CHAPTER – 5

ANTIMICROBIAL STUDIES

5. A. GENERAL:

The antimicrobial agent is the chemical substance which is inhibiting the growth of microorganism is known as antimicrobial agent. Although a wide range of chemicals have these properties if a sufficiently high concentration is used, the term is restricted to those compounds that are effective at concentration which is suitable for practical applications [1]. It is suitable to subdivided antimicrobial agents into various groups according to the action and purposes for which they are employed. Subdivision can be based upon the group of microorganism affected. Thus, antimicrobial agents which inhibit the growth of bacteria are called bacteriostatic or bactericidal and antimicrobial agents, which inhibit the growth of fungi are called fungi static or fungicidal [1].

The wide range of techniques is available for the antimicrobial assay. The principle of all these tests is similar, viz. the preparation of concentration gradient of the compound in a nutrient medium and observation of the growth of the microbial cultures, when the medium is seeded with the microorganism and incubated. Various variables are involved in the sensitivity and assay tests, such as size of the incolumns, nature of the culture medium, presence of the antimicrobial agents, and concentration of the agar in the medium, time of incubation and composition of the antimicrobial agents [1].

Metal ions play vital role in a vast number of biological processes [2,3]. Inorganic compounds play important role in organism [4].Biochemist have begun to investigate the molecular details of enzymes and other biologically active compounds, particularly metal chelates. The inorganic chemists on the other hand, have solely begun to recognize the similarities between compounds they work with, and biologically important compounds containing metal ions. These two trands have merged into active research i.e. bioinorganic chemistry [2].

This discipline is rapidly bridging the gap between traditional inorganic chemistry and biochemistry. The emergence of bioinorganic chemistry is rated to the general development of science. Biochemistry have evaluated to the point where biological process can be understood and explained in terms of molecules and electrons. Inorganic chemistry has developed concepts, theories and techniques that are both sophisticated and general enough to be applied to such chelate phenomenon as biological processes can be well interpreted by inorganic chemistry in involving concepts, theories and general sophisticated techniques.

Micronutrients required for the growth are supplied in proper amounts via natural processes under normal conditions. It has been known since centuries that blood contains ions [4]. Unfortunately, techniques were not accurate enough to establish the correlation between the presence of an in micronutrient and its biological effect. This is one of the reasons why the recognition of the essentialility of some trace elements has been delayed so long. A general realization of the importance of a biological orientation in all fields of human endeavor has aroused interest in many inorganic chemists to study biological systems[2]. The presence of metal in biological matter approaching their detection limits has intrigued biologist gor generation, though efforts to ascertain their functional significance have often been frustrating. The remarkable acceleration of the rate of progress in this field is the result of conjoint advances in many disciplines. Major progress in isolating and characterizing the composition, structure and functions of metalloenzymes has immensely aided the deliration of the molecular basis of the biological role of metals. Simultaneously, the emerging knowledge has opened a new direction to experiments in biochemistry, physiology, pathology, nutrition and medicine and the resultant

understanding of metallobiochemistry has given hope that metals have played unrecognized role in diseases. The possibility that metals might become therapeutic agents has motivated much of the past efforts in this field [2].The metal ion with biologically active ligands are a subject of considerable interest. Some of the biologically active compounds act via chelation [2, 3, 5-7].

5. B. STUDIES ON ANTIMICROBIAL ACTIVITIES:

In recent years, nitrogen heterocyclic have gained importance on account of their varied types of biological activities[3-15].Pyarazole depravities have been reported to possess antidiuretic [16], antihelminthic [17], hypoglycemic [18], fungicidal [19], antituberculotic [20], antineoplastic [21] and anti fertility [22] activates.

Anti-inflammatory and analgesic activities of certain substituted pyrazoles have been reported in the literature [23].Certain pyrazoles have been found active against *Staphylococcus aureus* and *Escheritia coli* [24]. Anti-cancer properties of some pyrazole derivatives are also known [25].

Nanda et al. [33] tested the twenty six sulphur and halogen free 5-pyrazolone compounds for their fungicidal activity against the fungus *Pyricularia oryae*.They reported that twenty-five out of twenty-six chemicals tested show significant fungicidal activity. They also suggested the correlation between structure and activity.

Mohanty et al. [34] determine the fungicidal activity of twenty one 5-pyrozolone compounds using the spore germination tests at various concentrations. They also reported that out of 21 compounds eight compounds inhibit the growth of *Pyricularia oryzae* spore germination. They found that the replacement of carbonyl oxygen atom by sulphur in pyrazolone nucleus enhances the fungicidal activity. They also reported

that the 4-nitroso derivative of the oxygenated compound is more active than its sulphur analogs.

Galabov et al. [35] examined the antiviral activity of some derivatives of 3-methyl-1-phenyl-5-pyrazolone as well as their metal chelates with zinc,copper,iron and manganese.

Jolly et al. [36] synthesized a series of new pyrazolones and their antimicrobial activity against *E.coli, S.facealis, K.pneuoniae, S.aureus, C.neoformeus, T.mentagrophytes, C.albicans and A.fumigatus* has also been reported.

Mittra et al. [37-42] reported more than five research paper on fungicidal activity using various depravities of 2-pyrazolin-5-ones, which possess significant activity against the fungi *pyricularia oryzae and Helmenthosporium oryzae.*

Ibrahim et al. [43] reported the synthesis, characterization and antibacterial properties of cobalt (II), nickel (II), copper (II), cadmium (II) and mercury (II) chelates of three depravities of 4-acetylhydrazono-2-pyrazolin-5-one (thione).

Shivarma et al. [44] studied the synthesis and biological activity of some 4-(5-aryl-2-furfurylidene)-1,3-disubstituded-2- pyrazolin-5-ones. They evaluated antibacterial activity against both Gram-positive and Gram-negative bacteria.

Jinzhou et al. [45] synthesized and evaluated antibacterial activities of five novel bis-schiff base chelates. They reported that metal chelates have high antibacterial activities.

Patel and Thaker reported that 4-benzoyl and 4-acetyl-3-methyl-1-phenyl-2-pyrazolin-5-one are known to have promising antibacterial, antiviral and antifungal activities [46].

El-Emary et al. [47] reported the synthesis and biological screening of new 1, 3-diphenyl-pyrazolonos with different heterocyclic moieties at positin-4.

Rana and Kharodawala [48-49] reported synthesis, characterization and antimicrobial activities of some transition metal chelates of heterocyclic ketoxime ligands. They reported that metal chelates inhibit the growth of *E.coli, B.subtilis, A.niger and T.longbrachiatum.*

Rather et al. [50] reported to hydrazones are process antibacterial activity against pathogenic bacteria i.e. *Klebsiella and Pseudomonas* and to non pathogenic bacteria i.e. *E.coli and Staphylococcus aureus.*

Benzoyl hydrazones are known to process antimicrobial activity [51-53]. 4-acyl-2-pyrazolin-5-one is reported to process antibacterial activity [54-55].

Sandra et al. [56] reported that thiosemicarbazones chelate showed a higher activity but the ligand had same activity against *E.Coli.*

M. Revinsiddappa et al. [57] reveals that the antimicrobial activity could be mainly due to the structure of the chelates and also the oxidation state of the metal ions.

Brief survey of the literature cited above on the antimicrobial activities of 4-acetyl-2-pyrazolin-5-one depravities suggested that the most of the 4-acyl-2-pyrazolin-5-ones ane their metal chelates are active against various microbial cultures. It is well known that the heterocyclic compounds exhibit bacterial, fungicidal and insecticidal activities [58]. Such a study is also highly useful to evaluate the possibilities of the use of metal chelates against microorganism [58]. So it was thought worthwhile to study and antimicrobial activities of the synthesized ligands and their metal chelates during the present investigation.

5. C. PRESENT WORK:

An attempt has been made to evaluate the antimicrobial activities of the synthesized ligands and their chelates of VO(II),Cr(II),Fe(II),Fe(III),Co(II),Ni(II),Cu(II) and Zn(II) against the bacterial strains such as *Escherichia coli and Bacillus subtilis* and Yeast strains such as *Saccharomyces cerevisiae* and fungal strains such as *Aspergillus niger* following the literature procedures[59-63].

5. D. EXPERIMENTAL:

5. D.1 MATERIALS:

All materials used in the present study were of the Best quantity.

(i) N-broth i.e. Nutrient broth medium (Titan Biotech Ltd., Delhi).

(ii) Sabouraud's dextrose broth medium (Titan Biotech Ltd., Delhi)

(iii) Antibacteriological grade Agar-agar (Qualigens-Glaxo, Mumbai)

5. D.2. MICRO ORGANISM:

Bacterial [*Escherichia coli and Becillus subtilis*] and fungal [*Aspergillus nigar*] and yeast [*Saccharomyces cerevisiae*] cultures were tested with ligands (I-IV) and their metal chelates.

The effect of the ligands (I-IV) and their metal chelates in the growth media were investigated by standard microbiological parameters. Concentration of the test compounds were kept constant (500_{ppm}) during all the experiments. The bacterial, fungal and yeast cultures were maintained on Nutrient-agar, Potato dextrose-agar and YEDP culture-slants respectively and were sub cultured every fortnight and stored at 0-5^{o}C temperature.

5. D.3. MEDIA COMPOSITION:

For the growth and test for the bacterial cultures, N-broth medium dissolved in distilled water and P^H was adjusted to 7.3 + 0.2 and sterilized at 15 psi steam pressure for 15 min in autoclave. For the preparation of the culture-tubes, 3.0 g agar-agar powder and 2.5 g N-broth medium were added in 100 ml distilled water.

For the growth and test for fungal cultures , Sabouraud's dextrose broth medium(Titan Biotech Ltd., Delhi),For the preparation of the culture tube, 3.0 g agar-agar powder and 3.0 g Sabouraud's dextrose broth medium were added in 100 ml distilled water and sterilized at 15 psi steam pressure for 15 min in autoclave.

For the growth of yeast cultures following composition of the medium were used.

Composition of the medium for Rhodotorula minuta:

1. Glucose - 2gm
2. Peptone - 1.25gm
3. KH_2PO_4 - 0.5 gm
4. $MgSO_4.7H_2O$ - 0.2gm
5. Distilled water - 100ml

Composition of the medium for *P. stipitis* MGYP:

1. Malt extract - 0.5gm
2. Glucose - 1 gm
3. Yeast Extract - 0.5gm
4. Peptone - 0.3 gm
5. Distilled Water - 100 ml

5. D.4. INOCULAM PREPARATION:

Bacterial cultures:

A loopful of cell mass from pregrown culture-tube (slant) Inculated into a sterile N-broth-tubes containing 15ml medium and incubated at 37°C for 24 hrs to get sufficient cell density (i.e. 1 x 10^8 cells / ml).

Fungal culture:

Well sporulated culture-tube of fungal cultures was used for preparation of spore suspension. About 5.0 ml of sterile distilled water containing few drops of twenty-80 solution was added to the culture-tube (slant) and growth was scraped with sterile nichrome wire-loop and collected in sterile tube. Spore suspension thus obtained was inoculated in the inoculum medium as 5% (v/v) in conical flasks and incubated at a room temperature on rotary shaker for 40 hours for the fungal cultures.

Yeast cultures:

A well pregrown slant of yeast culture was used for the preparation of incolums. 5.0 ml of sterile distilled water containing few drops of twin-80 solution was added to the slants and growth was scrapes with sterile nichrome wire loop and collected in sterile tube.

Inoculum thus obtained was inoculated in the test medium as 5% (V/V) in conical flasks and incubated at room temperature on rotary shaker (200 rpm) for 40 hrs for yeast cultures.

5. D.5. ANTIMICROBIAL ASSAY:

Antimicrobial assay was carried out by agar cup method which is based in the principle that the chemical substance in solution can diffuse though the agar seeded with test culture and produce concentration

gradient. Microbial growth is inhibited within is directly related to the minimum inhibitory concentration of the particular chemical agent against a specific organism.

For the agar cup diffusion method, the test compounds containing the ligand with metal chelates and bacterial and fungal and yeast culture were introduce in to the cups created by cork borer (0.85 cm) in the solidified nutrient agar , potato dextrose agar and Sabouraud's in the Petri plates. The test compound was introduced in to the cups and the plates were incubated at 37°C, 28 °C and 30 °C for bacterial, fungal and yeast cultures respectively. Microbial growth was determined by measuring the diameter the zone of inhibition. The degree of effectiveness was measured by determining the diameter of the zone of inhibition caused by the compound. Affectivity was classified in to three zones on the bases of the diameter of zone of inhibition:

+++ : Most effective

++ : Moderate effective

\+ : Slightly effective

_ : Non effective

5. E RESULT AND DISCUSSION:

Most of the compounds were active against microorganism the results are as under Table : 5.1 - 5.4.

The diamine ligand H_2BPPz-en without metal is slightly effective against *E.coli, B.subtillies, A nigar* and *S.cerevisiae.* The Cr(III),Fe(III),Co(II) and Zn(II) chelates of H_2BPPz-en are moderately effective against *B.subtillies* and *S.cerevisiae.* The Mn(II),Fe(II) and Cu(II) metal chelates of H_2BPPz-en are also moderately effective against *A.niger,* its VO(II),Mn(II) and Fe(II) metal chelates are non effective

against *S.cerevisiae*. The Cr(III),Mn(II),Fe(III),Co(II),Cu(II) and Zn(II) chelates of H_2BPPz-en chelates are also non effective against *E.coli.* While rest of the metal chelates of the diamine ligand H_2BPPz-en are slightly effective against *E.coli, B.subtillies, A nigar* and *S.cerevisiav.*

The diamine ligand H_2BPPz-mph without metal is non effective against *E.coli, B.subtillies, A nigar* but slightly effective against *S.cerevisiav.* The Cr(III) chelates of H_2BPPz-mph are also moderately effective against *B.subtillies*. The VO(II) chelates of H_2BPPz-mph are slightly effective against *E.coli, B.subtillies, A nigar* and *S.cerevisiav,* its Co(II) Chelates is slightly effective against *E.coli.* The Fe(II),Co(II),Ni(II),Cu(II) and Zn(II) chelates of H_2BPPz-mph are also slightly effective against *A.nigar*. While rest of the metal chelates of the diamine ligand H_2BPPz-mph are non effective against *E.coli, B.subtillis, S.cerevisiae and A.niger*.

The diamine ligand H_2BPPz-pph without metal is non effective against *E.coli*, but slightly effective against *B.subtillies*, *A.niger and S.cerevisiae.* The Mn(II) and Fe(II) chelates of H_2BPPz-pph are moderately effective against *B.subtillies and S.cerevisiae,* its Cu(II) chelates is also moderately effective against *B.subtillis*. The VO(II),Cr(III),Ni(II),Cu(II) and Zn(II) chelates of H_2BPPz-pph are slightly effective against *S.cerevisiae and A.nigar*. The Co(II) chelates of H_2BPPz-pph is non effective against *A.nigar* but slightly effective against *E.coli, B.subtilles and S.cerevisiae.* The Fe(III) chelates of H_2BPPz-pph is slightly effective against *E.coli and B.subtillis*. While rest of the metal chelates of the thiosemicarbazone ligand H_2BPPz-pph are non effective against *E.coli, B.subtillis, S.cerevisiae and A.niger.*

Among all the compound used H_2BPPz-pph and its chelates are found to be best antibacterial compound. The control treatments are non-effective against *Escherichia coli* and *Aspergillus niger* slightly effective

against the growth of *B.subtillus and Saccharronyces cerevisiae.* The antimicrobial activity of some metal chelates was found higher than that of corresponding ligand.

The diamine ligand H_2BPPz-benz without metal is slightly effective against *E.coli, B.subtillies, A.Niger and S.cerevisiae.* The Fe(III),Ni(II),Cu(II) chelates of H_2BPPz-benz are moderately effective against *B.subtillies and S.cerevisiae,* its VO(II) metal chelates are slightly effective against *B.subtillis, S.cerevisiae, A.niger.* The Fe(II) and Zn(II) chelates of H_2BPPz-benz are slightly effective against *B.subtillis and A.niger.* The Ni(II),Fe(II),Cr(III),Cu(II) and VO(II) chelates of H_2BPPz-benz is slightly effective against *A.niger.* While rest of the metal chelates of the diamine ligand H_2BPPz-benz are non effective against *E.coli,B.subtillis,S.cerevisiae and A.niger.*

TABLE-5.1

ANTIBACTERIAL ACTIVITY OF THE H_2BPP_Z-en (control-DMF)

Compounds	*E.coli*	*B.subtillis*	*S.cerevisiae*	*A.niger*
H_2BPP_Z-en	–	+	+	+
$[VO(BPP_Z\text{-en})(H_2O)]$	+	+	–	–
$[Cr(BPP_Z\text{-en})(H_2O)OAc]$	–	++	++	–
$[Mn(BPP_Z\text{-en})(H_2O)_2]$	–	+	–	++
$[Fe(BPP_Z\text{-en})(H_2O)_2]$	+	+	–	++
$[Fe(BPP_Z\text{-en})(H_2O)OAc]$	–	++	++	–
$[Co(BPP_Z\text{-en})(H_2O)_2]$	–	++	++	–
$[Ni(BPP_Z\text{-en})(H_2O)_2]$	+	+	+	+
$[Cu(BPP_Z\text{-en})(H_2O)_2]$	–	+	+	++
$[Zn(BPP_Z\text{-en})(H_2O)_2]$	–	++	++	–

TABLE-5.2

ANTIBACTERIAL ACTIVITY OF THE H_2BPP_Z-mph (control-DMF)

Compounds	*E.coli*	*B.subtillis*	*S.cerevisiae*	*A.niger*
H_2BPP_Z-mph	–	–	+	–
[VO(BPP_Z-mph)(H_2O)]	+	+	+	+
[Cr(BPP_Z-mph)(H_2O)OAc]	–	++	–	–
[Mn(BPP_Z- mph)(H_2O)$_2$]	–	–	++	+
[Fe(BPP_Z- mph)(H_2O)$_2$]	–	+	–	+
[Fe(BPP_Z- mph)(H_2O)OAc]	–	–	–	–
[Co(BPP_Z- mph)(H_2O)$_2$]	+	+	++	+
[Ni(BPP_Z- mph)(H_2O)$_2$]	–	+	–	+
[Cu(BPP_Z- mph)(H_2O)$_2$]	–	+	++	+
[Zn(BPP_Z- mph)(H_2O)$_2$]	–	+	–	–

TABLE-5.3

ANTIBACTERIAL ACTIVITY OF THE H_2BPP_Z-pph (control-DMF)

Compounds	*E.coli*	*B.subtillis*	*S.cerevisiae*	*A.niger*
H_2BPP_Z-pph	–	+	+	+
[VO(BPP_Z-pph)(H_2O)]	–	–	+	+
[Cr(BPP_Z- pph)(H_2O)OAc]	–	–	+	+
[Mn(BPP_Z- pph)(H_2O)$_2$]	–	++	++	–
[Fe(BPP_Z- pph)(H_2O)$_2$]	–	++	++	–
[Fe(BPP_Z- pph)(H_2O)OAc]	+	+	–	–
[Co(BPP_Z- pph)(H_2O)$_2$]	+	+	+	–
[Ni(BPP_Z- pph)(H_2O)$_2$]	–	–	+	+
[Cu(BPP_Z- pph)(H_2O)$_2$]	–	++	+	+
[Zn(BPP_Z- pph)(H_2O)$_2$]	–	–	+	+

TABLE-5.4

ANTIBACTERIAL ACTIVITY OF THE H_2BPP_Z-benz (control-DMF)

Compounds	*E.coli*	*B.subtillis*	*S.cerevisiae*	*A.niger*
H_2BPP_Z-benz	+	+	+	+
$[VO(BPP_Z\text{-benz})(H_2O)]$	–	+	+	+
$[Cr(BPP_Z\text{- benz})(H_2O)OAc]$	–	–	+	+
$[Mn(BPP_Z\text{-benz})(H_2O)_2]$	–	+	–	–
$[Fe(BPP_Z\text{- benz})(H_2O)_2]$	–	+	–	+
$[Fe(BPP_Z\text{- benz})(H_2O)OAc]$	–	++	++	–
$[Co(BPP_Z\text{- benz})(H_2O)_2]$	–	–	–	–
$[Ni(BPP_Z\text{- benz})(H_2O)_2]$	–	++	++	+
$[Cu(BPP_Z\text{- benz})(H_2O)_2]$	–	++	++	+
$[Zn(BPP_Z\text{- benz})(H_2O)_2]$	–	+	–	–

SUMMARY:

The studied on antimicrobial activities of the ligand and their metal chelates are described in chapter-V.

All the ligands and their metal chelates prepared during present investigation are tested for their antibacterial, antifangal, antiyeast activity against *Escherichia coli* , *Bacillus subtilis, Saccharomyces cerevisiae and Aspergillus niger* .It is observed that all the synthesized ligands and their metal chelates effect the growth of microorganisms and resulted in to inhibitory effect .Moderate effective inhibition was shown by the metal chelates of H_2BPP_Z-en followed by the metal chelates of H_2BPP_Z-mph,H_2BPP_Z-pph and H_2BPP_Z-benz.

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