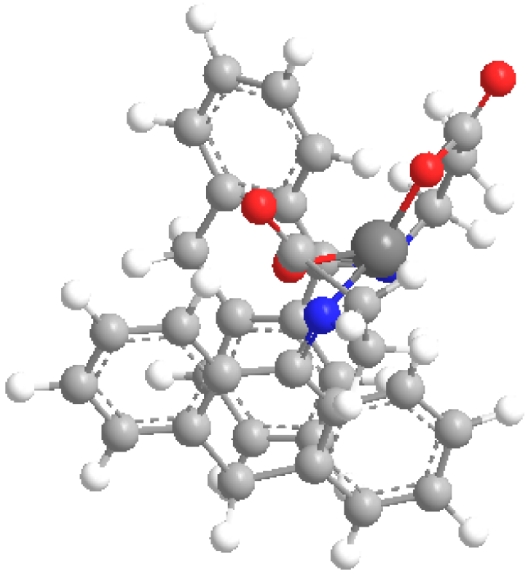
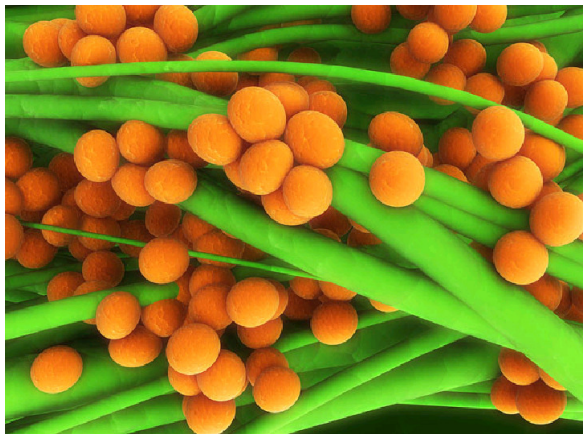
PART III
ANTIMICROBIAL STUDIES

Shaju. K. S. “Evaluation of metal evaluation binding capacity of azomethine class of compounds, electrochemical investigations on corrosion and their biological studies ” Thesis. Department of Chemistry, St. Thomas College Thrissur, University of Calicut, 2014



PART III

ANTIMICROBIAL STUDIES



CHAPTER 1

INTRODUCTION AND REVIEW

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatments is the main cause of this problem[1,2].Although the discovery and development of new antibiotics and chemotherapeutic agents have provided the tools for most wonderful control of infectious diseases caused by various microorganisms,the development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need[3].

Bacteria

The bacteria are microscopic organisms with relatively simple and primitiveforms of prokaryotic type. Danish physician Christian Grams discovered thedifferential staining technique known as Gram staining, which differentiates thebacteria into two groups “Gram positive” and “Gram negative”. Gram positivebacteria retain the crystal violet and resist decolourization with acetone or alcoholand hence appear deep violet in colour; while gram negative bacteria, whichloose the crystal violet, are counter-stained by safranin and hence appear red incolour.

Grampositive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope) and as a result are stained purple by crystal violet, whereas gramnegative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by the safranin.

The bacterial strains considered in the research work are both gram positive and gram negative. The important clinical pathogens associated with human body are *Staphylococcus aureus, Bacillus subtilis, Bacillus thuringiensis, Enterobacteraerogenes, Escherichia coli* and *Proteus vulgaris*. Among these *S.aureus, B.subtilis*and*B. thuringiensis*are gram positive bacteria while*E.aerogenes, E.coliand P. vulgaris*are gram negative bacteria.

Staphylococcus aureus

Family	Genus	Species
Micrococcaceae	*Staphylococcus*	*S. aureus*

The word staphylococcus is derived from the Greek language (Staphylo= bunch of grapes; Coccus = a grain or berry), while the species name isderived from Latin language (aureus = golden).

Basic habital of *S.aureus* is the anterior naves, though it is also a normal flora of human skin and of the respiratory and gastrointestinal tracts. The individual cells are 0.8 to 0.9 μ in diameter. They are oval or spherical, nonmotile, non-capsulated, non-sporulating strains with ordinary aniline dyes and are gram-positive, typically arranged in groups or irregular clusters like branches of groups in pus, seen single or in pairs. They easily grow on nutrient agar; the optimum temperature for the growth is 35^{o}C. *S. aureus* grows rapidly and produce circular (1-2 mm) endive edge, convex, soft, glistening colonies having a golden yellow pigment. *S. aureus* can tolerate moderately high concentration of NaCl, hence they can be selectively isolated on the nutrient medium containing 7.5 % sodium chloride.

It is able to ferment mannitol to organic acid. *S. aureus* also produce the coagulase which is able to clot citrated plasma. It produces the enzymes catalase, hyaluronidase as well as other virulent factors like hemolysins, leucocidins, enterotoxins and exofoliatin.*S. aureus* can cause a range of illness, from minor skin infections, such as pimples, impetigo, boils (furuncles), folliculitis, carbuncles and scalded skin syndromlife threateningdiseases such aspneumonia, meningitisosteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis.It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections

Bacillus subtilis

Family	Genus	Species
Bacillaceae	*Bacillus*	*B.Subtilis*

"Bacillus subtilis cells are rod-shaped, gram positive bacteria that are naturally found in soil and vegetation. *B. subtilis* grows in the mesophilic temperature range. The optimal temperature is 25-35^{0}C. Stress and starvation are common in this environment; therefore, *B. subtilis* has evolved a set of strategies that allow survival under these harsh conditions. One strategy, for example, is the formation of stress-resistant endospores. Unlike several other well-known species, *B. subtilis*has been historically classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct"[4].

"B.subtilis is readily present everywhere; the air, soil and in plant compost. It is predicted that it spends most of it time inactive and in spore

form. When the bacterium is active though, it produces many enzymes. One enzyme contributes to the plant degradation process. *B.subtilis* can also be found in the human body, mostly on the skin or in the intestinal tract. However it is very rare for this bacterium to colonize on the human body.*B. subtilis* is only known to cause disease in severely immunocompromised patients and can conversely be used as a probiotic in healthy individuals[5]. It rarely causes food poisoning"[6].

Along with enzymes, *B.subtilis* also produces a toxin called subtilisin. Subtilisin can cause allergic reactions if there is repeated exposure in high concentrations. This only poses a risk to fermentation plants that use high quantities of subtilisin.

Bacillus thuringiensis

Family	Genus	Species
Bacillaceae	*Bacillus*	*B.thuringiensis*

"*B.thuringiensis* (Bt) is a grampositive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. It is approximately 1 µm in width and 5 µm in length[7]. It grows at body temperature and produces a diamond-shaped crystal from its crystal proteins (Cry proteins) and uses it to fend off insects, predatorsand other pathogens.*B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on leaf surfaces, aquatic environments, animal feces, insect rich environments, flour mills and grain storage facilities"[8]

"During sporulation, many bacterial strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action. This has led to their use as insecticides and more recently geneticallymodified crops using Bt genes. Many crystal-producing Bt strains, though, do not have insecticidal properties"[9].

"B. thuringiensis is closely related to *B. cereus*, a soil bacteriumand *B. anthracis*, the cause of anthrax: the three organisms differ mainly in their plasmids. Like other members of the genus, all the three are aerobes capable of producing endospores. Upon sporulation, *B.thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins (called crystal proteins or Cry proteins), which are encoded by cry genes[10]. In most strains of *B. thuringiensis*, the cry genes are located on a plasmid (in other words, *cry* is not a chromosomal gene in most strains)"[11-13].

Enterobacteraerogene

Family	Genus	Species
Enterobacteriaceae	*Enterobacter*	*E. aerogenes*

"E. aerogenes is a gram

negative, oxidasenegative, catalasepositive, citrate positive, indole negative and rod-shaped bacterium. *E.aerogenes* is a nosocomicaland pathogenic bacterium that causes opportunistic infectionsincluding most types of infections.Infections commonly attributed to *E. aerogenes* are respiratory, gastrointesntinal and urinary tract infections, specifically cystits, in addition to wound, bloodstream and central nervous system infections. The majority is sensitive to most antibiotics designed for this bacteria class, but this is

complicated by their inducible resistance mechanisms, particularlylactamase which means that they quickly become resistant to standard antibiotics during treatment, requiring change in antibiotic to avoid worsening of the diseases like sepsis.

Some of the infections caused by *E. aerogenes* result from specificantibiotictreatments, venous catheter insertions and/or surgical procedures. *E. aerogenes* is generally found in the humangastrointestinal tract and does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygienic chemicals and soil. The bacterium also has some commercial significance – the hydrogen gas produced during fermentation has been experimented usingmolasses as the substrate".

Escherichia coli

Family	Genus	Species
Enterobacteriaceae	*Escherichia*	*E. coli*

"Escherichia coli[14](commonly abbreviated *E. coli*), is a gramnegative, rod-shaped bacterium thatis commonly found in thelower intestine of warm-blooded organisms (endotherms). Although *E. coli* in human large intestine can assist with waste processing and food absorption, some strains of *E. coli* can cause severe infections in many animals, such as humans, sheep, horses, dogs, etc. The one that only found in humans is called enter aggregative*E. coli*. Urinary tract infection, for example, can be caused by ascending infections of urethra. Such infections can be found in both adult male and female and some infants can be infected as well.

The bacterium can be grown easily and inexpensively in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

Proteus vulgaris

Family	Genus	Species
Enterobacteriaceae	*Proteus*	*P.vulgaris*

P.vulgaris is a rod-shaped, gramnegative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. It is grouped with theentero bacteriaceae and is an opportunistic pathogen of humans. It is known to cause urinary tract infections and wound infections.

P. vulgaris obtains energy and electrons from organic molecules. It ferments glucose, sucrose, galactose, glycerol and occasionally maltose with gas production, but never lactose; it liquefies gelatin, casein, and blood serum, curdling milk with acid production. It is not limited to any specific temperature range, but good growth occurs at 20°C and 30°C, while growth is poor at 37°C.

P. vulgaris has two interesting features. The cells are highly mobile and swarm across the surface of the agar plates, forming a very thin film of bacteria. When the cells stop and undergo a cycle of growth and division, the swarming periods are interspersed with periods and the colony has a distinct zonation. The other feature is that *P. vulgaris* can produce urease and degrade

urea to ammonia. By alkalinizing the urine, *P. vulgaris* makes the environment more suitable for its survival".

Aspergillusniger

Family	Genus	Species
Trichocomaceae	*Aspergillus*	*A. niger*

"*A. niger*is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is acommon contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould")[15]. Some strains of *A. niger* has been reported to produce potent mycotoxins called ochratoxins[16] but other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true *A. niger*strains do produce ochratoxin A[17]. It also produces the isoflavone orobol".

Growth of bacteria

"Growth of Bacteria is the orderly increase of all the chemical constituents of the bacteria. Multiplication is the consequence of growth. Death of bacteria is the irreversible loss of ability to reproduce.

Bacteria are composed of proteins, carbohydrates, lipids, water and trace elements.

Factors required for bacterial growth

The requirements for bacterial growth are:-

(A) Environmental factors affecting growth

(B) Sources of metabolic energy

A. Environmental factors affecting growth

1. **Nutrients:** Nutrients in growth media must contain all the elements necessary for the synthesis of new organisms. In general the following must be provided : (a) Hydrogen donors and acceptors, (b) Carbon source, (c) Nitrogen source, (d) Minerals : sulphur and phosphorus, (e) Growth factors: amino acids, purines, pyrimidines; vitamins, (f) Trace elements: Mg, Fe, Mn.
2. **pH of the medium:** Most pathogenic bacteria grow best in pH 7.2-7.4.
3. **Gaseous requirement**

   (a) ***Role of oxygen***: Bacteria may be classified into four groups on oxygen requirement.

   (i) Aerobes: They cannot grow without oxygen, e.g. *Mycobacterium tuberculosis.*

   (ii) Facultative anaerobes: These grow under both aerobic and anaerobic conditions. Most bacteria are facultative anaerobes, e.g. *Enterobacteriaceae.*

   (iii)Anaerobes: They only grow in absence of free oxygen, e.g. *Clostridium*,*Bacteroides*.

   (iv)Microaerophils grow best in oxygen less than that present in the air, e.g. *Campylobacter.*

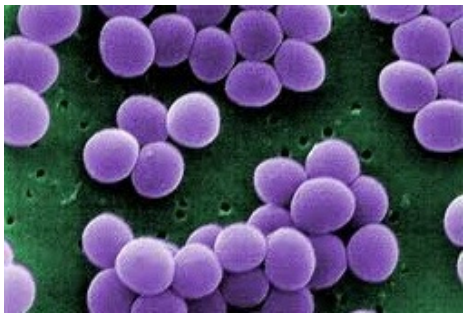
(b) ***Carbon dioxide*:** All bacteria require CO_2 for their growth. Most bacteria produce CO_2. *N. gonorrhoeae* and *N. meningitides* and *Br abortus* grow better in presence of 5 per cent CO_2.

4. **Temperature:** Most bacteria are mesophilic. Mesophilic bacteria grow best at 30-37°C. Optimum temperature for growth of common pathogenic bacteria is 37°C. Bacteria of a species will not grow but may remain alive at a maximum and a minimum temperature.
5. **Ionic strength and osmotic pressure**
6. **Light:** Optimum condition for growth is darkness.

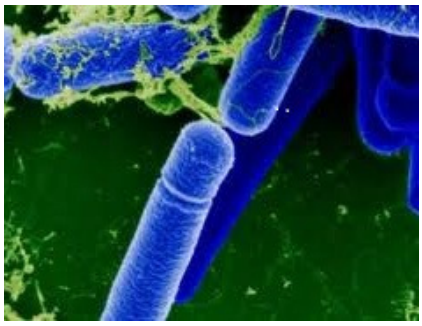
B. Sources of metabolic energy

Mainly three mechanisms generate metabolic energy. These are fermentation, respiration and photosynthesis. An organism to grow, at least one of these mechanisms must be used".

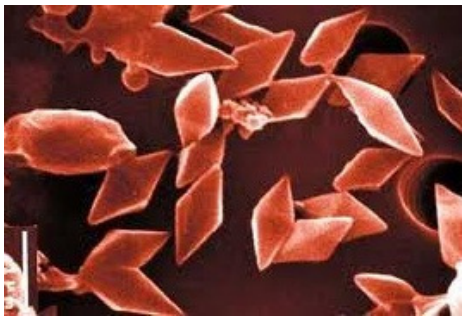
A suitable culture medium for the growth of bacteria should provide all the requirements mentioned above. Water is an unavoidable component for the growth of living cells. The energy required for the bacterial growth may be light from sun or lamps, inorganic substances like ammonia, carbon monoxide or sulphur and organic matters such as fats, proteins, sugars, etc. Without the source of energy, bacteria will die quickly. Protein, nucleic acids, ammonia and nitrogen gas are considered as the main sources of nitrogen, while carbon dioxide, carbon monoxide, methane etc. are the important source of carbon. In addition to these sources, trace amounts of transition metals such as Fe, Zn, Co etc. are essential for the bacterial growth and will be obtained from some enzymes.



Staphylococcus aureus



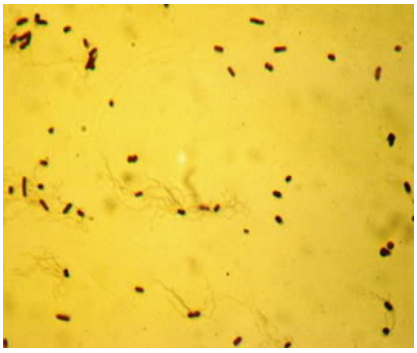
Bacillus subtilis



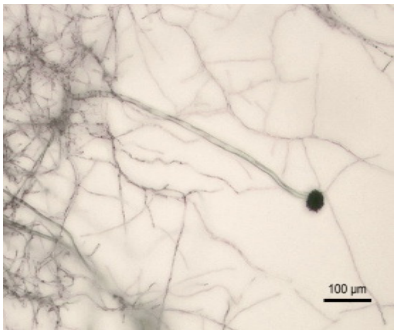
Bacillus thuringiensis

Enterobacteraerogenes

Escherichia coli



Proteus vulgaris



Aspergillusniger

Fig. 3.1: Micrographs of bacteria and fungus

Schiff base and metal complexes as antimicrobial agents - A review

Schiff bases have been pointed to as promising antibacterial agents. "Lots of researchers studied the synthesis, characterization and structure activity relationship (SAR) of Schiff bases. Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum of pharmacological activities with a wide variety of biological properties.The development of a new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemist[18]. They are known to exhibit a variety of potent activities. The pharmacologically useful activities include antibacterial, anticonvulsant, antiinflammatory, anticancer, anti-hypertensive, anti-fungal, antipyretic, antimicrobial, anti-HIV, cytotoxic activity, hypnotic and herbicidal activities[19]. Metal complexes of Schiff bases have been reported as radiopharmaceuticals for cancer targeting and agrochemicals"[20].Literature survey revealed that Schiff bases and their complexes possess marked antibacterial activity.

"Some antimicrobial Schiff bases were reported by Sinha et.al[21] against *Basillussubtilis, Pseudomonas fluroscence, Staphylococcus aureus, Aspergillusniger, Candida albicans*and *Trichophytonrubrum*. The anti microbial activity data shows that 3-(3H-imidazol-4-yl)-2-[(1H-indol-3-yl methylene)-amino]-propionic acidand 2-[(1H-indol-3-yl methylene)-amino]-pentanedioic acidwere inhibited the growth of*S. aureus*and*P. fluorescence* which may be due to higher aromaticity of naphthalein".

Elzahanyet.al[22] have synthesized "some transition metal complexes with Schiff bases derived from 2- formylindole, salicylaldehyde and N-amino rhodanine. The free ligands and their metal complexes were screened for

antimicrobial activities against *Bacillus cerens, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*and *Candida albicans*. The results indicated that the ligands do not have any activity, where as their complexes showed more activity against the same organisms under identical experimental conditions".

The synthesis and antimicrobial activity of a series of "Schiff bases derived from the condensation of 5-chloro-salicylaldehyde and primary amines have recently been reported by Shi et. al[23].Among them (*E*)-4-chloro-2-[(4-fluro-benzylimino)methyl] phenol exhibited favorable anti-microbial activity.Isatinderived Schiff bases have also been reported[24] to possess antibacterial activity".

Yousifet. al[25]"reported some tetra Schiff bases of 1,2,4,5-tetra-(5-amino-1,3,4-thiadiazole-2-yl) benzene and among the synthesized compounds, 1,2,4,5-tetra[5-(4-nitrobenzylideneamino)-1,3,4-thiadiazole-2-yl] benzene was found to be the most potent antimicrobial activity".

Schiff bases with "a 2,4-dichloro-5-fluorophenyl moiety are also effective in the inhibition of bacterial growth. Schiff bases from this class completely inhibited the growth of *S.aureus*, *E. coli*, *P.aeruginosa"*and *K. pneumoniae*[26].Kundariyaet.al[27] presented "a series of novel antimicrobial Schiff bases of 1H-pyrazole [3, 4-*b*] pyridine-3-amine. The substituted Schiff bases against *C. albicans*show highly promising activity".

Debnathet. al[28]"prepared some pyran derivative showing weak activity against grampositive bacteria andno activity against gramnegative bacteria. Further, cyanopyran Schiff's bases showed weak activityagainst grampositive and gramnegative bacteria but lack in antifungal activity. The zones of

inhibition for the pyran and cyanopyran showed lower than the corresponding Schiff's bases, indicating that Schiff bases of pyran and cyanopyran possess more potential antibacterial activity".

Venkateshet. al[29]"synthesized some novel Schiff base complexes of metal ion and reported their antimicrobialand antifungalactivities. Among them Cu^{2+} and Zn^{2+} metal complexes of (*E*)-4-(1-(2, 4-dihydroxyphenyl) ethylidene amino) benzenesulfonamideshowedexcellent activity". Singh et. al[30]"reported the Schiff bases of 5-bromothiophene-2-carboxaldehyde and screened them *invitro*against some bacteria and fungi.Synthesis and pharmacological studies of novel schiff bases of 4-hydroxy 6-carboxyhydrazino benzofuran was reported" by Raoet. al[31].

"4-Chloro-2-oxo-2H-chromene-3-carbaldehyde was reacted with different anilines in rectified spirit to yield a series of Schiff bases of the type 4-chloro-3-(substituted-phenylimino)methyl)-2H-chromen-2-one[32]. These co-mpounds were characterized on the basis of their spectral (IR, ^{1}H NMR) data and evaluated for antimicrobial activity *in vitro* against fungi, gram positive and gram negative bacteria".

Aanandhiet.al[33] have "reported the synthesis of a series of 1-(5-substituted-2-oxoindolin-3-ylidene)-4-(substituted-pyridin-2-yl)thiosemi-carbazide derivatives. These compounds were screened for *in vitro* antibacterial and antifungal activity against *B.subtilis,S. aureus, E.coli, P. aeruginosa, C. albicans,* and *A. niger.* All the compounds were reported to exhibit moderate to good antibacterial and antifungal activity".

Wadheret al.[34]"reported a series of Schiff base of 4, 4'-diaminodiphenylsulphone and substituted 2-azetidinone. Molecular docking

was carried out to identify potential inhibitor of AmpC enzyme of *E. Coli* HKY28 and also screened for antibacterial activity using *S. aureus* and *E. coli"*.In continuation,Wadheret.al[35] reported "some Schiff bases of azetidinone and 4-thiazolidinonederivatives with the aid of structure based computer aided drug designing (CADD). It was founduseful to understand the probable binding of paraaminosalicylic acid on the active site of AmpC enzyme ofHKY28 which will suggest the better insight in the designing of novel analogues as a probable antimicrobialagent".

"Metal complexes of Schiff bases derived from 2-furancarboxaldehyde and o-phenylenediamine and 2-thiophenecarboxaldehyde and 2-amino-thiophenol was reported by Geindyet. al[36]. The synthesized ligands, in comparison to their metal complexes were also screened for their antibacterial activity against bacterial species, *Escherichia coli, Pseudomonas aeruginosa*and *Staphylococcus Pyogones*as well as *fungi (Candida)*".

Mishra et. al[37]"reported new bidentate or tridentate Schiff bases and their VO(II) and Co(II) complexes. Some of the complexes have been screened for their antimicrobial activity on different species ofpathogenic bacteria/fungi, *E. coli, S. aureus, S. fecalis, A. niger,* and*T. polysporum*. All the complexes show higheractivity than the free ligand.A series of novel Schiffs bases of indazolone derivatives were reported by Muthumaniet. al[38] and evaluated the antimicrobial activity against grampositive andgramnegative bacteria".

"Some Schiff bases of 2-amino-5-aryl-1,3,4-thiadiazole derivatives with different aromatic aldehyde were reported and evaluated for their

analgesic, antiinflammatory, antibacterial (*S. aureus* and *E. coli)* and antitubercular activity (*Mycobacterium tuberculosis)"*by Pandey et. al[39]

Balujaet. al[40]"compared Schiff bases derived from sulphonamide and acetophenone and copper, nickel, cobalt and iron complexes of them for antibacterial and antifungal activity. The results concluded that presence of metal causes more inhibition i.e. more activity.Out of the four metals studied, cobalt and iron were found to have more antimicrobial activity".

Hamilet. al[41]"reported Co(II) and Cu(II) complex with Schiff bases derived from o-phenylenediamine and 2-hydroxyacetophonone and evaluated antibacterial activity. Results concluded that more antibacterial activity was shown by complexes than the free Schiff bases".Ronadet. al[42]"prepared a series of 7-(2-substituted phenylthiazolidinyl)-benzopyran-2-one derivatives ofSchiff bases and evaluated antibacterial and antifungal activities against various bacterial and fungal strains. The result showed that compounds exhibited potential antibacterial and antifungal activity as thatof standard antibiotics ciprofloxacin and griseofulvin".

Cheng et. al[43]"reported the synthesis of a series of peptide Schiff bases (PSB). The inhibitory activitiesagainst *Escherichia coli*were investigated *in vitro* andmolecular docking simulation were carried out. Top 10 PSB compounds which possess both good inhibitory activity and well binding affinities were picked out and their antibacterial activities against gramnegative and grampositive bacterial strains were tested, expecting to exploit potent antibacterial agent with broad-spectrum antibiotics activity.The results demonstrated that compound*N*-(3-(5-bromo-2-

hydroxybenzylideneamino)propyl)-2-hydroxybenzamide acted as a potential antibiotic".

"A series of biologically active pyrazinederived Schiff base ligands have been synthesized byChohan et.al[44]by the condensation reaction of 2-aminopyrazine with salicylaldehyde and acetamidobenzylaldehyde. Then their Co(II), Ni(II) and Zn(II) complexes have been prepared. The biological evaluation of the simple uncomplexed ligand in comparison to their complexes has been determined against bacterial strains namely *Escherichia coli, Staphylococcus aureus*and *Pseudomonas aeruginosa.*

Some new (N-indolidene-DL-glycine, N-indolidene-DL-alanine and N-indolidene-DL-valine) amino acid Schiff bases were prepared by Sari[45] by the condensation of indole- 3-carboxaldehyde with DL-glycine, DL-alanine and DL-valine. Antimicrobial activities of the amino acid Schiff bases have been tested against four different microorganisms.

Antibacterial and antifungal activities of mixed ligand transition metal complexes of Cu^{2+}, Ni^{2+} and Co^{2+} ions with Schiff base ligands derived from the condensation of o-hydroxy benzaldehyde with amino phenols and nitrogen donor amine bases was reported" by Islam et. al[46].

"Synthesis, characterization and electrochemical behaviour of Cu(II), Co(II), Ni(II) and Zn(II) complexes derived from acetylacetone and p-anisidine was reported by Raman and coworkers[47]. These authors have observed that the complexes synthesized by them show fairly good antimicrobial activity.

New Schiff bases of the type, 2-[4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N1-(substitutedmethylene)acetohydrazides were synthesized" by

Sathyanarayana[48]via"the condensation of aryl/hetero aromatic aldehydes with 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetohydrazides under conventional and microwave conditions and characterized by IR, ^{1}H NMR and mass spectral data. The synthesized compounds have been screened for antimicrobial activity".

Nair and coworkers[49]"have studied the synthesis and antibacterial activity of some Schiff base complexes derived from 4-ethyl-6-{(*E*)-1-[(3-nitrophenyl)imino]ethyl}benzene-1,3-diol and 4-ethyl-6-{(*E*)-1-[(2-nitrophenyl)imino]ethyl}benzene-1,3-diol.The Schiff bases showed greater activity than their metal complexes.

Synthesis of Schiff bases of napthathiazol-2-amine and metal complexes of 2-(2'-hydroxy)benzylideneaminonapthathiazole as potential antimicrobial agent was reported" by Faizul and coworkers[50].

Rajendran and Karvembu[51]"have reported the synthesis of Schiff bases derived from 3-amino-2H-pyrano [2,3-*b*]quinolin-2-ones. The synthesized Schiff base compounds were screened against the fungalstrains, such as*Aspergillusniger*and *Fusarium sp.* A series of 4-substituted-emoni-methyltetrazolo [1,5-a]quinoline with appropriate aromatic amine by refluxing in dioxane. They evaluated for their antiinflammatory and antimicrobial activities[52].

The *invitro* antibacterial and antifungal activities of five different amino acid Schiff bases derived from the reaction of 2-hydroxy-1-napthaldehyde with glycine, L-alanine, L-phenylalanine, L-histidine, L-tryptophanandthe manganese(III) complexes of these bases were investigated"

by Sakiyanet. al[53] .The *in vitro* activities against some gram positive and gram negative bacteria and fungi were determined.

Matharasiet. al[54]"reported some aloin Schiff bases as antibacterial agents with *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Schiff bases bearing sulfur atom and their corresponding aglycones exhibited stronger activity towards bacteria".

The antifungal behavior of the Schiff base,anthracenecarboxaldehyde L-histidine and its complexes with Cu(II),Co(II),Mn(II) and Ni(II) has been studied by Devi et. al[55].

"A series of transition metal series complexes of Co(II), Ni(II), Cu(II), Mn(II) and Fe(III) have been synthesized by Kulkarni et. al[56] with Schiff base derived from isatinmonohydrazone and fluvastatin. The Schiff bases and their complexes have been screened for their *invitro* antibacterial (*Escherichia Coli, Staphylococcus aureus,Pseudomonas aeruginosa*and *Bacillus subtilis*) and antifungal (*Aspergillusniger*and*PencilliumChrysogenum*) activities.

Antimicrobial activities of the Schiff bases prepared from DL-amino acids (DL-glycine, DL-alanine) and halo aldehydes (5-chloro-2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde) and their Cu(II) and Ni(II) complexes were estimated by Sari et. al[57] for six bacteria, such as *Bacillus cereus* RSKK 863, *Staphylococcus aureus* ATCC 259231, *Micrococcus luteus*NRLL B-4375, *Escherichia coli* ATCC 11230, *Aeromonashydrophila*106, *Pseudomonas aeroginosa*ATCC 29212 and the yeast *Candida albicans*ATCC 10239".

The synthesized cobalt complex with histidine ligandwas evaluated by Sahaet. al[58] for "*invitro* antibacterial and antifungal activity against the multidrug resistant pathogens, such as *Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus, Aspergillusniger, Aspergillusflavus*and *Candida albicans*. The metal complex showed the significant antibacterial and antifungal activity and the results were comparable with the activity of commercial antibiotics".

Raiet. al[59]"synthesized some new Co(II), Ni(II) and Cu(II) complexes with tridentate Schiff base ligand, 3-amino-2-ethylquinazoline-4(3H) thiosemicarbazone (AEQT). The Schiff base and their complexes have been screened for their antibacterial effect by disc diffusion method.

A new series of four transition metal complexes of a Schiff base derived from salicylaldehyde and glycine, viz. [N-salicylideneglycinatodiaqua cobalt(II) dimer] $(SGCo)_2$, [N-salicylideneglycinato-di-aqua-nickel(ll)dimer] $(SGNi)_2$, [N-salicylideneglycinato-aqua-copper(II)] (SGCu) and [N-salicylideneglycinatodiaqua zinc(II) dimer] $(SGZn)_2$ have been synthesized by Islam et. al[60]. These compounds were screened for *in vitro* antibacterial activities against six pathogenic bacteria, such as *Shigellasonnei, Escherichia coli, Bacilussubtilis, Sarcinalutea, Staphylococcus aureus*and *Pseudomonas arioginosa*. The antibacterial activity was determined by the disc diffusion method using DMSO as solvent".

A novel Schiff base ligand and its Ni(II) and Cu(II) complexes were synthesized by Chaudhary[61]and "screened for their *invitro* antibacterial activity against four bacterial pathogens (*E. coli, B.subtilis*, *S.aureus* and *P.*

vulgaris). The results of these studies revealed that the free ligand and its metal complexes showed significant antibacterial potency.

The symmetrical tetradentate Schiff base complexes were prepared by the reaction of the Schiff base ligand derived from 2-hydroxy-1-naphthaldehyde and 5-amino-1-naphthol (1:1 molar ratio) and the metal salt in 2:1 molar ratio. The ligand and its complexes with Cu(II), Zn(II), Ni(II) and Mn(II) ions were screenedusing nutrient agar as medium bySubramanianand Sakunthala[62]for their antibacterial activities against one gram positive bacteria and two gram negative bacteria (*Staphylococcus aureus, Klebsielapneumoniae*and*Escherichia coli*). The zones of inhibition of the synthesized compounds were compared with the free ligand and the standard streptomycin and have been discussed.

New $[ML_2(H_2O)_2]$ complexes, where M= Co(II), Ni(II), Cu(II) and Zn(II) while L corresponds to the Schiff base ligand, were synthesized by condensation of cefotaxime with salicylaldehyde*in situ* in the presence of divalent metal salts in ethanolic medium by Reiss et al.[63]All the synthesized complexes were tested for *in vitro* antibacterial activity against some pathogenic bacterial strains, namely *Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus"*.

Scope of present investigation

The review of the literature showed that Schiff base compounds have been shown to be promising leads for the design of more efficientantimicrobial agents.It was proved that chelation of ligand with a metal will increase its biological activity. A wide spectrum of references listed clearly show that there are only few reports of antimicrobial activity of

polynuclearSchiff base complexes of amino acids and no notable work has been reported on the antimicrobial studies of transition metal complexes of Schiff bases derived from anthracene-9(10H)-one and amino acids.

In the present work, a variety of transition metal complexes derived from six potential Schiff base ligands 3-(anthracen-9(10H)-ylidene-amino)propanoic acid [A9Y3APA], (S)-2-(Anthracen-9(10H)-ylideneamino)-5-guanidinopentanoic acid [A9Y5GPA], (S)-2-(anthracen-9(10H)-ylidene-amino)-3-(1H-imidazole-4-yl)propanoicacid (A9Y3IMPA), (S)-2-(anthracen-9(10H)-ylideneamino)-3-(1H-indole-3-yl)propanoic acid (A9Y3INPA), (S)-2-(anthracen-9(10H)-ylideneamino)-3-phenylpropanoic acid [A9Y3PPA],and (R)-2-(anthracen-9(10H)-ylideneamino)-3-mercapto-propanoicacid [A9Y3MPA]were synthesized and their ability towards the growth inhibition of bacteria and fungi were investigated. Some clinically important bacteria such as*S. aureus, B. subtilis, B. thuringiensis, E. aerogenes, E.coli and P.vulgaris* were used to evaluate the growth inhibition activity of these metal complexes. The results generated were compared with the antibacterial data obtained for standard antibiotics such as erythromycin, streptomycin, gentamicin, ampicillin and penicillin-G. Antifungal activity of these Schiff bases and their transition metal chelates were also subjected to study using the fungus *A. niger*.

CHAPTER 2

MATERIALS AND METHODS

The synthesized Schiff base ligands and their complexes were screened *in vitro* for their microbial activity studies. The micro organisms used were supplied from the stock collection of the Department of Biotechnology, Mercy College, Palakkad, Kerala.

All the glass wares used were borosil. They were washed thoroughly and rinsed with double distilled water. All the tubes and the petridishes were sterilized at 100^0C before preparing the samples using an autoclave.

Antimicrobial agents (AMA)

The following compounds were screened as antimicrobial agents (AMA)

1. A9Y3APA, A9Y5GPA and A9Y3IMPA ligands and their Mn(II), Fe(III), Co(II), Cu(II) and Zn(II) complexes.
2. A9Y3INPA and A9Y3MPA ligands and their Mn(II), Fe(III), Cu(II) and Zn(II) complexes .
3. A9Y3PPA and its Cr(III), Mn(II), Fe(III), Ni(II), Cu(II) and Zn(II) complexes

General methods of antimicrobial sensitivity testing

"Antimicrobial susceptibility testing methods are divided in two types based on the principle applied in each system. They include:

1. Dilution technique
2. Disc diffusion technique

In dilution technique measures the minimum inhibitory concentration (MIC) and can also be used to measure the minimum bactericidal

concentration (MBC) which is the lowest concentration of antimicrobial required to kill the bacteria.

In disc diffusion technique, a disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial agent. This is placed on a plate of sensitivity testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism" is inhibited[64-68] at a distance from the disc that is related to the sensitivity of the organism.

The antibacterial property of the compounds was tested by disc diffusion method as described by Kirby-Bauer method[69].

"The required media for Kirby-Bauer method:

(a) Mueller-Hinton sensitivity agar (MHA)

Composition of MHA:

Beef infusion	-	300gm
Casein acid Hydrolysate	-	17.5gm
Starch	-	1.5gm
Agar	-	17gm
Distilled Water	-	1000ml
pH	-	7.3

Dissolved the ingredients in sufficient quantity of distilled water. Then it was sealed with a sterilized cotton plug and autoclaved at 121^0C for 15 minutes. Cooled for 30minutes and pour the media into 90mm diameter sterile petridish to a depth of 4mm (about 25ml/plate) and kept inside the laminar flow hood chamber. The plates were allowed for solidification and dried.

(b) Nutrient broth for the inoculum preparation

Peptone	-	10g
Beef Extract	-	5g
Yeast extract	-	5gm
NaCl	-	5g
Distilled Water	-	1L
pH (25^0C)	-	7.5 ±0.02

Dissolved the ingredients by heating with distilled water. Dispense each test tube with 5-7 ml amount of the medium which is plugged with non absorbing cotton and autoclaved at 121^0C for 15 minutes".

Preparation of stock solution of AMA for antibacterial screening

Schiff bases and their complexes (AMA) accurately weighed and dissolved in DMSO to yield the required concentration of 100, 250 and 500 µg/disc, using sterile glassware.

Preparation of sample discs

Paper disc method was employed for antimicrobial screening. Samples were applied to paper disc having 5mm diameter (Whatman No: 1) with the help of a micro pipette. Also paper discs were made with DMSO inorder to study the activity of the solvent against each micro organism. The discs were kept in an incubator for 24 hours at 37^0C.

Antibiotic and antifungal standards

The choice of antibiotics and antifungal to be included in sensitivity test will depend on the test organisms, range of locally available and local prescribing policies. Commercially available standards such as erythromycin, streptomycin, gentamicin, ampicillin and penicillin-G were used as standard

antibiotics against all the bacterial strains. Fluconazole and Amphotericin-B were used as the antifungal standards.

Bacterial strains used

1) *Staphylococcus aureus* — *Gram positive*
2) *Bacillus subtilis* — *Gram positive*
3) *Bacillus thuringiensis* — *Gram positive*
4) *Enterobacter aerogenes* — *Gram negative*
5) *Escherichia coli* — *Gram negative*
6) *Proteus vulgaris*. — *Gram negative*

Kirby-Bauer method procedure

1. "Inoculum preparation

   Using a sterile wire loop, touch 3-5 well isolated colonies of test organism (from stock culture) and emulsifies the sterile nutrient broth and incubates the broth at 37^0C for 1hr in an incubator.

2. After the incubation, take out the broth and inoculate the broth into MHA medium using a sterile swab by streak the swab evenly the surface of the medium in three directions rotating the plates approximately 60^0C to ensure the even distribution.

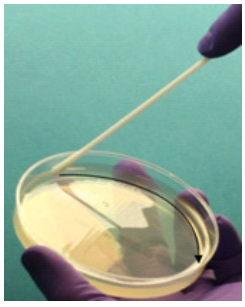


Fig. 3.2: Inoculation of MHA plate

3. Allow to dry 3-5minutes.
4. Using a sterile forceps place the appropriate antimicrobial disc (AMA) evenly distributed on the inoculated plates.

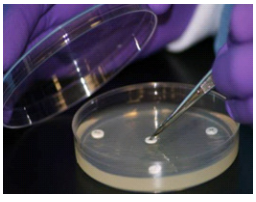


Fig. 3.3: Placement of paper discs

5. Within 30 minutes of applying the disc, invert the plate and incubate it aerobically at 35^0C for 16-18 hrs overnight. Also run a standard plate along with the test plate.
6. After the overnight incubation, examine the test and standard plate growth. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm.

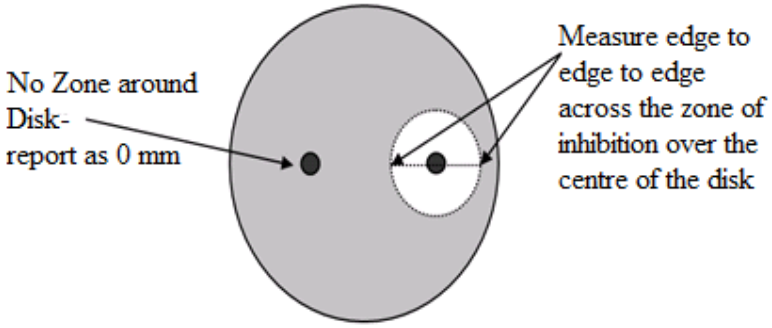


Fig. 3.4: Measurement of zones of inhibition

The inhibition zone formed by the AMA disc against the particular test bacterial strain determined the antibacterial activity of the synthesized ligand and complexes. Therefore, the diameters of zones showing complete inhibition (mm) were compared with standard drug (antibiotic) disc".

All inhibitory tests were performed in triplicate. An average of two independent readings for each compound was recorded.

Antibiotic sensitivity in fungus

The Schiff bases and the complexes (AMA) were also screened their antifungal activities. The fungus used for the studies was *Aspergillus niger*. Disc diffusion method was employed for the activity study.

Disc diffusion technique (Kirby-Bauer method):

"The medium used for the sensitivity testing was the potato dextrose agar (PDA). The sensitivity procedure was done as same as the bacterial antibiotic sensitivity method.

1) potatoes (sliced washed unpeeled) - 200g
2) Dextrose - 20g
3) Agar - 20 g in 1000mL distilled water

Preparations of media and agar plate

200g potato tubers were chopped into small pieces and transferred into a beaker containing 100ml of distilled water. The contents were boiled for twenty minutes and filtered with four folded muslin cloth. This filtrate is called potato extract. 20g dextrose and 15g agar were transferred into the extract and dissolved by gentle heating. This medium was transferred into a measuring cylinder of 1L capacity and made up to 1000ml using distilled water. Then pH of the medium was adjusted to 5.6 by using 0.1N HCl or 0.1N NaOH drop wise. This medium was taken in conical flasks covered by cotton plugs and sterilized in autoclave at 121^0C for 15 minutes. About 20ml of sterilized PDA medium was poured into sterile petridishes and allowed to set".

Preparation of sample discs

Stock solutions of the AMA were prepared as stated earlier. Paper disc method was employed for AMA inoculation. These samples were applied to paper disc having 5mm diameter (Whatman No: 1) with the help of a micro pipette. The discs were kept in an incubator for 24 hours at 37^0C. Before using

the working stock of sample disc, it should be allowed to keep at room temperature about 1hr and protect it from direct sun light.

Antifungal standards

Fluconazole and Amphotericin-B were used as the antifungal standards.

Procedure

The colonies of *Aspergillus niger* (from stock) was spread on the medium using cotton swab method.

- After some time place the antifungal disc having the concentration 100, 250 and 500µgdisc^{-1}.
- The paper disc containing the solvent DMSO was also tested.
- Incubate at 37^{0}C for one week.
- After the incubation analyze for the fungal growth inhibition by measuring the diameter of inhibition zone and the mean of the three replicates are taken.
- Standard antifungas were screened for their growth inhibition activity

.

CHAPTER 3

ANTIMICROBIAL STUDIES ON SCHIFF BASES AND THEIR COMPLEXES

The use of metal chelates as antimicrobial agents is of great importance in the pharmaceutical science due the emergence of drug resistant bacteria. Schiff base complex continues to attract many researchers because of their wide application in antimicrobial activity and pharmacological application. In this context, the present chapter discusses the evaluation of antimicrobial activity of synthesized Schiff bases and their metal complexes. The synthesized six Schiff base ligands A9Y3APA, A9Y5GPA, A9Y3IMPA, A9Y3INPA, A9Y3PPA, A9Y3MPA and their complexes were screened *in vitro* for their microbial activity studies against gram positive (*S. aureus, B. thuringiensis* and *B. subtilis)* and gram negative *(P. vulgaris, E. coli* and *E. aerogenes)* at various concentrations 100µgdisc^{-1}, 200µgdisc^{-1} and 500µgdisc^{-1}. Their zones of inhibition in mm (including the size of the discs) have been recorded after 24 h and tabulated in respective tables. For comparison, the inhibitive power of standard antibiotics was also screened. The zone of diameter of DMSO was subtracted from all the diameter of the zones in order to nullify the effect of DMSO. The details of the results are described below.

Antibacterial studies on the Schiff base A9Y3APA and its metal complexes

Table 3.1 exhibits the antimicrobial activity (diameter of zone of inhibition in mm) of the Schiff base, 3-(anthracen-9(10H)-ylideneamino) propanoic acid [A9Y3APA] and its transition metal complexes. From the table, while comparing the results of ligand and complexes, it is clear that the

zones of clearance of all the complexes were higher than that of ligand. In most of the cases, when the concentration of AMA increases, the ligand and complex become more toxic to microorganisms. The ligand, Mn(II) and Zn(II) complex were not toxic towards *P. vulgaris* for the concentration of 100 and 200µgdisc^{-1}. At a lower concentration of 100µgdisc^{-1} the ligand was also inactive towards *E. coli* and *E. aerogenes*. The maximum activity showed by ligand A9Y3APA was 8mm towards *B. Subtilis* at a concentration of 500µgdisc^{-1}. The maximum zone of inhibition values exhibited by the complexes Mn(II), Fe(III), Ni(II), and Zn(II) were 13mm, 11mm, 12mm and 11mm respectively towards *B. subtilis* at a concentration of 500 µgdisc^{-1}. Copper complex exhibited high zone of clearance towards all microbial growth than that of other complexes. The Cu(II) complex was more active towards *B. thuringiensis* and a zone of clearance of 22mm was shown at a concentration of 500µgdisc^{-1}.

All the complexes exhibited zone of inhibition at a concentration of 500µgdisc^{-1} towards microorganisms even to *S. aureus, B. subtilis* upon which ampicillin and penicillin-G are inactive. Majority of the compounds showed low activity than the standard antibiotics used such as erythromycin, streptomycin and gentamicin. The zone of inhibition of Cu(II) complex towards *B. Subtilis* were comparable with the values of erythromycin at all concentrations. The activity of Cu(II) complex at a concentration of 500µgdisc^{-1} was comparable with standard antibiotic streptomycin at a concentration of 500µgdisc^{-1} towards *B. thuringiensis.* Figure 3.5 shows the antibacterial activity of Cu(II)-A9Y3APA at 100µgdisc^{-1} against *S. aureus*.

Antibacterial studies on the Schiff base A9Y5GPA and its metal complexes

The zone of inhibition values of the Schiff base (S)-2-(Anthracen-9(10H)-ylideneamino)-5-guanidinopentanoic acid [A9Y5GPA] and their metal complexes are described in the table 3.2. From the table it is evident that the ligand was inactive towards *E. coli* and *E. aerogenes* for the concentration of 100 µgdisc^{-1} and *P. vulgaris* for the concentration of 100 and 200µgdisc^{-1}. In many cases the diameter of zone of inhibition increases with increase in concentration of the ligand and complexes. The maximum zone inhibition shown by ligand A9Y5GPA was only 8mm towards *B. subtilis* at a concentration of 500µgdisc^{-1}. All the complexes were responsive to all microorganism and the complexes show greater activity than ligand, except in the case of Mn(II) complex. Among the complexes studied, Cu(II) complex showed the highest antimicrobial activity. The maximum zone of inhibition value of 18mm was exhibited by Cu (II) complex against the growth of *S. aureus* at a concentration of 500µgdisc^{-1}. Other complexes viz. Mn(II), Fe(III), Co(II) and Zn(II) showed maximum zone values of 12mm, 12mm, 16mm and 11mm respectively at a concentration of 500µgdisc^{-1} towards *S. aureus.* Figure 3.6 shows the antibacterial activity of Cu(II)-A9Y5GPA at 100µgdisc^{-1} against *S. aureus.*

Compared to the inhibitive power of antibiotics, the zone of inhibition values of ligand and complexes were very low.

Antibacterial studies on the Schiff base A9Y3IMPA and its metal complexes

The results of growth inhibition of microorganisms by the application of Schiff base (S)-2-(anthracen-9(10H)-ylideneamino)-3-(1H-imidazole-4-

yl)propanoic acid (A9Y3IMPA) and its metal complexes are tabulated in the table 3.3. The table clearly establishes that all the ligand and complexes inhibited the growth of microorganism. The complex and ligands were more active towards gram positive bacteria. The ligand showed maximum zone of inhibition towards *S. aureus* i.e., 15mm at a concentration of 500µgdisc^{-1}. The complexes showed more inhibitive activity than a ligand A9Y3IMPA. Mn(II) complex showed maximum zone value of 16mm towards *S. aureus* and *B. subtilis* at a concentration of 500µgdisc^{-1}. Fe (III), Co(II), Cu(II) and Zn(II) complexes showed greater inhibition values against *B. subtilis* and a zone of 17, 18, 22 and 16mm respectively were exhibited at a concentration of 500µgdisc^{-1}. Co(II), Cu(II) and Zn(II) complexes showed same growth inhibition value, i.e., 11mm at the concentration of 500µgdisc^{-1} against *P. vulgaris.*

The ligand and complexes were better antibacterial agents than ampicillin and penicillin-G against most of the microorganisms. The growth inhibition values of Co(II) and Cu(II) complexes were comparable with the values of standard antibiotic erythromycin, towards microorganism *B. subtilis* at all concentrations. Also the activity of Cu(II) complex towards *E. aerogenes* can be comparable with the inhibitive ability of standard antibiotic streptomycin at all concentrations.

Antibacterial studies on the Schiff base A9Y3INPA and its metal complexes

The antibacterial response of the Schiff base (S)-2-(anthracen-9(10H)-ylideneamino)-3-(1H-indole-3-yl)propanoic acid (A9Y3INPA) and their metal complexes are tabulated in the table 3.4. The growth inhibition response values clearly indicate that ligand and the metal complexes were toxic against

the microorganisms. The ligand showed maximum activity towards the growth of *B. subtilis* i.e., 17mm of diameter zone of inhibition at a concentration of 500µgdisc^{-1}. All the complexes showed higher activity than the ligands with respect to microorganism and concentration. Mn(II) and Zn(II) complexes showed maximum growth inhibition diameter of 18mm towards *B. subtilis* at a concentration of 500µgdisc^{-1}. The Fe(III) and Cu(II) complexes showed more toxicity against *B. subtilis* and the growth inhibition values were 16mm and 23mm respectively at a concentration of 500µgdisc^{-1}. In general the ligand and complexes were more active towards gram positive bacteria.

The activity of Mn(II), Fe(III) ,Cu(II) and Zn(II) complexes were comparable with the activity of standard antibiotic erythromycin towards *B. subtilis* at all concentrations. Also the zone of inhibition value of standard antibiotic streptomycin was comparable with the value of Cu(II) complex towards *E. aerogenes* at all concentrations. Activity of ligand and complexes were higher than the activity of standard antibiotic ampicillin and penicillin-G towards all microorganisms except *B. thuringiensis.* Figure 3.7 shows the antibacterial activity of Cu(II)-A9Y3INPA at 100µgdisc^{-1} against *S. aureus*.

Antibacterial studies on the Schiff base A9Y3PPA and its metal complexes

The diameter of zone of clearance of Schiff base, (S)-2-(anthracen-9(10H)-ylideneamino)-3-phenylpropanoic acid [A9Y3PPA] and their transition metal chelates against various gram positive and gram negative bacteria are presented in the table 3.5. From the results it is evident that ligand A9Y3PPA was inactive towards *P. vulgaris* and *E. coli* at a concentration of 100µgdisc^{-1}. The ligand showed maximum inhibition zone value of 10mm at a concentration of 500µgdisc^{-1} against *B. subtilis.* The Cr(III) complex was

inactive towards *E. coli* at a concentration of 100µgdisc^{-1}. The Mn(II) and Zn(II) complexes were also inactive towards *E. aerogenes* at a concentration of 100µgdisc^{-1}. All the complexes except Zn(II) showed maximum activity against *B. subtilis* at a concentration of 500µgdisc^{-1}. The maximum zone of inhibition values of Cr(III) and Fe(III) are 12mm and Mn(II) shows 13mm. The Ni(II) and Cu(II) complexes showed high inhibition zone of 19mm. The maximum inhibition of growth activity values of Zn(II) complex was 12mm at a concentration of 500µgdisc^{-1} towards *B. thuringiensis*.

The activity of Cu(II) complex was comparable with the growth preventing efficiency of the standard antibiotic, erythromycin towards *B. subtilis* and *E. aerogenes* at a concentration of 500µgdisc^{-1}. Also the activity of Ni(II) complex was comparable with the activity of erythromycin towards *B. subtilis* at a concentration of 500µgdisc^{-1}.

Antibacterial studies on the Schiff base A9Y3MPA and its metal complexes

The zone of inhibition of Schiff base (R)-2-(anthracen-9(10H)-ylideneamino)-3-mercaptopropanoic acid [A9Y3MPA] and their metal complexes against various microorganisms are illustrated in the table 3.6. From the table it is clear that both ligand and complexes possesses activity against different microorganisms. The ligand showed maximum zone of inhibition of 18mm towards *B. subtilis* at a concentration of 500µgdisc^{-1}. Significant activity was shown by almost all complexes towards *B. subtilis* at a concentration of 500µgdisc^{-1}. The growth of inhibition values corresponding to Mn(II), Fe(III), Cu(II) and Zn(II) complexes were 19mm, 21mm, 24mm and 20mm respectively at a concentration of 500µgdisc^{-1} towards the growth of *B. subtilis*.

The antibacterial activity of ligand and the Cu(II) complex were comparable with the activity of antibiotic erythromycin at all concentrations towards *B. subtilis*. The activity of Mn(II), Fe(III), Cu(II) and Zn(II) complexes were comparable with the activity of antibiotic streptomycin at all concentrations towards *E. aerogenes*. The activity of Cu(II) at the concentration of 500µgdisc^{-1} was same as the activity of streptomycin and gentamicin at the concentration of 100µgdisc^{-1} towards *B. subtilis.* The activity of Mn(II) and Cu(II) complexes at the concentration of 500µgdisc^{-1} were comparable with activity of standard antibiotic erythromycin, streptomycin and gentamicin at the concentration of 100µgdisc^{-1} towards the bacterial species *E. coli*. Figure 3.8 shows the antibacterial activity of Cu(II)-A9Y3MPA at 100µgdisc^{-1} against *S. aureus*

Antibacterial studies on the antibiotics

The results of zone of inhibition of antibiotics against each microorganism at different concentrations (100µgdisc^{-1}, 200µgdisc^{-1}, 500µgdisc^{-1}) are listed in the table 3.7. From the table it is evident that majority of the standard antibiotics exhibited appreciable activity against the studied bacterial strains. But ampicillin and penicillin-G showed significant amount of antibacterial activity only against *B. thuringiensis.* Also ampicillin and penicillin-G are inactive towards *S. aureus, B. subtilis, E. aerogenes, E. coli* and *P. vulgaris* at all concentrations. Figure 3.9 shows the antibacterial activity of streptomycin at 100µgdisc^{-1} against *S. aureus*

Discussion

The *in vitro* biological evaluation of complexes against various pathogenic bacterial strains showed that metal complexes are having higher

antimicrobial activity than free ligands. All the complexes studied, exhibited low to moderate activity against *S. aureus, B. thuringiensis* , *B. subtilis, P. vulgaris, E. coli* and *E. aerogenes.* Complexes of A9Y3APA, A9Y5GPA were found to be less active against certain bacterial species even at highest concentration of 500µgdisc^{-1}. Moderate activity was registered in the case of complexes with A9Y3IMPA, A9Y3IMPA, A9Y3PPA and A9Y3MPA for the highest concentration.

Copper complexes exhibited higher antibacterial activity than that of other metal complexes. In general these complexes were more toxic against gram positive bacteria than gram negative bacteria.

The enhancement in the antibacterial activity of many Schiff bases may be rationalized on the basis that it mainly possess C=N bond. The mode of action may involve the formation of a hydrogen bond through the azomethane nitrogen atom with the active centres of the cell constituents, resulting in interference with the normal cell process. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or the difference in ribosomes of microbial cells. It has also been proposed that concentration plays a vital role in increasing the degree of inhibition; as the concentration increases, the activity also increases[70].

Most of the complexes exhibited higher activity than the ligand against studied microorganisms and this activity enhanced on coordination with the metal ions. The antibacterial activity of complexes is influenced by its stability. Lower the stability of the complexes, greater will be the antibacterial activity. This is probably because they have more free ions in the solution,

which can enhance the cooperative interaction between the metal ions and the ligands[71,72].

The enhanced activity of the complexes over the ligand can be explained on the basis of Tweedy's chelation theory[73,74]. It is known that chelation tends to make the ligand act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand. In a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands, and there may be π-electron delocalization over the whole chelation[75]. This increases the lipophilic character of the metal chelate and favours its permeation through the lipid layer of the bacterial membranes. There are also other factors which increase the activity namely, solubility, conductivity and bond length between the metal and the ligand[76] which may explain the values registered in the present case.

The cell of the microbes, in the case of gram + ve is single, layered and in the case of gram –ve is multilayered structure. The weak antibacterial activity against gram negative bacteria was ascribed to the presence of the outer membrane which possesses hydrophilic polysaccharides chains as a barrier to the complexes.

Antifungal studies

Antifungal activity of all the complexes and ligands were determined at three different concentrations 100, 200, 500µgdisc^{-1} on fungus *A. niger*. Plate disc method was adopted for the studies.

From the antifungal studies it was concluded that all Schiff bases and their transition metal chelates were quite inactive against the growth of the fungus *A. niger* at all concentrations.

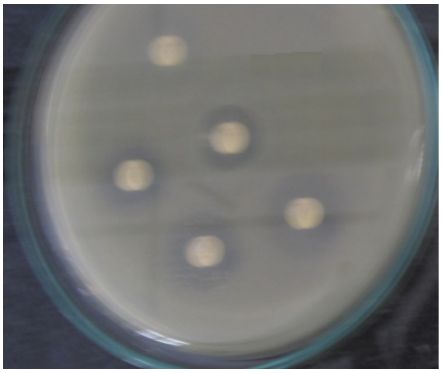


Fig. 3.5: Antibacterial activity of Cu(II)-A9Y3APA at 100µgdisc^{-1} against *S.aureus*

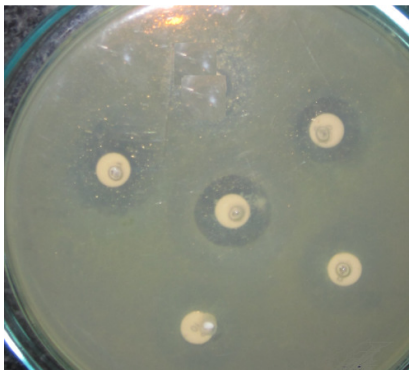


Fig. 3.6: Antibacterial activity of Cu(II)-A9Y5GPA at 100µgdisc^{-1} against *S.aureus*

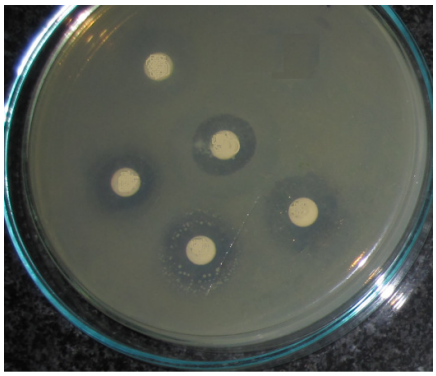


Fig. 3.7: Antibacterial activity of Cu(II)-A9Y3INPA at 100µgdisc^{-1} against *S.aureus*

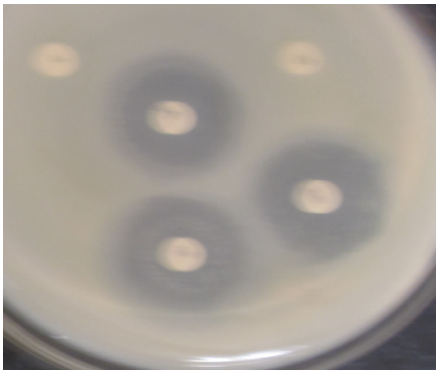


Fig. 3.8: Antibacterial activity of Cu(II)-A9Y3MPA at 100µgdisc^{-1} against *S.aureus*

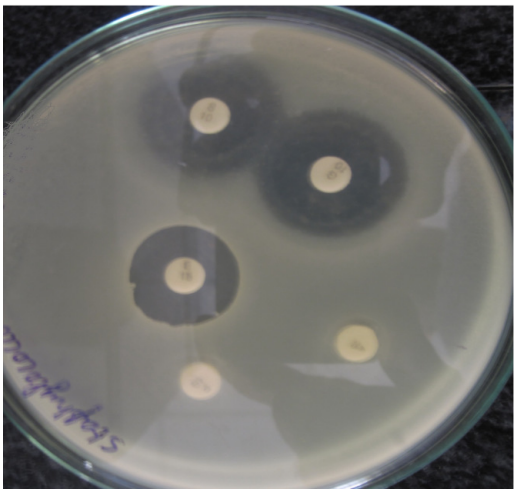


Fig. 3.9: Antibacterial activity of streptomycin at 100µgdisc^{-1} against *S.aureus*

Table. 3.1. Antibacterial activity of the Schiff base, 3-(anthracene-9(10H)-ylidene amino) propanoic acid (A9Y3APA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})																	
	S. aureus			***B. thuringiensis***			***B. subtilis***			***P. vulgaris***			***E. coli***			***E. aerogenes***		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y3APA	1	3	4	2	5	7	4	7	8	0	0	1	0	2	3	0	2	3
Mn(II) complex of A9Y3APA	3	6	9	4	8	11	6	12	13	0	0	3	1	5	4	2	4	5
Fe(III) complex of A9Y3APA	2	5	10	5	7	9	5	9	11	2	6	7	1	5	8	1	3	7
Ni(II) complex of A9Y3APA	3	8	9	6	9	10	9	11	12	4	3	6	2	5	7	1	4	9
Cu(II) complex of A9Y3APA	6	17	18	9	20	22	11	18	19	7	14	15	3	10	12	4	8	9
Zn(II) complex of A9Y3APA	4	6	9	5	7	8	6	8	11	0	0	4	3	4	6	1	3	5

Table. 3.2. Antibacterial activity of the Schiff base, (S)-2-(Anthracen-9(10H)-ylideneamino)-5-guanidinopentanoic acid [A9Y5GPA] and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			***B. thuringiensis***			***B. subtilis***			***P. vulgaris***			***E. coli***			***E. aerogenes***		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y5GPA	1	4	7	1	3	5	2	5	8	0	0	2	0	1	4	0	3	4
Mn(II) complex of A9Y5GPA	3	6	12	1	5	9	4	6	10	2	4	6	0	3	8	1	5	7
Fe(III) complex of A9Y5GPA	5	8	12	2	7	11	3	6	9	1	3	7	1	5	7	2	5	5
Co(II) complex of A9Y5GPA	7	11	16	4	8	12	3	7	10	1	4	8	2	4	8	1	5	9
Cu(II) complex of A9Y5GPA	10	14	18	8	10	16	6	8	13	2	5	8	3	7	9	1	8	11
Zn(II) complex of A9Y5GPA	2	8	11	3	6	7	5	7	9	1	5	6	1	3	5	1	5	7

Table. 3.3. Antibacterial activity of the Schiff base, (S)-2-(anthracen-9(10H)-ylideneamino)-3-(1H-imidazole -4-yl)propanoic acid (A9Y3IMPA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})																	
	S. aureus			*B. thuringiensis*			*B. subtilis*			*P. vulgaris*			*E. coli*			*E. aerogenes*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y3IMPA	7	10	15	5	9	11	7	11	14	4	7	8	3	6	8	5	7	9
Mn(II) complex of A9Y3IMPA	9	12	16	7	11	14	8	13	16	6	9	12	4	8	10	6	9	11
Fe(III) complex of A9Y3IMPA	9	11	12	6	10	13	10	15	17	5	8	9	3	9	9	6	8	10
Co(II) complex of A9Y3IMPA	10	12	15	8	12	14	11	14	18	6	9	11	5	11	13	5	8	9
Cu(II) complex of A9Y3IMPA	11	14	17	11	13	15	13	19	22	9	10	11	8	12	14	7	11	13
Zn(II) complex of A9Y3IMPA	9	12	14	8	11	14	8	12	16	5	8	11	4	9	10	5	9	11

Table. 3.4. Antibacterial activity of the Schiff base, (S)-2-(anthracen-9(10H)-ylideneamino)-3-(1H-indole-3-yl)propanoic acid (A9Y3INPA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (μgdisc^{-1})																	
	S. aureus			***B. thuringiensis***			***B. subtilis***			***P. vulgaris***			***E. coli***			***E. aerogenes***		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y3INPA	8	10	12	9	11	13	10	14	17	6	9	10	4	8	11	5	7	9
Mn(II) complex of A9Y3INPA	9	11	14	10	13	16	11	16	18	8	10	11	6	9	13	6	8	10
Fe(III) complex of A9Y3INPA	9	11	14	10	12	14	11	15	16	8	9	13	5	7	8	6	9	11
Cu(II) complex of A9Y3INPA	11	13	18	12	16	21	15	19	23	9	11	12	10	12	15	8	10	13
Zn(II) complex of A9Y3INPA	10	12	13	11	13	15	12	16	18	7	10	12	5	11	13	6	8	10

Table. 3.5. Antibacterial activity of the Schiff base (S)-2-(anthracen-9(10H)-ylideneamino)-3- phenylpropanoic acid (A9Y3PPA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})																	
	S. aureus			*B. thuringiensis*			*B. subtilis*			*P. vulgaris*			*E. coli*			*E. aerogenes*		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y3PPA	1	4	7	1	6	8	3	7	10	0	1	2	0	3	5	1	3	4
Cr(III) complex of A9Y3PPA	2	6	8	3	8	11	4	9	12	1	3	5	0	5	7	1	6	7
Mn(II) complex of A9Y3PPA	4	8	12	2	7	12	6	9	13	1	4	7	1	6	8	0	4	5
Fe(III) complex of A9Y3PPA	1	5	9	3	8	11	4	8	12	2	2	3	1	4	5	1	4	5
Ni(II) complex of A9Y3PPA	2	9	12	5	9	15	5	11	19	1	6	11	2	7	9	1	5	7
Cu(II) complex of A9Y3PPA	8	10	15	8	11	14	9	13	19	7	9	11	3	8	12	3	9	12
Zn(II) complex of A9Y3PPA	1	6	9	3	10	12	6	11	11	2	4	5	1	5	8	0	5	6

Table. 3.6. Antibacterial activity of the Schiff base, (R)-2-(anthracen-9(10H)-ylideneamino)-3- mercaptopropanoic acid (A9Y3MPA) and its transition metal complexes

Compound	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})																	
	S. aureus			***B. thuringiensis***			***B. subtilis***			***P. vulgaris***			***E. coli***			***E. aerogenes***		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
A9Y3MPA	8	10	14	9	10	16	11	15	18	4	7	9	3	8	11	6	7	10
Mn(II) complex of A9Y3MPA	9	13	20	10	11	18	13	16	19	5	10	14	5	9	14	7	9	12
Fe(III) complex of A9Y3MPA	8	11	18	10	12	17	12	15	21	5	8	11	4	9	11	7	8	12
Cu(II) complex of A9Y3MPA	13	15	22	11	13	21	15	19	24	8	9	12	6	12	15	9	11	13
Zn(II) complex of A9Y3MPA	10	12	19	10	11	17	11	14	20	5	7	10	4	10	12	7	9	11

Table. 3.7. Antibacterial activity of the standard antibiotics and the solvent DMSO

Compound	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})																	
	S. aureus			***B. thuringiensis***			***B. subtilis***			***P. vulgaris***			***E. coli***			***E. aerogenes***		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
DMSO	2	2	4	3	3	4	2	4	5	0	0	0	2	3	4	3	4	4
Erythromycin	15	19	24	28	32	34	11	14	15	22	25	32	15	17	20	10	11	11
Streptomycin	22	24	27	20	26	30	24	28	32	19	27	32	15	17	22	7	9	10
Gentamicin	22	24	25	25	25	25	24	25	27	22	25	28	14	17	20	14	15	17
Ampicillin	0	0	0	28	32	34	0	0	0	0	0	0	0	0	0	0	0	0
Penicillin-G	0	0	0	20	21	24	0	0	0	0	0	0	0	0	0	0	0	0

SUMMARY

Antimicrobial sensitivity of different azomethine compounds (A9Y3APA, A9Y5GPA, A9Y3IMPA, A9Y3INPA, A9Y3PPA and A9Y3MPA) chelated with different transition metals Cr(III), Mn(II), Fe(III), Ni(II), Cu(II) and Zn(II) was studied by Kirby Bauer paper disc diffusion method against gram positive (*S. aureus, B. thuringiensis* and *B. subtilis)* and gram negative *(P. vulgaris, E. coli* and *E. aerogenes)* bacteria and fungus *A. Niger* at various concentrations 100µgdisc^{-1}, 200µgdisc^{-1} and 500µgdisc^{-1}. Antibacterial activity of standard antibiotics like erythromycin, streptomycin, gentamicin, ampicillin and pencillin-G and standard antifungals like fluconazole and amphotericin-B were also screened for comparison. Antimicrobial activity was assessed by measuring the growth inhibition zone diameters.

For almost all tested complex, the diameters of the inhibition zone were superior to those exhibited by the ligand, suggesting that antimicrobial activity of the complexes are clearly superior to that of ligand. The ligand A9Y3MPA exhibited higher diameter zone of inhibition (18mm) than that of other ligands towards *B. subtilis* at the concentration of 500µgdisc^{-1}. The lowest activities were showed by the ligands A9Y5GPA and A9Y3APA towards *B. subtilis* . Among the complexes of various ligands studied, Cu(II) complex of A9Y3MPA shows highest zone of inhibition (24mm) towards *B. subtilis* at the concentration of 500µgdisc^{-1}. The activities of all the copper complexes of the ligands, except that of A9Y5GPA and A9Y3PPA, were comparable with standard antibiotics erythromycin towards *B. subtilis* at all concentrations. Also the activities of Co(II) complex of A9Y3IMPA , Mn(II),

Fe(III), Zn(II) complexes of A9Y3INPA and A9Y3MPA were comparable with standard antibiotics erythromycin towards *B. subtilis* at all concentrations. The activity of Cu(II) and Ni(II) complex of A9Y3PPA (19mm each) were comparable with the growth preventing efficiency of the standard antibiotic erythromycin (15mm) towards *B. subtilis* at a concentration of 500µgdisc^{-1}.

The inhibitive activity of Cu(II) complexes of A9Y3IMPA (7mm,11mm.13mm) and A9Y3INPA (8mm,10mm.13mm) were comparable with standard antibiotic streptomycin (7mm, 9mm, 10mm) towards *E. aerogenes* at 100µgdisc^{-1}, 200µgdisc^{-1} and 500µgdisc^{-1} concentrations respectively. Also the activity of Mn(II), Fe(III), Cu(II) and Zn(II) complexes of A9Y3MPA were comparable with the activity of antibiotic streptomycin at all concentrations towards *E. aerogenes*. The activity of Cu(II) complex of A9Y3MPA (24mm) at the concentration of 500µgdisc^{-1} was same as the activity of streptomycin and gentamicin at the concentration of 100µgdisc^{-1} towards *B. subtilis.* The activity of Mn(II) and Cu(II) complexes of A9Y3MPA (14mm & 15mm) at the concentration of 500µgdisc^{-1} were comparable with activity of standard antibiotic erythromycin, streptomycin and gentamycin (15mm, 15mm and 14mm) at the concentration of 100µgdisc^{-1} towards the bacterial species *E. coli*.

The *in vitro* biological evaluation of complexes against various pathogenic bacterial strains shows that metal complexes exhibited higher antimicrobial activity than free ligands. Such increased activity of the metal complexes can be explained on the basis of 'Chelation theory'. Chelation could enhance the lipophilic character of the central metal atom, which

subsequently favors its permeation through the lipid layers of the cell membranes and blocking the metal binding sites on enzymes of microorganisms. Gram positive bacteria are more susceptible to ligands and complexes than the gram negative bacteria.

Investigations on the antifungal activity of the newly synthesized Schiff bases and their metal complexes pointed out that all the studied compounds are quite inactive towards the growth of the fungal species *A. niger*.

REFERENCES

1) F. Baquero, *J. Antimicrob. Chemother.,* 39(Suppl.A),**1997**, 1
2) M.N. Alekshun, S.B. Levy, *Cell.,* 128 (6), **2007**,1037
3) R.L.B. Unmet, *Biochem. Pharmacol*., 71 (7), **2006**, 991
4) M. M. Nakano, N. Zuber, *Annu. Rev. Microbiol.,* 52,**1998**, 165
5) M. R. Oggioni, G. Pozzi, P. E. Valensin, P. Galieni, C. Bigazzi, *J. Clin. Microbiol.,* 36 (1), **1998**, 325
6) K. J. Ryan, C. G. Ray, *Sherris Medical Microbiology,* McGraw Hill, (4th ed.), **2004**
7) H. Sakai, M.T. Howlader, Y. Ishida, A. Nakaguchi, K. Oka, , K. Ohbayashi, M. Yamagiwa, T. Hayakawa, *J. Biosci. and Bioeng.*, 103, **2007**, 381
8) N. D. Rand, Doctor of Philosophy thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa, **2009**
9) J. Y. Roh, J. Y. C. Li, M. S. Jin, B. R. Je, *J. Microbio. and Botech.,* 17(4), **2007**, 547
10) N. Circkmore. *"Bacillus thuringiensis Toxin Nomenclature"*, **2008**
11) H. D. Donald, *Biotech. Genetic Engg. Reviews*, 2 ,**1984** 341
12) C. C. Beegle, T. Yamamoto, *Canadian Entomologist*, 124 (4), **1992**, 587
13) X. Jian, L. Qin, Y. Xiang-dong, Z. Shu-de, *Entomological J. East China,* 15(1), **2006**, 53
14) *Oxford English Dictionary* (3rd ed.), Oxford University Press, (2001)
15) R. A. Samson, J. Houbraken, R. C. Summerbell, B. Flannigan, J. D. Miller, *Microorganisms in Home and Indoor Work Environments*, New York, **2001**, 287

16) M. Abarca, M. Bragulat, G. Castellá, F. Cabañes, *Appl. Environ. Microbiol.,* 60 (7), **1994**, 2650

17) E. Schuster, N. Dunn-Coleman, J. C. Frisvad, P. W. Van Dijck, *Appl. Microbio. Biotech.,* 59, **2002**, 426

18) B. S. Kumar, K. G. Parthiban, *Asian J. Pharm. Clin. Res.,* 4, **2011**, 137

19) N. Shahabadi, S. Kashanian, F. Darabi, *Eur. J. Med. Chem.,* 45, **2010**, 4239

20) S. Ershad, L. A. Sagathforoush, G. K. nezhad, S. Kangari, *Int. J. Electrochem. Sci.,* 4, **2009**, 846

21) D. Sinha, A. K. Tiwari, S. Singh, G. Shukla, P. Mishra, H. Chandra, A. K. Mishra, *Eur. J. Med. Chem.,* 43, **2008**, 160

22) E. A. Elzahany, K. H. Hegab, S. K. H. Khalil, K. N. S. Youssef, *Austr. J. Basic. Appl. Sci.,* 2(2), **2008**, 210

23) L Shi, H. M. Ge, S. H. Tan, H. Q. Li, Y. C. Song, H. L. Zhu, *Eur. J. Med. Chem.,* 42(4), **2007**, 558

24) S. N. Pandeya, D. Sriram, G .Nath, C. E. De, *Eur. J. Pharm. Sci*., 9(1), **1999**, 25

25) E. Yousif, E. Rentschler, N. Salih, J. Salimon, A. Hameed,; M. Katan, *J. Saudi Chem. Soc.,* **2011**,1

26) M. S. Karthikeyan, D. J. Prasad, B. Poojary, K. S. Bhat, B. S. Holla, N. S. Kumari, *Bioorg. Med. Chem.,* 14(22), **2006**, 7482

27) D. S. Kundariya, B. M. Bheshdadia, N. K. Joshi1, P.K. Patel, *Int. J. Chem. Tech. Res.,* 3, **2011**, 238

28) S. Debnath, V. Mallareddy, S. Y. Manjunath, M. F. Saleshier, *Int. J. Pharm. Sci. and Nanotech.,* 3, **2010**, 1153

29) P. Venkatesh, *Asian J. Pharma. Health Sci.,* 1**, 2011**, 8

30) K. Singh, P. Puri, Y. Kumar, C. Sharma, K. R. Aneja, *Bioinorg. Chem. And Appl.,* **2011**, Article ID 654250,1

31) G. K. Rao, K. N. Venugopala, P. N. S. pai, *J. Pharm. and Toxi.*, 2(5), **2007**, 481

32) S. Bairagi, A. Bhosale and M. N. Deodhar, *E-Journal of Chemistry.*, 6(3), **2009**, 759

33) M. V. Aanandhi, S. George, V. Vaidhyalingam, *ARKIVOC.,* (xi), **2008**, 187

34) S. J. Wadher, M. P. Puranik, N. A. Karande, P.G. Yeole, *Int. J. Pharm. Tech. Res.* 1, **2009**, 22

35) S. J. Wadher, N. A. Karande, S. D. Sonawane, P.G. Yeole, *Int. J. Chem. Tech. Res.* 1, **2009**, 1303

36) G. G. Mohamed, M. M. Omar, A.M. Hindy, *Turk. J. Chem.*, 30, **2006**, 361

37) A. P. Mishra, M. Soni, *Metal-Based Drugs*., 7**, 2008**,1

38) P. Muthumani, R. Meera, S. Venkatraman, Murugan, P. Devi, *J. Chem. Pharm. Res.*, 2, **2010**, 433

39) A. Pandey, D. Dewangan, S. Verma, A. Mishra, R. D. Dubey, *Int. J. Chem. Tech. Res.,* 3, **2011**, 178

40) S. Baluja, A. Solanki, N. Kachhadia, *J. Iranian Chem. Soc.,* **3, 2006**, 312

41) A. M. Hamil, K. M. Khalifa, A. AL-Houni, M. M. El-ajaily, *Rasayan. J. Chem.,* 2, **2009**, 261

42) P. M. Ronad, M. N. Noolvi, S. Sapkal, S. Dharbhamulla, V.S. Maddi, *Eur. J. Med. Chem.,* 45, **2010**, 85

43) K. Cheng, Q. Z. Zheng, Y. Qian,; L. Shi, J. Zhao, H. L. Zhu, *Bioorg. & Med. Chem.,* 17**, 2009**, 7861

44) Z.H. Chohan, S. Mushtaq, *Pakistan J. Pharm. Sci.,* 13(1), **2000**, 21

45) N.G.U. Sari, *J. Sci.*, 16(2), **2003**, 283

46) M.S. Islam, M.A Farooque, M.A.K. Bodruddoza, M.A. Mosaddik , M.S. Alam, *Online J. Biol. Sci.*, 1(8), **2001**, 711

47) N. Raman, V. Muthuraj, S. Ravichandran, A. Kulandaisamy, *Indian Acad. Sci(Chem.Sci).*, 115, **2003,** 161

48) V.S.V. Sathyanarayana, P. Sreevani, A. Sivakumar, V. Vijayakumar, *ARKIVOC*., (xvii), **2008**, 221

49) R. Nair, A. Shah, S. Baluja , S. Chanda, *J. Serb. Chem.Soc.*, 71(7), **2006**, 733

50) A. Faizul, S. Satendra, K.S. Lal, P. Om, *J. University Sci. B.*, 8(6), **2007**, 446

51) S. P. Rajendran, R. Karvembu, *Indian J. Chem.,* 41(B), **2002**, 222

52) S. Bawa, S. Kumar, *Indian J. Chem.,* 48(B), **2009**,142

53) I. Sakiyan, E. Logoglu, S. Arslan, N. Sari, N. Sakiyan, *Biometals.*, 17, **2004**, 115

54) D. S. P. Matharasi,; I. A. A. Yousef, S. Gopi, S. Kumar, B.Vijayakumar, S. Kanan, C. Gunasekar, S. Narasimhan, *Eur. J. Sci. Res.* 43, **2010**, 297

55) G. Indiradevi, G. Parameswaran, V. Veena, *Asian J. Chem.*, 16(1), **2004**, 493

56) A. D. Kulkarni, S. A. Patil, P. S. Badami, *Int. J. Electrochem. Sci*.,4,**2009,** 717

57) N. Sari, P. Gürkan, S. Cete, and I. Sakiyan, *Russian J. Coord. Chem.,* 32(7) **2006***,* 511. doi**:** 10.1134/S1070328406070086

58) S. Saha, D. Dhanasekaran, S. Chandraleka, A. Panneerselvam, *Phy. Chem. and Tech*., 7(1), **2009**, 73. doi: 10.2298/FUPCT0901073S

59) B.K. Rai, R. Kumari, *Oriental J. Chem.*, 29(3), **2013**, 1163

60) M. N. Islam, S. M. S. Shahriar, M. K. Islam, M. Jesmin, M. M. Ali, J. A. Khanam, *Int. Lett. Chem., Phy., and Astr.* 5, **2013**, 12

61) N. K. Chaudhary, *Archi. of Appl. Sci. Res.,* 5 (6), **2013**, 227

62) P. Subramanian, M. Sakunthala, *World J. Pharmacy and Pharmaceutical Sci.,* 2 (5), **2013**, 2753

63) A. Reiss, M. C. Chifiriuc, E. Amzoiu, C. I. Spînu, *Bioinorg. Chem. and Appli.*, **2014**, Article ID 926287, DOI: org/10.1155/2014/926287

64) C. Limban, M. C. Chifiriuc, *Int. J. Molec. Sci.*, 12(10), **2011**, 6432

65) C. Limban, A. V. Missir, I. C. Chirita, *Chemical Papers*, 65 (1), **2011**, 60

66) C. Limban, L.Marutescu, M. C. Chifiriuc, *Molecules*, 16 (9), **2011**,7593

67) R. Olar, M. Badea, D. Marinescu, *Euro. J. Medi. Chem.*, 45(7), **2010**, 2868

68) G. M. Nitulescu, C. Draghici, M. C. Chifiriuc, L. Marutescu, C. Bleotu, A. V.Missir, *Medi. Chem. Res.*, 21, **2012**, 308

69) A. W. Bauer, W. M. M. Kirby, J. C. Sherries, M. Truck, *Am. J. Clin. Path.,* 44, **1966**, 493

70) Z. H. A. El-Wahab, M. M. Mashaly, A. A. Salman, B. A. El- Shetary, A. A. Faheim, *Spectrochim. Acta A*, 60 (12), **2004**, 2861

71) A. Marcu, A. Stanila, O. Cozar, L. David, *J. Optoelectron. Adv. M* 10(4), **2008**, 830

72) A Stanila, A Marcu, D Rusu, M Rusu, L David., *J. Mol. Struct*. 834, **2007**, 364

73) B.G. Tweedy, *Phytopathalogy,* 55, **1964**, 90

74) A. K. Sharma, S. Chandra, *Spectrochim. Acta A*, 78 (1), **2011**, 337

75) Z. H. Chohan, M. Arif, M. A. Akhtar, C. T. Supuran, *Bioinor. Chem. and Appli.*, **2006**, Article ID 83131

76) K. R. K. Reddy, P. Suneetha, C. S. Karigar, N. H. Manjunath, K. N. Mahendra, *J. Chil. Chem. Soc.*, 53(4) **2008**, 1653