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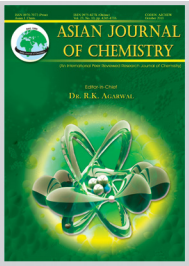

Antifungal Screening of Some Transition Metal Ferrocyanides Against *Aspergillus niger* and *Candida albicans*

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Synthetic and antimicrobial aspects of some clinical pathogen inhibitor transition metal ferrocyanides complexes were carried out. Ferrocyanides of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were synthesized by known Kourim's procedure. Antifungal screening of these metal ferrocyanides was carried out against *Aspergillus niger* and *Candida albicans* using paper disc and food poisoning technique. Cobalt ferrocyanides showed significant potential activity against *A. niger* (72 %) and cadmium ferrocyanide showed maximum growth inhibition against *C. albicans* (85 %) respectively.

Key Words: *Aspergillus niger*, *Candida albicans* and Transition metal ferrocyanides.

INTRODUCTION

Many transition metal ions in the living systems work as enzymes or carriers in macrocyclic ligand field environment. Therefore meaningful research in this direction might generate simple models for biologically occurring metallo enzymes[1] and thus will help in developing our understanding of biological systems. These ligands are also of theoretical interest as they are capable of furnishing an environment of controlled geometry and ligand field strength[2-5]. A literature survey reveal that a number of polydentate macrocyclic ligands and their metal complexes have been reported[6].

Transition metal ferrocyanides are well known for their ion exchange[7-9] catalytic and adsorption properties[10, 11]. There are few report on antitumor activity of some platinum complexes[12]. Studies on their antimicrobial properties have not been reported yet.

Keeping in view the importance of the subject some transitional metal ferrocyanides were examined for their antifungal activity.

EXPERIMENTAL

Collection of fungal culture: Two test organisms, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) were collected from Plant Pathology Laboratory, University of Rajasthan, Jaipur, Rajasthan. The fungal cultures (*A. niger and C. albicans* were maintained on Saboraud Dextrose Agar (SDA), incubated at 25 ºC. The inoculated medium was incubated at 25 ºC for 2 days for the *C. albicans* and 3 days for *A. niger*.

Synthesis of metal ferrocyanides: Manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were prepared following the Kourim's procedure[13]. A solution of potassium ferrocyanide (167 mL, 0.1 M) was added to solution of desired metal salt (500 mL, 0.1 M) with constant stirring at room temperature. A slight excess of metal salt solution markedly improves the coagulation of the precipitate. The reaction mixture was heated on a water bath at 80 ºC for 3-4 h and allowed to stand at ambient temperature for 24 h. The precipitate was filtered under vacuum and washed thoroughly with double distilled water. It was dried in an oven at 60 ºC. The dried product was ground and sieved to 100 mesh sizes. The coloured powers of metal complexes were stable in air. These were characterized on the basis of elemental analysis, IR spectroscopy and magnetic susceptibility measurement (Tables 1-3).

Screening of metal complexes for fungicidal activity: Paper disc method was used for initial screening of antifungal potential of metal complexes chosen for present investigations. This method was based on diffusion capacity of test chemical(s) through agar medium. Fungal plug were placed at the center of assay plate containing sterilized SDA and allowed to grow. After circular growth of about 2-3 cm diameter four sterilized paper disc (two loaded with 20 mL aqueous suspension of metal ferrocyanides and two with same amount of pure solvent) were placed at equal distance from center in order to see the effect of metal ferrocyanides on the growth of fungal pathogens. Inhibition zones were measured after 1 to 3 days of incubation depending upon the growth of pathogen. Dumb bell shaped growth of fungus was observed in case of metal ferrocyanides containing growth inhibitory component(s).

TABLE-1
ELEMENTAL ANALYSIS DATA OF METAL FERROCYANIDECOMPLEXES

Complexes	Elemental analysis (%): Found (calcd.)				
	Metal	Fe	C	H	N
$Mn_2[Fe(CN)_6].3H_2O$	28.56 (29.23)	14.66 (14.86)	20.67 (19.17)	1.69 (1.61)	22.59 (22.36)
$Co_2[Fe(CN)_6].2H_2O$	32.12 (32.22)	15.30 (15.27)	19.65 (19.70)	1.11 (1.10)	21.16 (22.97)
$Ni_2[Fe(CN)_6].5H_2O$	27.85 (27.93)	13.00 (13.28)	16.51 (17.14)	2.22 (2.30)	18.79 (19.19)
$Cu_2[Fe(CN)_6].7H_2O$	27.10 (27.32)	12.10 (12.01)	14.75 (15.49)	3.13 (3.03)	18.12 (18.07)
$Zn_2[Fe(CN)_6].3H_2O$	32.84 (32.95)	14.10 (14.08)	17.74 (18.16)	1.51 (1.45)	20.40 (21.18)
$Cd_2[Fe(CN)_6]$	50.12 (51.47)	12.58 (12.79)	17.71 (16.50)	0.26 (0.00)	20.38 (19.24)

TABLE-2
INFRARED SPECTRAL DATA OF METAL FERROCYANIDE COMPLEXES

Complexes	Adsorption frequencies (cm^{-1})				
	ν(HOH)	ν(C≡N)	HOH bending	ν(Fe-C)	ν(metal-N)
$Mn_2[Fe(CN)_6]\cdot3H_2O$	3701	2070	1631	592	451
$Co_2[Fe(CN)_6]\cdot2H_2O$	3724	2083	1609	592	465
$Ni_2[Fe(CN)_6]\cdot5H_2O$	3697	2091	1611	592	463
$Cu_2[Fe(CN)_6]\cdot7H_2O$	3845	2090	1621	592	503
$Zn_2[Fe(CN)_6]\cdot3H_2O$	3685	2080	1600	603	496
$Cd_2[Fe(CN)_6]$	3724	2071	1623	590	508

TABLE-3
MAGNETIC MOMENTS OF METAL
FERROCYANIDE COMPLEXES

Metal hexacyanoferrate(II)	μ_{eff} (BM) theoretical value	μ_{eff} (BM) experimental value
$Mn_2[Fe(CN)_6]\cdot3H_2O$	5.92	6.21
$Co_2[Fe(CN)_6]\cdot2H_2O$	3.87	4.36
$Ni_2[Fe(CN)_6]\cdot5H_2O$	2.83	2.99
$Cu_2[Fe(CN)_6]\cdot7H_2O$	1.73	2.45
$Zn_2[Fe(CN)_6]\cdot3H_2O$	0.00	0.81
$Cd_2[Fe(CN)_6]$	0.00	0.90

Food poisoning technique was used to find per cent inhibition. For this purpose 0.375 % (w/v) spread to each Petri-dish after pouring the sterilized medium, while in control treatment equal amount of pure solvent was added. The fungal plug was placed at the centre of Petri-dish. Growth of fungus was recorded after one to three days depending upon the growth of pathogen. The percent inhibition was calculated using the formula of Vincent[14].

$$\text{Inhibition (\%)} = (C\text{-}T)/C \times 100$$

where, C is the growth in control in mm and T is growth in treatment in mm. All the experiments were carried out in triplicate in randomized block design and average value was used for interpretation of results.

RESULTS AND DISCUSSION

Antifungal screening of metal ferrocyanides taken for present study and are synthesized has been reported in (Table -4).

The molecular formula of synthesized metal complexes established on the basis of data obtained from elemental analysis are $Mn_2[Fe(CN)_6].3H_2O$, $Co_2[Fe(CN)_6]\cdot2H_2O$, $Ni_2[Fe(CN)_6]\cdot5H_2O$, $Cu_2[Fe(CN)_6]\cdot7H_2O$, $Zn_2[Fe(CN)_6]\cdot3H_2O$ and $Cd_2[Fe(CN)_6]$ respectively.

In case of metal ferrocyanide cobalt and cadmium ferrocyanide have showed maximum growth inhibition 72-85 % against both the pathogens. Manganese and copper ferrocyanide were not effective against *A. niger* and both were showed activity up to 25-45 % against *C. albicans*. Zinc ferrocyanide was not showed any inhibition against *C. albicans* but it showed 20 % fungicidal potential against *A. niger*. Manganese and cobalt cause 25-30 % inhibitions against *C. albicans*. Nickel ferrocyanide was not exhibiting any activity against both the pathogens.

TABLE-4
ANTIFUNGAL SCREENING OF TRANSITION
METAL FERROCYANIDES

Metal ferrocyanides	*A. niger*		*C. albicans*	
	Inhibition zone (mm)	Inhibition (%)	Inhibition zone (mm)	Inhibition (%)
$Mn_2[Fe(CN)_6]\cdot3H_2O$	-	-	6	25
$Co_2[Fe(CN)_6]\cdot2H_2O$	17	72	7	30
$Ni_2[Fe(CN)_6]\cdot5H_2O$	-	-	-	-
$Cu_2[Fe(CN)_6]\cdot7H_2O$	-	-	10	45
$Zn_2[Fe(CN)_6]\cdot3H_2O$	5	20	-	-
$Cd_2[Fe(CN)_6]$	12	55	18	85

There are few reports on synergistic effect of antimicrobial activity of metal ferrocyanide with botanicals[15]. These complexes have also been reported to adsorb biomolecules[16,17]. There may be the possibility of adsorption of active ingredient at the surface transitional metal ferrocyanides. Thus concentration and shift life of active botanicals may increase and may result in increased activity (biopotentiation). Further studies in this direction are in progress.

The result lends credence to the folkloric use of these transition ferrocyanides in treating microbial infection and shows that ferrocyanides of cobalt, cadmium and copper could be exploited for new potent antifungal agents.

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