 100µl solution of different ferrocyanides of metals except in the centre vials. Centre vial were dispensed with 100 µl of antibiotics with their respective fungus as positive control. Plates incubated at 37ºC for 16 hours. The antifungal activities were assayed by checking the diameter of the inhibition zone formed around the well.

Following antibiotics were used as positive control for antifungal activity

Fungus	Positive control
Byssochlmys fulva	D.H_2O
Aspergillus Niger	D.H_2O
Aspergillus fumigatus	D.H_2O

3. Results and Discussion

The identification of crystalline materials and analysis of unit cell dimensions of metal ferrocyanides X-RAY diffraction technique is used. Ferrocyanide of Vanadium, Chromium, Zirconium, Silver and Cadmium were characterized by X-ray diffraction (XRD) analysis. D-

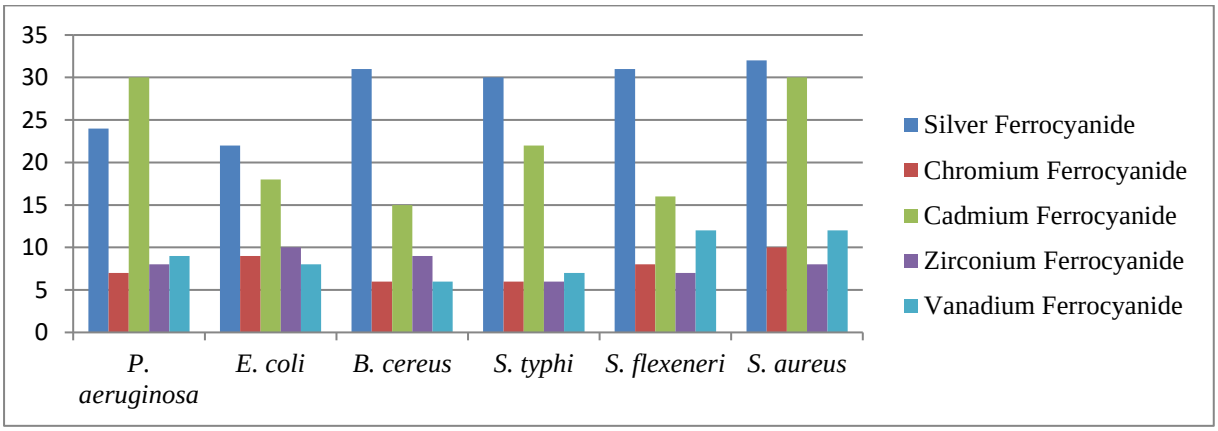
spacing (Å) observed for cadmium ferrocyanide 3.57042, 3.47058, 2.52951, 2.26396, 2.10968, 2.08891, 2.03121, 1.79215, 1.60399, 1.55485, 1.509, 1.4094 showed good agreements with the published data PC-PDF file respectively. The relative intensity was found to be 100 % in case of 2 θ (24.9184) angle in between the d spacing 3.57042and d-Spacing [Å] reported in PCPDF database 3.53031 respectively. D-spacing (Å) observed for chromium ferrocyanide was recorded as 62621, 3.33776, 2.66179, 2.47651, 181371, and 1.67112 which showed good agreement with the published data PC-PDF file. The relative intensity was found to be 100% in case of 2 θ (26.6864) angle in between the d spacing 3.33776 and d-Spacing [Å] reported in PCPDF database3.34661. The d-Spacing (Å) observed for silver ferrocyanide have been found to be 3.68278, 2.99063, 2.33616, 1.83933, and 1.72917 showed very closely resemblance to the data present in PC-PDF file. The relative intensity was found to be 100% in case of 2 θ (29.8519) angle in between the d spacing 2.99063 and d-Spacing [Å] reported in PCPDF database2.89061. The d-spacing (Å) values of vanadium ferrocyanide were recorded as 3.58382, 3.41280, 2.52913 and 1.79869 have been showed good agreement with the published data PC-PDF file. The relative intensity has been found to be 100% in case of 2 θ (35.4646) angle in between the d spacing 2.52913 and d-Spacing[Å] reported in PCPDF database2.52810. D-spacing (Å) observed for zirconium ferrocyanide3.53834, 3.12934, 2.50668, 2.21638, 1.81198 and 1.40459 showed good agreements with the published data PC-PDF file. The relative intensity was found to be 100% in case of 2 θ (28.5001) angle in between the d spacing 3.12934 and d-Spacing [Å] reported in PCPDF database 3.11829.

All five synthesized transition metal ferrocyanides have been screened for antibacterial activity against six bacterial strains namely *P.aeruginosa, E.coli, B.cereus, S.typhi, S.flexneri* and *S.aureus* . The results are displayed in (Table-1 and Fig. 1).

Table-1 Antibacterial activity of transition metal ferrocyanides

Name of metal ferrocyanide	**Inhibition zone in mm**					
	P.aeruginosa	***E.coli***	***B.cereus***	***S.typhi***	***S.flexneri***	***S.aureus***
Vanadium ferrocyanide	9	8	6	7	12	12
Chromium ferrocyanide	7	9	6	6	8	10
Zirconium ferrocyanide	8	10	9	6	7	8
Silver ferrocyanide	24	22	31	30	31	32
Cadmium ferrocyanide	3	18	15	22	16	30

Figure 1 Graphical representation of antibacterial activity of metal ferrocyanides

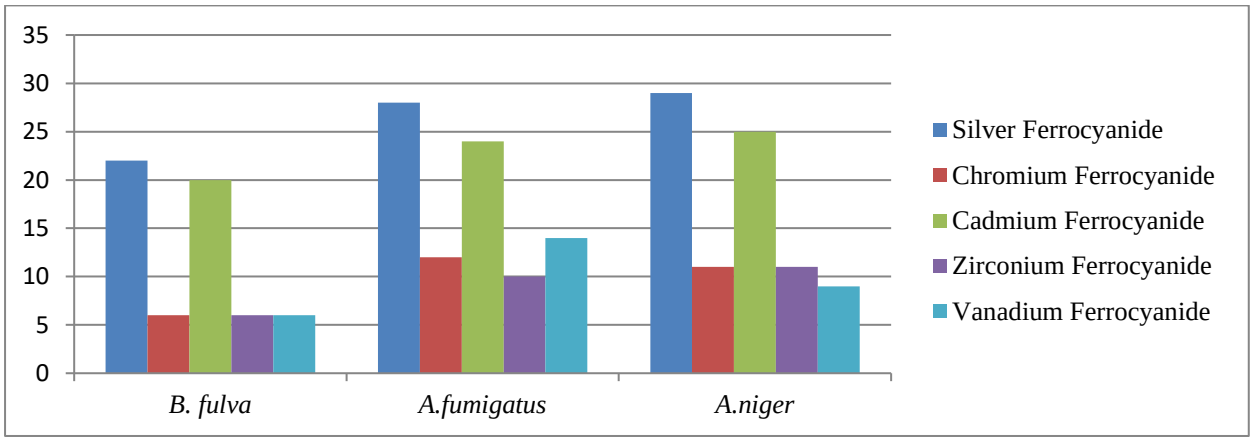


Silver ferrocyanide showed maximum bactericidal potential up to 22-32mm against all the bacterial strains taken for present investigation. While cadmium and Zirconium ferrocyanide have shown significant growth inhibition up to 3-30 mm against all the pathogens of bacteria. Vanadium ferrocyanide has been found to possess minimum antibacterial activity against all the test pathogens. Chromium ferrocyanide has ranging from 6-10mm significant activity against *P. aeruginosa, E. coli, B. cereus, S. typhi, S. flexneri* and *S. aureus* all test pathogens. The fungicidal activities of ferrocyanides of Vanadium, Chromium, Zirconium, Silver and Cadmium have been carried out.

Table-1 Antifungal activity of transition metal ferrocyanides

Name metal feerocyanides	**Growth Inhibition in (mm)**		
	B. fulva	***A. fumigatus***	***A. niger***
Vanadium ferrocyanide	6	14	9
Chromium ferrocyanide	6	12	11
Zirconium ferrocyanide	6	10	11
Silver ferrocyanide	32	28	29
Cadmium ferrocyanide	20	24	25

Figure 2 Graphical representation of antibacterial activity of metal ferrocyanides



It is found that Silver ferrocyanide and Zirconium ferrocyanide possess maximum and minimum fungicidal property against all the fungal strains taken in the present investigation. It is clear from (Table-2 and Fig.2) that the Silver ferrocyanide recorded maximum growth inhibition up to 32mm against *B. fulva* and also Cadmium ferrocyanide showed significant growth inhibition 20-25 mm against all the fungal strain taken for present investigation while Vanadium ferrocyanide and Chromium ferrocyanide showed antmycotic potential up to 6-14 mm against the same fungal pathogen

Table-3 Statistical Analysis of bacterial strains

Correlations							
Name of Bacteria		***P. aeruginosa***	***E. coli***	***B. cereus***	***S. typhi***	***S. flexneri***	***S. aureus***
P.aeruginosa	Pearson Correlation	1	.567	.810	.599	.821	.433
	Sig. (2-tailed) P-value		.319	.097	.286	.088	.467
	N	5	5	5	5	5	5
E.coli	Pearson Correlation	.567	1	.941*	.989**	.892*	.958*
	Sig. (2-tailed) P-value	.319		.017	.001	.042	.010
	N	5	5	5	5	5	5
B.cereus	Pearson Correlation	.810	.941*	1	.941*	.960**	.847
	Sig. (2-tailed) P-value	.097	.017		.017	.010	.070
	N	5	5	5	5	5	5
S.typhi	Pearson Correlation	.599	.989**	.941*	1	.934*	.977**

	Sig. (2-tailed) P-value	.286	.001	.017		.020	.004
	N	5	5	5	5	5	5
S.flexneri	Pearson Correlation	.821	.892*	.960**	.934*	1	.864
	Sig. (2-tailed)	.088	.042	.010	.020		.059
	N	5	5	5	5	5	5
S.aureus	Pearson Correlation	.433	.958*	.847	.977**	.864	1
	Sig. (2-tailed)	.467	.010	.070	.004	.059	
	N	5	5	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table-4 Statistical Analysis of fungal strains

Name of Fungus		**Correlations**		
		B. fulva	***A. fumigatus***	***A. niger***
B. fulva	Pearson Correlation	1	.967**	.973**
	Sig. (2-tailed)		.007	.005
	N	5	5	5
A. fumigatus	Pearson Correlation	.967**	1	.966**
	Sig. (2-tailed)	.007		.007
	N	5	5	5
A. niger	Pearson Correlation	.973**	.966**	1
	Sig. (2-tailed)	.005	.007	
	N	5	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

4. Statistical Analysis

Statistical analysis was performed using the statistical software SPSS Statistics IBM Base 22.0. Descriptive statistics, Shapiro-Wilk tests for normality and Sig. (2-tailed) P-value at 0.05 and 0.01 level applied in the present investigation. Understanding and description of the data was done through descriptive statistics and Shapiro-wilk test for confirming the assumption for the parametric methods. Sig. (2-tailed) P-value was applied to look the significance mean difference between all the bacterial strains. Value was taken in to consideration reported in (Table-3 and Table-4) shown significant (2-tailed) p-value in all the microbial strains taken for present investigation.

5. Conclusion

Establishment of resistance of antimicrobial agents of several microbial strain is the greatest challenges that scientists are facing now a days. Research should be continued to the development of novel compounds and evaluated against different microbial strains. The drugs based on metal are among the most promising complexes that can be used in that direction. These complexes have also been reported to adsorb biomolecules. This is because there may be the possibility of adsorption of active ingredient at the surface transitional metal ferrocyanides because of the presence of pores on the surface. Thus concentration, shelf life and biocidal activity of adsorbed active agents may increase and result in increased synergistic effect with various synthetic and natural compounds. These types of studies may be helpful in development of antimicrobial formulations against various harmful microorganisms.

6. References

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