Chapter-VII

Antimicrobial Activity
The control of microorganisms is critical for the prevention and treatment of disease. Microorganisms also grow on and within other organism and microbial colonization can lead to disease, disability and death. Thus the control or destruction of microorganisms residing within the bodies of humans and other animals is of great importance and it is achieved by using antimicrobial agents.

An antimicrobial agent is any substances that act against microorganism i.e. chemical substance, which inhibits the growth of the microorganism, or destroy it. A wide range of chemicals exhibits this property when used in a sufficiently higher concentration. However the term is usually restricted to those substances that are effective at concentration suitable for practical applications. The chemicals at a low concentration should have a broad spectrum of antimicrobial activity. On the basis of the action and purpose for which the antimicrobial agent are employed the subdivision into different group is possible. Subdivision can be based upon the group of microorganism affected like antibacterial, antifungal, antiprotozoal, antiviral and antineoplastic, chemotherapeutic agents all more and less specific for treatments of disease caused by specific pathogenic agents [1, 2].

Fungi are plant-like organisms that lack chlorophyll. Fungi are on one of the five kingdoms of life. Many fungi are beneficial and useful edible mushrooms would be an example of these while some are harmful some fungi can infect plants and people. There are over 10,000 species of fungi. Since they do not have chlorophyll, fungi must absorb food from other, since they do not use light to make food, fungi can live in damp and dark places. Fungi are supposed to “Eat” things when they are dead but sometimes they start eating when the organism is still alive most commonly, fungi grow as pathogen on the skin of animals or people this is sometimes called symptom.

Fungi irritation to the nose and causes allergies. Over 37 million people have allergies and many of them are caused by fungi.
can get some fungi known as penicillium ans stachybotrys. They float in the air and can cause watery eye and breathing problems.

Finally, fungi can be helpful and not helpful, but they all are important and required in life. Fungi are on of the earth big recycles without because of them we could not like and some times humans die because of them, but they are important and required in life. Fungi also cause a number of plant and animal diseases in humans ringworm, athletes foot and several more serious disease are caused by fungi. Because fungi are generally similar to animals other organisms. This make fungal desease very difficult to treat plant and disease caused by fungi include -root, smuts and leaf root and stem root may cause several damage.

Several key steps must be completed for an antimicrobial agent to successfully inhibits or kill the infecting microorganisms.

1) The agent must be in active form. This is ensured through the pharmacodynamic design of the drug, which takes in to account the route through which the patient will receive the agent e.g. orally, intramuscularly, intravenously.

2) The agent must also be able to achieve sufficient levels or concentration at the site of interaction so that it has a chance to exert an antimicrobial effect.

Large numbers of chemical compounds have the ability to inhibits the growth and metabolism or have ability to kill them. Depending upon its mode of action and its utility, these chemicals compounds are used for preparation of disinfectant, antiseptic, sanitizer, germicide, bacteriostatic and antimicrobial agents. However there are wide ranges of chemical compounds but only very few of them are truly chemotherapeutic because all of them do not show selective toxicity. They must kill or inhibit the microbial pathogen without damaging the host cells.
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Different chemicals have different mode of actions on the host immune system of the common mechanism include:

1) Disruption of cells
2) Denaturation of proteins
3) Inactivation of enzymes
4) Damage to nucleic acids
5) Oxidation of cellular constituents

The chemothepeutic agent like disinfects can be either cidal or static. The antimicrobial agent (antibacterial and antifungal) are often described as:

1) **Bacteriostatic or fungistatic** : The antimicrobial agent having the property of inhibiting bacterial or fungal multiplication. The term bacteriostatic or fungistatic describes a drug that temporarily inhibits the growth of microorganisms.

2) **Bactericidal or fungicidal** : The antimicrobial agent having the property of killing bacteria or fungi. The term bacteriostatic or fungicidal describes a drug that attaches to its receptor and causes complete destruction and death of the microorganism.

For the substance of antimicrobial activity the following three conditions must be fulfilled.

1) The substance or compound to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated.

2) Favorable conditions like, nutritional media, temperature, incubation time etc. must be provided to offer a minimum opportunity for optimum growth of the organism in absence of antimicrobial agents.

3) There should be a have been proposed and adopted for the measurement of antimicrobial activity [4] Like agar–agar diffusion method [5,6] and paper–disc diffusion method [7,8].
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LITERATURE SURVEY

An extensive amount of work on coordination compound of transition elements with various Schiff base ligand derived from variety of aldehydes and amines have been done due to their industrial and biological applications [9, 10]. A number of Schiff base complexes reported to be a great utility in pharmacological and biological aspects [11,13], Kulkarni et al [14] have studies antimicrobial activity of juglons and their chelates and reported that stronger the hydrogen bonding greater is the activity. Tiwari et al [15,16] synthesized the metal chelates of 8-hydroxy quilline-5(p-tolyl)sulphonamide and studied their bacteriastatic properties by using cup plat agar diffusion technique S.aureus and E. coli. The antibacterial activity of ligand was found to enhance on complexation with metal. Shanet et al [17] prepared new prepared Schiff base compounds and screened for antibacterial activity using cup plate method at 100-500 ppm against S.aureus and E. coli. From the results of that azomethine linkage is an essential requirement for such activity.

Literature survey reveals that the Schiff bases seen to poeseses antitubercular [19], anticancer [20], antitumor [21], bacteriostatic fungicidal [22] actylcholine sterease inhibitors [23] medicinal and agrochemical activity [24].

Pancholi et al [25] investigated the effect of coordination of Cu(II), Ni(II), Mn(II), Zr(II), VO(IV) and UO_2 (VI) with 2,4-dihydroxy acetophenoneoxime-thioureao-trioxane on the growth of various bacteria, fungicide, yeast, so as to determine the active principle responsible for antimicrobial activity of polymer samples. The results suggest that variation in the structure on coordination affects the growth of microorganism and may results into either inhibitory effect, stimulatory effect on reduction in toxicity of metal ion towards some microorganism. By using the metal the polychelates of 2-hydroacetophenone oxime-thioureao-trioxane resin were tested P. fluorescences, E. coli B. subtilis and S.marcescens bacteria culture. The result suggest that all the compounds
show moderate inhibition on the bacteria culture. The antimicrobial activity of bivalent Co(II), Ni(II) and Cu(II) polychelates with thiohene-2-aldehyde-4-cholo-1-bromoanilline against E.coli, S.typhi, S.aureus and B. subtilis was reported. The chloro series compounds showed moderate to better activity while Cu(II) chelate are more effectively than Ni(II) and Co(II) chelates. The antimicrobial properties of the metal complexes derived from Schiff bases of phenyl butazone or oxyphenetylbutnye and o-aminophenol by cup plate method in DMF solvent against. The ligand and their complexes are found to posse good antibacterial activity. It was also observed that oxyphebutazone and metal complexes are much more toxic then their parent ligand itself S.typhi and E.coli more active and B. subtilis by single disc method and observed that metal complexes are more active than Schiff bases. Similar observation were reported by Rao et al [28] and Purohit et al [29].

Antimicrobial active of Schiff base complexes of Cu(II), Zn(II), Cd(II) and Hg(II) have been reported by El-Mnakhy et al [30]. The antimicrobial study of the ligand and complexes was evaluated by using the diffusion method at different concentration against B. subtilis, S. aureus and E. coli. The free ligand and their metal complexes exhibited significant inhibitory effects against gram negative bacteria, much enhanced effect against gram positive bacteria especially the Hg (II) and Cu(II) complexes of SAH CBAH and BAH at all concentration. All the complexes of Cd(II) and Cu(II) are showed as low inhibitory effect, complete inhibition growth was observe after turbidity measurement for 24 h for bacteria at 50 ppm Cd for B megatherium at 100 ppm Cd for B megatherium at 100ppm Cd enterobacteria spectrophotometric and at 500ppm Cd for entrobacter aerogenous and B cereus. The gram negative bacteria were more talent to Cd than the gram positive. In general the activity for free ligand and CHAH complexes against both types of bacteria was found superior but the gram positive was found resist and responded lower than gram negative. The results revealed that the heavy metal complexes, improve the antimicrobial activity.

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Singh et al [31] have reported biological activity of the Ti(III) Zr(IV) synthesis from ketone and semicarbazide hydrochloride / thiacarbazide. The antimicrobial activity of ligand has been evaluated against the bacteria S.aureus, E.coli and P. aeruginosa by disc diffusion technique in DMSO at different concentrations. The zones of inhibitions were showed that all the carbazide are physically active.

**EXPERIMENTAL :**

For evolution of invitro antimicrobial activity the following three conditions must be fulfilled first, the substance to be evaluated must be brought in to intimate contact with the test organism against which the activity is to be estimated, secondly favorable conditions (temp, nutritional media, incubation time etc.) must be provided to offer maximum opportunity for optimum growth of the organism in the absence of antimicrobial agent and thirdly there should be method for measuring the antibacterial response obtained by antimicrobial agent[32]

Different method have been proposed and adopted for the measurement of antibacterial activity [33]. These are five methods.

1) Agar streak dilution method.
2) Agar diffusion (cup, paper plate, disc, cylinder) methods.
3) Turbidmetric method.
4) Special dilution method.

Special method (specific for measuring the action of a specific substance).

In the present study we have used disc diffusion method and following microorganisms were used.

1) *Escherichia coli* (Gram Negative).
2) *Salmonella aero genes* (Gram Negative).
3) *Aerobatic aero genus* (Gram Negative).
4) *Bacillus subtile*
5) *Bacillus megatherium* (Gram Positive).
6) *Proteus Vulgaris* (Gram Negative).
7) *Staphylococcus aureus* (Gram Positive).
A) Media used for antibacterial activity
   i) Nutrient agar
      Medium Composition
      Beef Extract - 3.0g
      peptone - 5.0
      NaCl - 5.0
      Agar- 25.0
      Distilled Water- 1000ml.
      $p^H$ 6.9
   ii) Nutrient broth
      Medium Composition
      Beef Extract - 3.0g
      peptone - 5.0
      NaCl - 5.0
      Distilled Water- 1000ml.
      $p^H$ - 6.9

   Both the above mentioned media used were of bacteriostatic grade.
The above media were to be suitable for the growth of all screen organism
used in the present wrote.
The antifungal activity of the synthesized complexes was
determined by disc diffusion method the following microorganisms were
used in the present study
   i) A. niger
   ii) P. citrinum
   iii) S. cerevisiae

B) Media preparation:
Czapak-Dox media was used for antifugual activity.

Composition
Sucrose - 30grm.
Sodium nitrate - 2grm.
Dipotassium sulphate - 1grm.
Magnesium sulphate - 0.5grm.
Potassium Chloride - 0.01grm
Ferric Chloride - 0.01grm.
Agar - 20grm.
$P^H$ - 5.8.

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C) **Slant Preparation:**

Nutrient agar media was dissolved in distilled water and sterilized by autoclaving at 121°C, 15 lbs for 20 minutes. About 5ml of molten media was transformed as optically in previously sterilized test tube. The test tube were then plugged lightly and placed in slanting position to cool and solidly.

D) **Stock Culture:**

Culture was growth on the nutrient agar slants by incubating them for 24 h at 37°C.

E) **Culture Dilution:**

One loopful to stock culture was added to 5ml nutrient broth media for inoculation. This inoculated broth was incubated for 24 hours at 37°C. For all experimentally purpose 24 hours fresh diluted culture of broth the organism were used.

F) **Preparation of Samples:**

Making an aqueous solution of samples usually tests the antimicrobial activity. However metal complexes prepared in the present study were insoluble in water and common organic solvents but formed suspension in Dimethyl sulphoxide (DMSO). Hence to study the antimicrobial activity of metal complexes their suspensions were prepared by using by DMSO. Dimethyl sulphoxide may have some antimicrobial activity therefore blank of Dimethyl sulphoxide was taken and tested as control.

In order to check the potency of the complexes the solutions were prepared with ~ 1mg/ml of this solution was added to 5ml of the nutrient broth solution containing the organisms to be tested. The tubes with organisms and medium with solvents (DMSO) were used as controls. These tubes were kept for incubation at 37°C for 24 hours. Most of the metal complexes under study showed total inhibition of the test culture (Ecoli, S typhi, A aureus, B subtited B megatherium, P valganis and S.aureus) within 24 hours of incubation. The tube containing metal complexes showing of inhibition (i.e. Antimicrobial activity) was clear. Thus for all
the antimicrobials screenings the concentration of 1mg/ml were used which is the range of the substance to be used as antibiotic.

G) Disc Diffusion method

The sterilized media was poured in to sterilized Petri sterile poetry plates. The coordination complexes were dissolved in Dimethyl sulphaoxide (DMSO) with concentration of 5 mg/ml and disc were impregnated in the solution were spread on the media. Then the plates were kept for incubation in oven at 37°C. After 24h of incubation zone of inhibition were measures.

Similarly a concentration of 1mg/ml was used and results were recorded by measuring the diameter of zones organisms sensitive to compound was inhibited from growing in circular zone around the paper disc.

H) Determination by MIC (Minimum inhibitory concentration):

Minimum inhibitory concentration of the antifungal coordination compounds was also determined. Metal complexes of cadmium chloride was used for MIC determination. The metal ion complexes of cadmium was dissolved in DMSO with concentration of 2mg, 1mg, 0.5mg, 0.25mg, 0.125mg, 0.0625mg, 0.0312mg, 0.0156mg and 0.0076 mg/ml and DMSO was used a control.

Sterilized Czapek-Dox agar slants were prepared and incubated for 4-5 days and after incubation results were obtained .By observing the tubes the inhibitory concentration, which inhibits growth of organisms, were determined.

I) Reading of Results:

The reading and interpretations of results were done according to Cappuccino and Sherman [34]. The diameter of the zone of inhibition of growth, including 6 mm diameter of the disc was measure by the plate against a ruler.
I) **Disc diffusion Test:**
The sterilized nutrient agar media was poured into the sterile petri plates 24 h old broth culture of *E. coli*, *S. aureus* and *P. aeruginosa* were used. The sterilized disc were impregnated into sample solution of concentration of 0.5mg/ml and 1mg/ml. Culture of bacteria was spread on agar plates under aseptic conditions. After spreading disc was kept on agar surface plates were incubated at 37°C for 24h. Disc moistened with Dimethyl sulphoxide used as a control.

**Results and Discussion:**
The inhibition effect of the ligands (BNPSAP, BNBSAP, BIPSAP, and BNESAP) and metal chelates enhancing their biological activity and summarized in table 7.1. Four ligands and their metal complexes have been studied for their antifungal activities.

**Following four ligands have been used in present investigation.**
1) 4,4′-bis[(N-Propane salicylaldehyde)amine-5) azo] biphenyl. (BNPSAP)
2) 4,4′-bis [(N-Butane salicylaldehyde)amine-5) azo] biphenyl. (BNBSAP)
3) 4,4′-bis [(I-Propane salicylaldehyde)amine-5) azo] biphenyl. (BIPSAP)
4) 4,4′-bis [(N- Ethane salicylaldehyde)amine-5) azo] biphenyl. (BNESAP)

**Biological studies of BNPSAP and their metal complexes:**

**Antimicrobial -**
Antimicrobial screening of BNPSAP and its chelate polymers *S. aureus*, *E. coli*, *P. aeruginosa* strain were carried out. The results evince that the Mn(II) chelate and the ligand exhibits bacteriostatic behavior (resistant) toward all the bacteria strains used Fe(II), Co(II), Ni(II), Cu(II) and Zn(II). Chelates polymers are found to show low to moderate bactericidal (reasoned) nature against most of the bacteria strain (table 7.1) and are found to be bacteriostatic (resistant) towards the other. The bischelating ligand and complexes found to be bacteriostatic against *P. aeruginosa* polychelate of Cd(II) is found to posses fairly good
antibacterial activity as compared to other chelate polymer of BNPSAP ligand other chelate Zn(II) and Cd(II) show moderate sensitivity against *P. aeruginosa* culture while a significant sensitivity towards *S. aureus.*

**Antifungal:**

The ligand and its metal complexes were screened for their antifungal activity against *A. niger* and *P. citrinum,* both show positive response against *S. cerevisiae.* The test organism show more activity towards Fe(II) and Cu(II) complexes.

**Biological studies of BNBSAP and its metal complexes:**

**Antimicrobial -**

All the chelate polymers and the ligand show antimicrobial behaviour. They are found to be bacteria static against *E. coli* strain. The ligand BNBSAP is found to show low sensitivity against *S. aureus, E. coli,* *P. aeruginosa* resistant against other bacteria Mn(II) chelate show low sensitivity against, *E. coli* and, *P. aeruginosa.* The chelate polymers of Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with BNBSAP show low bactericidal behavior towards the other shown in (table 7.2).

**Antifungal:**

The ligand BNBSAP and its complexes do not response as antifungal agent against *A. niger* and, *P. citrinum* but it show antifungal activity against *S. cerevisiae* a compared to Co(I), Cd(II) and Mn(II) complexes of Ni(II), Fe(II), Cu(II) and Zn(III) show good responses at concentration of 05 mg/ml.

**Biological studies of BIPSAP and its metal complexes:**

**Antimicrobial -**

All the chelate polymers with BIPSAP ligand were screened for their antimicrobial activity a significant activity is shown by the ligand against *S. aureus* strain and also a low sensitivity against all the other bacteria.
except *E. coli* where it exhibits a bacteriostatic behavior Cu(II) and Zn(II) BIPSAP chelates show good zone of inhibition against *S. aureus* bacteria strain. In general all the chelates are show low to moderate activity against some strain of bacteria are resistant toward the other shown in table 7.3.

**Antifungal:**

The ligand BIPSAP and its complexes do not response as antifungal activity against *A. niger*, *P. citrinum* but they show antifungal activity *S. cerevisiae* as Co(II), Cd(II) and Mn(II) complexes of Ni(II), Fe(II), Cu(II) and Zn(II) show good response at concentration 0.5mg/ml.

**Biological studies of BNESAP and its metal complexes:**

**Antimicrobial** -

Bischelating ligand BNESAP and its chelate polymers were screened for their antibacterial studies. The chelates were found to show greater inhibition effect than the ligand BNESAP.

BNESAP ligand show a low sensitivity against the most resistant bacteria strain *E. coli* is found to be bacteriastatic (resistant) towards other bacteria Mn(II) and Fe(II) chelates are found to be resistant against all the bacteria strains. The chelate polymers of Co(II), Ni(II), Cu(II) and Zn(II) ions exhibits moderate bactericidal (sensitivity) property against most of all the bacteria strain and show a low sensitivity towards the other bacteria. The sensitivities of Cd(II) BNESAP chelate polymers found to be excellent as compared to other BNESAP chelate polymer. Cd(II) chelate show a significant sensitivity against *S. aureus* and *E. coli* and is resistant towards *P. aeruginosa*.

**Antifungal:**

The ligand BNESAP show moderate activity against *A. niger* while Ni(II) show response against *P. citrinum* at concentration of 1 mg/ml as compared BNESAP and Cd(II) complexes.
Table 7.1: Antibacterial and Antifungal activity of BNPSAP and its Polychelates.

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<th>Compounds</th>
<th>Antibacterial Activity</th>
<th>Antifungal Activity</th>
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<td></td>
<td>E. coli</td>
<td>S. aureus</td>
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<tr>
<td>BNPSAP</td>
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<td>Mn (II)</td>
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<td>Fe (II)</td>
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<td>Co (II)</td>
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<td>Ni (II)</td>
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<td>Zn (II)</td>
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<td>Cd (II)</td>
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R = 1-3mm  
S = 5-10mm

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Table 7.2: Antibacterial and Antifungal activity of BNBSAP and its Polychelates.

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S = 5-10mm

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Table 7.3: Antibacterial and Antifungal activity of BIPSAP and its Polychelates.

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<td>Cd (II)</td>
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R = 1-3mm  
S = 5-10mm
Table 7.4: Antibacterial and Antifungal activity of BNESAP and its Polychelates

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<td>Fe (II)</td>
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<td>Co (II)</td>
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<td>Zn (II)</td>
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<td>R</td>
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<td>Cd (II)</td>
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