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SYNTHESIS & ANTIMICROBIAL ACTIVITIES OF HYDRAZO METAL COMPLEX LIGANDS

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ABSTRACT

The d-Block metal ions have tendency to form the complexes. A series of transition metal complexes with Schiff bases, aromatic hydrazones have been quite extensively investigated. The chemistry of hydrazone complexes involving O,N,S donor ligands has received special attention because of their coordination capability, their pharmacological activity and their uses in analytical chemistry as metal extracting agents. It has recently been shown that the metal complexes are more potent and less toxic in many cases as compared to the parent compound. Considering these aspects, the present investigation deals with the synthesis of Fe(II), and Co(II) metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6-methoxy-phenol (Scheme-1) and their characterization by analytical, spectral and thermal studies. Hydrazo metal complex ligands are a group of heterocyclic compoundswhich have attracted much attention as a result of their varied pharmacological properties which include antibacterial,anticoagulants, antibiotic, antifungal, anticancer, and anti inflammatory and share an important place in this regards. In the current research study, complexes are characterized by elemental analysis, IR, NMR and Chromatographic evaluation was done by Thin Layer Chromatography in order to check purity[1-4]. Antimicrobial efficacy of synthesized compounds was assessed against selective bacteria such as *Becillum* and *E. Coli*[9-11] and antifungal activity against *Fusarium oxysporum* and *T.* Reesei[12-14] by using ager well diffusion method[5-8]. Results concluded that as chelation increases the anti-microbial potency therefore synthesized Hydrazo metal complex ligands have significant antibacterial and antifungal activity in comparison with free ligands.

Keywords: Metal Complexes, Biological interest, Pharmacological interest, Antimicrobial.

INTRODUCTION

The chemical science of macrocyclic nitrogen and sulphur contributor ligands and their buildings with change metal particles has been an intriguing and captivating region of research action everywhere throughout the world since most recent couple of decades. The proceeded with enthusiasm to multiply basic curiosities of such edifices is because of their wide application in restorative, biochemical, bioinorganic, condition, modern and photochemistry. Before said buildings have gotten much consideration as of late by virtue of their sound plan and blend in coordination science in light of their potential wide applications as practical materials, enzymatic response instrument and in bioinorganic science.

In organic framework, the metal particles are coordinated to ligands as opposed to existing as free particles. Connections, for example, intercalation [insertion of a molecule (or ion) into layered solids], hydrogen bonds, electrostatic, Vander Waals compel, etc. broadly existing in them makes significant impact on natural procedures. Contingent upon their fixation they either contribute towards the wellbeing of creature or cause harmfulness.

Almost certainly, edifices of copper particle share vital application in above said regions of research. The chelation of metal particle with nitrogen and sulphur contributor moieties merits referencing [15-17].

Their expanding physiological significance and their dynamic job in co-appointment science makes energy to combine, portray and improve this class of mixes. Broad basic and Physico-Studies of such mixes are pivotal for expanding the comprehension of the auxiliary knowledge, kind of holding and electronic communications between proximate metal focuses and included ligands. For the equivalent, we continue with the technique of union and synopsis of antibacterial and antifungal affectability testing by Well diffusion method. The reason for the well diffusion plate dissemination weakness test is to decide the affectability or opposition of pathogenic vigorous and facultative anaerobic microorganisms to different antimicrobial mixes. The nearness or nonattendance of development around the circles is a backhanded proportion of the capacity of that compound to inhibit that organism.

Considering these aspects, the present investigation deals with the synthesis of Fe(II), and Co(II) metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6- methoxy-phenol (Scheme-1) and their characterization by analytical, spectral and thermal studies. Hydrazo metal complex ligands are a group of heterocyclic compoundswhich have attracted much attention as a result of their varied pharmacological properties which include antibacterial,anticoagulants, antibiotic, antifungal, anticancer, and anti inflammatory and share an important place in this regards.

MATERIALS AND METHODS

LR/AR grade chemicals and reagents were used in standard operating procedures.

Preparation of hydrazone

2-Hydrazino-4,6-dimethyl benzothiazole

Hydrazine hydrate (4 ml of 80%) was taken in round bottom flask and kept in freezing mixture. Concentrated hydrochloric acid (4ml) was added to it. On shaking and cooling the above mixture ethylene glycol (20ml), 2-amino-4,6-dimethyl benzothiazole (0.02 mole, 3.56 gms) was added. The mixture was refluxed for 2.5 hours at 150-160°C. The solution was cooled at room temperature. The crystalline product was obtained. (Yield 71%, m.p.175°C.)

2-**[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6-methoxy-phenol:(DBYHMP)**

(4,6-dimethyl-benzothiazol-2-yl)-hydrazine (7 g, 0.005mole) was mixed with o-hydroxy-mmethoxybenzaldehyde (5.608 g, 0.005mol) in ethanol. The mixture was placed in round bottom flask and refluxed on water bath for 1 h. and then allowed to cool at room temp. The resulting light brown colored precipitate was filtered, washed several times with ethanol (Scheme-2). (Yield 80%, m.p.= 200° C)

Preparation of the complex

[Fe(DBYHMP)2]: The complex was prepared by refluxing 1:2 molar mixture of ethanolic solutions of the ligand and ferrous sulphate for 1 h, maintaining pH (~8) by adding alcoholic ammonia. The sparingly soluble, pale brown product was separated and washed with ethanol and the complex was dried over anhydrous calcium chloride in vacuum and tested with TLC (yield=69%).Chemical analysis. Found: Fe 7.82; C 57.65; H 4.57; N 11.90; O 9.08; S 9.15 %. FeC34H32N6O4S2requires: Fe 7.88; C 57.63; H 4.55; N 11.86; O 9.03; S 9.05 %.

Antimicrobial susceptibility testing: Here we had used well diffusion method to determine the antimicrobial nature of complex. We had tracked following steps :

Culture and Maintenance of clinical isolates: Pure cultures of Staphylococcus aureus, *Bacillus subtilis, Escherichia coli, Fusariumoxysporum, Trichoderma reesei* obtained from Medical College, Jaipur, India was used as indicator organisms. Each culture was further maintained on the same medium after every 48 h

of transferring. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay: In vitro antibacterial activity of the samples was studied against gram positive and gram-negative bacterial strains by the agar well diffusion method (Perez et al, 1990). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay: Anti-fungal activity of the samples was investigated by agar well diffusion method (Bonjaret al, 2005). The yeasts andsaprophytic fungi were subculture onto Potato dextrose agar, PDA (Merck, Germany) and respectively incubated at 37°Cfor 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBSand adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of theagar medium. The plates were dried at room temperature for 15min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

RESULTS AND DISCUSSION

Biological activity is basically an articulation portraying the gainful or antagonistic effect of medication on living issue[26-30]. Developments of more up to date, less expensive and increasingly powerful analogs of particles with officially very much perceived organic exercises from a key piece of research in the pharmaceutical field. Realizing alterations by controlling the parent structures serves to upgrade the

movement of the intense analogs and dispenses with unfriendly impacts or poisonous quality related with the parent tranquilize is the orientated objective of present science. The examination depicted here is a stage to accomplish such an objective.

Activity Index = Zone of inhibition / zone of inhibition of reference

The Activity can be found by = Activity = Activity index × 10

We are discussing here the complex of Fe(II) metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6- methoxy-phenol (Scheme-1). Hydrazo metal complex ligands are a group of heterocyclic compounds.. The diameter of zone of inhibition is given and diameter of well is 6 mm. The enhanced biological activity of the macrocyclic complexes can be explained based on Overtone's concept and Tweed's Chelation theory. According to this theory, it has been suggested that coordination reduces the polarity of the metal ion to a greater extent because of partial sharing of the positive charge of the metal ion with donor groups within the chelate ring. Further, this coordination process also increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes which subsequently enhances the penetration through the lipid layer of cell membranes and blocking of the metal binding sites in the enzymes of microorganisms thus destroying them more aggressively.

These complexes also perturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism. In addition to this, many other factors, such as solubility, dipole moment, conductivity, stability and geometry of the complexes which are influenced by the metal ion may be the possible reasons for the antimicrobial activities of these metal complexes.

Most commonly such agents inhibit or kill the microbes by following basic mechanism:

- Transition metal complexes bind the microbes or their metabolites through N O S donor.
- Damage the cell wall or inhibit of cell wall synthesis alters alternation of permeability of cytoplasmic membrane.
- Alternation of permeability of cytoplasmic membrane.
- Alternation physical state of protein and nucleic acids.
- Inhibition of enzyme action.
- Substrate competition with essential metabolites.

Following graphs are the interpretations for the antimicrobial study for the synthesized complex:

The antimicrobial screening of the synthesized hydrazone metal Complex [Fe(DBYHMP)2] were performed against two pathogenic bacteria *Bacillus subtilis, Escherichia coli* and two fungi namely *Fusarium oxysporum, Trichoderma reesei*. Since DMSO was used as a solvent, it was also screened against all organisms. The diameter of zone of inhibition induced by DMSO was 9mm. The results of the investigated samples were summarized in **Table 1** and **Table 2**. The anti-fungal activity results revealed that synthesised complex showed less activity as compared to standard. The inhibition of the complex against the bacteria and fungi are in the order :-

Becillum > E. Coli ; Fusarium Oxysporum > T. Reesei

Table 1: Antibacterial sensitivity of synthesized complex against bacteria

S.no	**Microorganism**	**Complex (in mg/ml)**			**Standard (in mm)**
		30	**60**	**90**	
1.	*Becillum*(in mm)	08	13	16	20
2.	*E. Coli*(in mm)	05	08	12	20

Table 2: Antifungal sensitivity of synthesized complex against fungus

S.no	**Microorganism**	**Complex (in mg/ml)**			**Standard (in mm)**
		30	**60**	**90**	
1.	*Fusarium oxysporum*	NIL	08	13	20
2.	*T. Reesei*	08	11	15	20

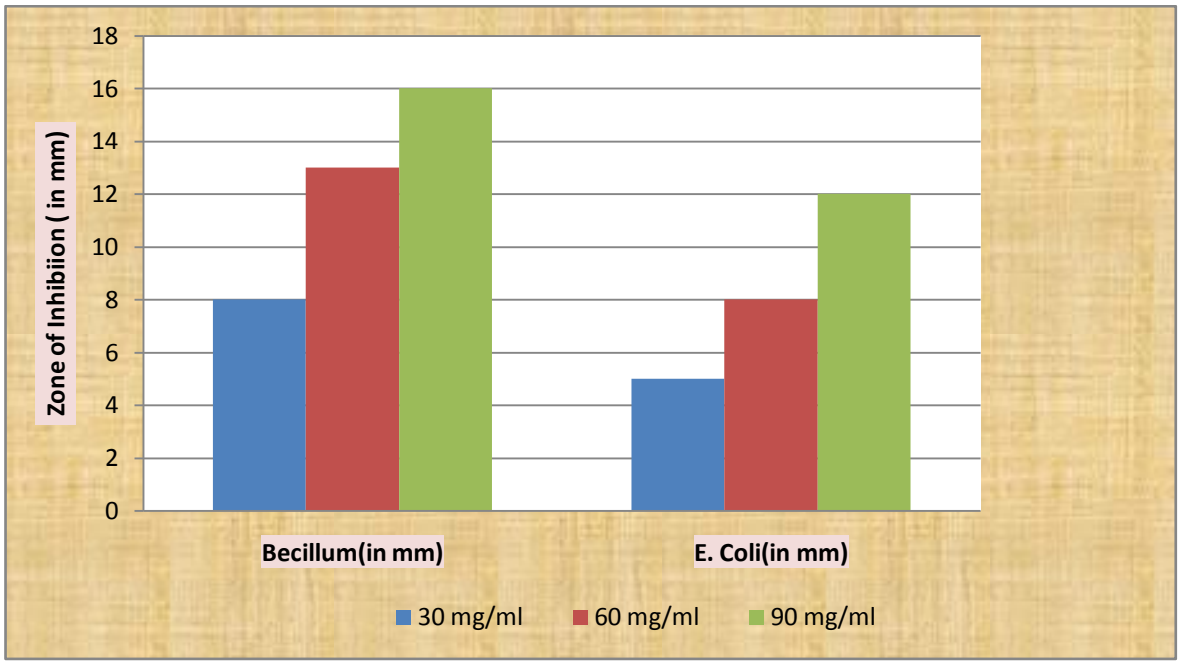

Fig 1: Antibacterial sensitivity of synthesised complex against bacteria

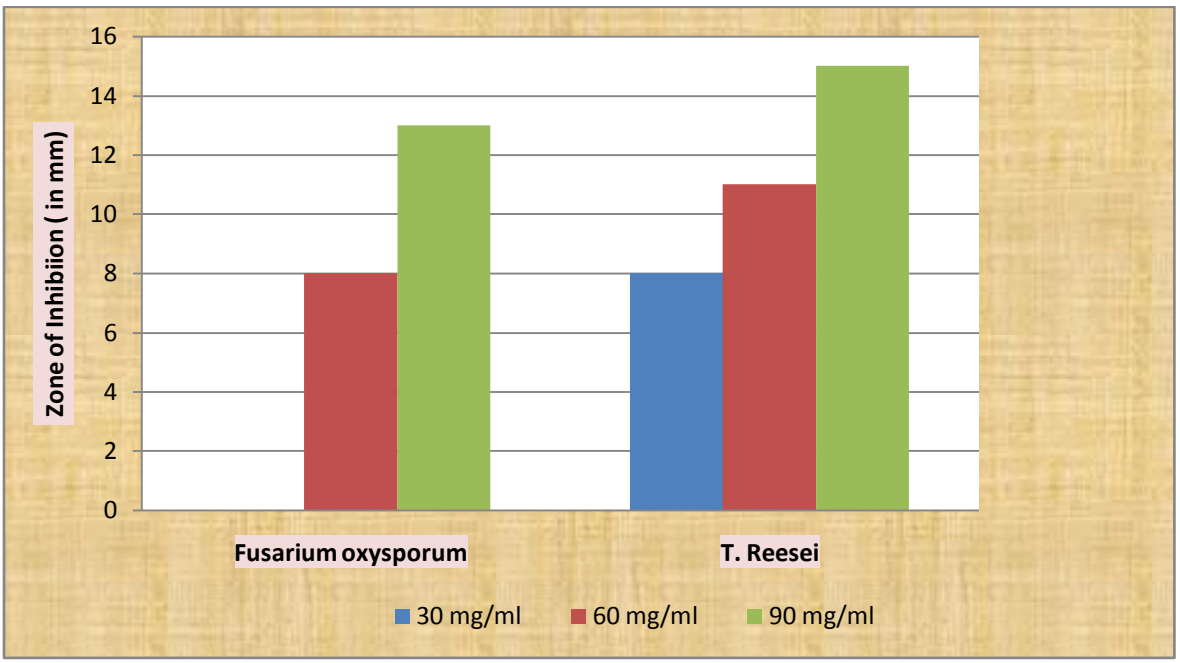

Fig.2: Antifungal sensitivity of synthesised complex against fungus

Fig.3a: Test disks presenting sensitivity of complex against different bacteria (Becillum & E. Coli)

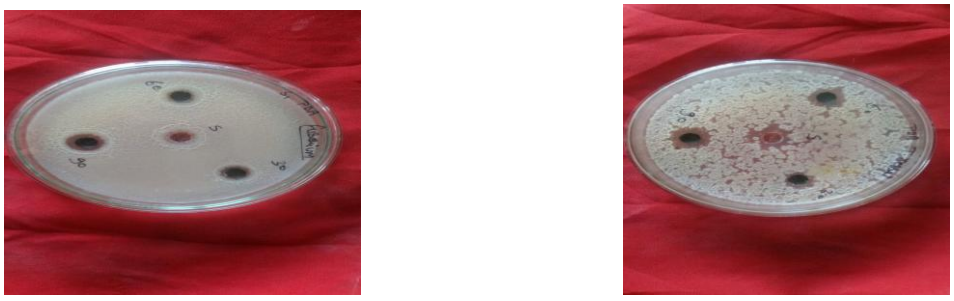

Fig.3.b : Test disks presenting sensitivity of complex against different fungi (*Fusarium* & *T. Reesei*)

CONCLUSION

The antibacterial and the antifungal activity of the synthesized hydrazone metal Complex [Fe(DBYHMP)2] have been evaluated by the well diffusion method. The results are expressed in millimeter. The two antimicrobial disks with ciprofloxacim (for anti-bacterial) and (ketokenazole for anti-fungal) were taken as standards and the sample disks were compared with it. A scrutiny of **Table 1** and **2** reveals that complexes are very much active as antibacterial and antifungal agent. Hence, we can conclude that hydrazone metal Complex [Fe(DBYHMP)2] containing compounds are able to enhance the performance.

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