CHAPTER - 5
ANTIMICROBIAL ACTIVITIES
5.1 General
Bactericidal drugs kill bacteria and bacteriostatic drugs slow or stop the bacterial growth. These definitions are not absolute; bacteriostatic drugs may kill some bacteria, and bactericidal drugs may not kill all of the bacteria in vitro. More precise quantitative methods identify the minimum in vitro concentration at which an antibiotic can inhibit growth (minimum inhibitory concentration, or MIC) or kill (minimum bactericidal concentration, or MBC).

5.1.1 Methods of antimicrobial susceptibility testing
Antimicrobial susceptibility testing methods are divided into three types based on the principle applied in each system. They include:

- Diffusion
- Dilution
- Diffusion and Dilution

- Stokes method
- Minimum Inhibitory Concentration
- E-Test method

- Kirby-Bauer method
  - i) Broth dilution
  - ii) Agar dilution

5.1.2 Diffusion methods
In the disk diffusion test (also called the Kirby Bauer test), disks containing an antimicrobial agent are placed on the surface of an agar plate containing a medium that has been inoculated with the disease agent being tested, which will grow and fill the disk. The antimicrobial agent diffuses into the medium, killing some of the disease agent around where the antimicrobial agent was inoculated, depending on how susceptible the disease agent is to the antimicrobial agent. The size of the area cleared of the disease agent shows how effective the antimicrobial agent is.

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the
NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures.

5.1.3 Dilution methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. The way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned by any of the dilution methods.

There are two methods of testing for MIC:

(a) **Broth dilution method (Double dilution method):**

The Broth dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also.

(b) **Agar dilution method:**

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate. If only one organism is to be tested e.g. *M. tuberculosis*, the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism. The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60 °C. Blood may be added and if ‘chocolate agar’ is required, the medium must be heated before the antibiotic is added.
5.1.4 Dilution and diffusion (E-Test)

E test also known as the epsilometer test is an ‘exponential gradient’ testing methodology where ‘E’ in E test refers to the Greek symbol epsilon (ε). The E test (AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation, a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy.

E test can be used to determine MIC for fastidious organisms like S. pneumoniae, β-hemolytic streptococci, N. gonorrhoeae, Haemophilus sp. and anaerobes. It can also be used for nonfermenting Gram negative bacilli (NFGNB) for e.g.-Pseudomonas sp. and Burkholderia pseudomallei.

5.2 Bacteria

Bacteria are the large group of unicellular microorganisms. There are approximately five nonillion (5×10^{30}) bacteria on earth typically a few micrometers in length, wide range of shapes, ranging from spheres to rods and spirals. Bacteria are ubiquitous in every habitat on earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the earth's crust, as well as in organic matter and the live bodies of plants and animals. Most people have been conditioned to think of bacteria as harmful, but the reality is that we live symbiotically with beneficial intestinal bacteria. An average person has about four pounds of bacteria in the body. Majority of these bacteria reside in digestive tract. Delicious cheese or yogurt people love to take so much is actually a product of helpful bacteria growing in milk. Examples of helpful bacteria also include; *E. coli* which helps in digestion and its rod shaped *rhizobium* that plays a vital role in nitrogen fixation, *Streptomycin* is also one
of them, which are mainly used in manufacturing of antibiotics. These organisms are also helpful in production of gases such as methane which is actually 20% of the natural gas deposit on Earth.

Bacteria are generally classified on three bases: (1) Their response to oxygen. (2) Source of their energy. (3) Cell wall structure.

1. Their response to oxygen

   A) Aerobic bacteria thrive in the presence of oxygen and require it for their continued growth and existence.

   B) Anaerobic bacteria that cannot tolerate gaseous oxygen, such bacteria live in deep underwater sediments and can cause bacterial food poisoning. The most notorious of the anaerobic pathogens are the clostridia—spore-forming, gram-positive bacilli found widely in dust, soil, and vegetation and as normal flora in mammalian gastrointestinal tracts.

   C) Facultative anaerobes, which prefer growing in the presence of oxygen, but can continue to grow without it.

2. Source of their energy

   A) Heterotrophs, derive energy by breaking down the complex organic compounds that they intake from the environment, this includes saprobic bacteria found in decaying material, as well as those that rely on fermentation or respiration.

   B) Autotrophs, fix carbon dioxide to make their own food source; they may be fueled by light energy (photoautotrophic), or by oxidation of nitrogen, sulfur, or other elements (chemoautotrophic) but chemoautotroph are uncommon, photoautotroph are common and quite diverse. Hence the ecosystem, both on land and in the water, depends heavily upon the activity of bacteria by their ceaseless labor. They complete cycles of nutrients such as carbon, nitrogen, and sulfur.

3. Cell wall structure

   A) Cell membrane provides cells, structural support, protection, and also acts as a filtering mechanism. A major function of the cell wall is to act
as a pressure vessel, preventing over-expansion when water enters the cell. They are found in almost all the type of cells of plants, bacteria, fungi, algae, but some arches animals and protozoa do not have cell walls. The materials in a cell wall vary from species to species. In plants, the strongest component of the complex cell wall is a carbohydrate called cellulose, which is a polymer of glucose. In bacteria, peptidoglycan forms the cell wall.

B) Gram-positive cells, peptidoglycan makes up as much as 90% of the thick, compact cell wall, which is the outermost structure of Gram + cells.

C) Gram– negative bacteria are more chemically complex, thinner and less compact. Peptidoglycan makes up only 5 – 20% of the cell wall, and is not the outermost layer, but lies between the plasma membrane and an outer membrane.

The following microorganisms have been used for the antibacterial study.

5.3 Gram-positive microorganism

5.3.1 Staphylococcus aureus (S. aureus):
Staphylococcus aureus is a bacterial species named from Greek σταφυλόκοκκος meaning the "golden grape-cluster berry". Also known as "golden staph" and Oro staphira, it is a facultative anaerobicGram-positivecoccalbacterium, which was first discovered in the pus of surgical abscesses by Sir Alexander Ogston in 1883 [1]. The golden appearance is the etymological root of the bacteria's name: aureus means "golden" in Latin. Staphylococcus aureus is a "Golden Cluster Seed" and also known as golden staph. S. aureus frequently colonizes the skin and has a niche preference for the anterior nares of the nose, with persistent nasal carriage occurring in 25–30% of the population. Staphylococcus aureus is one of the most prevalent organisms responsible for nosocomial infections and cases of community-acquired S. aureus infection have continued to increase despite widespread preventative measures [2].
S. aureus can cause a wide variety of diseases ranging from skin infections such as impetigo and folliculitis to life-threatening diseases such as severe haemorrhagic pneumonia, meningitis, toxic shock syndrome and septicaemia. 

*Staphylococcus aureus* is an opportunistic Gram-positive bacterium which causes a wide variety of diseases ranging from minor skin infections to potentially fatal conditions such as pneumonia, meningitis and septicaemia. In recent years, the emergence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA), defined as having a minimum inhibitory concentration for oxacillin of 4 µg/mL or greater, was first described in 1961. Methicillin-resistant *S. aureus* (MRSA), which has broad resistance to β-lactam antibiotics, including penicillins and cephalosporins [4].

In 1996, a *S. aureus* strain with intermediate resistance to vancomycin (VISA) (vancomycin MIC= 8µg /ml) was first isolated from a patient in Japan. In June 2002, the World’s first reported clinical infection due to *S. aureus* with high resistance to vancomycin (VRSA) (vancomycin MIC>128 µg /ml) was diagnosed in a patient in the USA. This isolate contain the vanA genes from enterococci and the methicillin-resistance gene mecA. Till today only five VRSA have been found all over the world. First in USA in 2002 , second in Michigan in 2002, third in Pennsylvania in 2002, fourth in New York in 2004, fifth in New York in 2005, and the sixth in Kolkata (India) in 2005 [5].
The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline and aminoglycosides has made the therapy of staphylococcal disease a global challenge. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA [6]. There is one ongoing trial comparing co-trimoxazole with vancomycin for MRSA bacteriaemia. Tigecycline (currently the only available intravenous tetracycline derivative in the UK) is a potentially useful drug for certain types of MRSA infection, although its low blood levels probably preclude its use for primary bacteraemia [7]. Today, S. aureus has become resistant to many commonly used antibiotics such as penicillins, cephalosporins, carbapenems, oxacilline, vancomycin, streptomycin, kamamycin and gentamicin [8].

5.3.2 Bacillus subtilis:

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium commonly found in soil [9]. Gram-positive organisms in general occupy a variety of habitats, ranging from soil and water to decomposing plants and mammalian gut or oral flora, thereby also being potentially pathogenic [10]. A member of the genus Bacillus, B. subtilis is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Unlike several other well-known species, B. subtilis has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct [11]. B. subtilis is only known to cause disease in severely immunocompromised patients, and can conversely be used as a probiotic in healthy individuals. It may contaminate food but rarely causes food poisoning [12].

In 1835, the bacterium was originally named Vibrio subtilis by Christian Gottfried Ehrenberg, and renamed Bacillus subtilis by Ferdinand Cohn in 1872 [13]. B. subtilis subsp. spizizenii strain gtP20b was isolated from the sediment...
at a 608-m depth in the Indian Ocean and from the layer close to the bottom surface of the ocean. The sampling was taken through a multicorer during the cruise of the research ship Sonne on expedition 130 in 1998 and stored at -20°C. [14].

**Bacillus subtilis**

*Bacillus subtilis* is a member of the Gram-positive bacteria of the genus *Bacillus* and has been used as a model organism to investigate differentiation, gene/protein regulation and cell cycle events in bacteria for more than a century [15]. *B. subtilis* is used to produce a variety of enzymes, including amylase, which is helpful in the de-sizing of textiles and starch modification for the sizing of paper. *B. subtilis* also produces the enzyme protease, including subtilisin, which is used in detergents and the leather industry. Perhaps more notably, *B. subtilis* is used to produce many antibiotics, such as difficidin, oxydifficidin, bacilli, bacillomyin B, and Bacitracin, which is helpful in treating bacterial skin infections and preventing infection in minor cuts and burns [16].
5.4 Gram-negative microorganism

5.4.1 Serratia marcescens:

*Serratia marcescens* is a species of Gram-negative bacterium in the family Enterobacteriaceae. *Serratia marcescens* was discovered in 1819 by Venetian pharmacist Bartolomeo Bizio, as the cause of an episode of blood-red discoloration of polenta in the city of Padua [17]. Bizio named the organism four years later in honor of Serafino Serrati, a physicist who developed an early steamboat; the epithet *marcescens* (Latin for "decaying") was chosen because of the pigment's rapid deterioration (Bizio's observations led him to believe that the organism decayed into a mucilage-like substance upon reaching maturity).

Until the 1950s, *S. marcescens* was erroneously believed to be a nonpathogenic "saprophyte" and its reddish coloration was used in school experiments to track infections. It has also been used as a simulant in biological warfare tests by the United States Military. Since 1950, *S. marcescens* has steadily increased as a cause of human infection, with many strains resistant to multiple antibiotics [18]. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. Other strains of *Serratia* include *Serratia plymuthica*, *Serratia liquefaciens*, *Serratia rubidaea* and *Serratia odorifera* and among all the strains. *S. marcescens* is known to causediscase [19].

![Serratia marcescens](image)
Due to its ubiquitous presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found growing in bathrooms (especially on tile grout, shower corners, toilet water line, and basin), where it manifests as a pink discoloration and slimy film feeding off phosphorus-containing materials or fatty substances such as soap and shampoo residue.

*S. marcescens* may also be found in environments such as dirt, supposedly "sterile" places, and the subgingival biofilm of teeth. Due to this, and the fact that *S. marcescens* produces a reddish-orange tripyrrole pigment called prodigiosin, *S. marcescens* may cause extrinsic staining of the teeth.

### 5.4.2 Escherichia coli:

The genera *Escherichia* and *Salmonella* diverged around 102 million years ago (credibility interval: 57–176 mya), which coincides with the divergence of their hosts: the former being found in mammals and the latter in birds and reptiles [20]. This was followed by a split of the escherichian ancestor into five species (*E. albertii*, *E. coli*, *E. fergusonii*, *E. hermannii* and *E. vulneris*). The last *E. coli* ancestor split between 20 and 30 mya [21].

In 1885, Theodor Escherich, a German pediatrician, first discovered this species in the feces of healthy individuals and called it *Bacterium coli commune* due to the fact it is found in the colon and early classifications of Prokaryotes placed these in a handful of genera based on their shape and motility. Following a revision of bacteria it was reclassified as *Bacillus coli* by Migula in 1895 and later reclassified in the newly created genus *Escherichia*, named after its original discoverer [22]. The genus belongs in a group of bacteria informally known as "coliforms", and is a member of the *Enterobacteriaceae* family ("the enterics") of the Gammaproteobacteria.

*Escherichia coli* commonly abbreviated *E. coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally
responsible for product recalls [23]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K₂ and by preventing the establishment of pathogenic bacteria within the intestine [24].

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_Escherichia coli_

_E. coli_ is a group of bacteria that populate the human (and animal) gut. _E. coli_ is usually thought of as a "good" bacterium; it lives in the intestines as part of the normal "gut flora." A number of genera within the family are human intestinal pathogens (e.g. _Salmonella, Shigella, Yersinia_).

Pathogenic strains of _E. coli_ are responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis).

_5.4.3 Pseudomonas aeruginosa:_

_Pseudomonas aeruginosa_ is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility [25]. An opportunistic human pathogen, _P. aeruginosa_ is also an opportunistic pathogen of plants. _P. aeruginosa_ is the type species of the genus _Pseudomonas_. The word _Pseudomonas_ means "false unit", from the Greek _pseudo-_ (Greek: 'false') and _monas_ (Latin: _monas_, from Greek: 'a single unit'). The stem word _mon_ was used early in the history of microbiology to
refer to germs, e.g. Kingdom Monera. The species name aeruginosa is a Latin word meaning "copper rust" as seen with the oxidized copper patina on the Statue of Liberty.

Although classified as an aerobic organism, P. aeruginosa is considered by many as a facultative anaerobe as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate as a terminal electron acceptor and in its absence it is also able to ferment arginine by substrate-level phosphorylation.

P. aeruginosa is frequently isolated from non-sterile sites (mouth swabs, sputum, and so forth) and under these circumstances; it often represents colonization and not infection. The isolation of P. aeruginosa from non-sterile specimens should therefore be interpreted cautiously and the advice of a microbiologist or infectious diseases physician should be sought prior of starting treatment. Often no treatment is needed.

When P. aeruginosa is isolated from a sterile site (blood, bone, deep collections), it should be taken seriously and almost always requires treatment.

Pseudomonas aeruginosa

P. aeruginosa is naturally resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment, particularly
through modification of a porin. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically.

In fact, *P. aeruginosa* is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed.

5.5 Literature survey

Boney V. K. J., et. al. reported antibacterial activities of 2-hydroxynaphthaldehyde, 2-aminophenol and its complexes with nickel and cobalt [26]. Antimicrobial activity of the ligands and their metal (Co(II), Ni(II), Cu(II) and Zn(II)) complexes against bacteria and fungus has been carried out by Shakru R. et. al. [27]. Antibacterial effect of Cu(II), Ni(II), Co(II), Mn(II) and Fe(III) complexes were studied by Munde A. S., et. al. [28]. In vitro antibacterial activity of macrocyclic complexes against five bacteria has been done by Sharma R., et. al. [29]. Sheikh R. A., et. al. [30] had done antifungal and antibacterial studies performing Minimum Inhibitory Concentration (MIC80), Disc diffusion assay and WST1 cell cytotoxicity assay. Varghese S., et. al. [31] reported the 2-hydroxybenzilidene-3-aminophenol Complexes and its antimicrobial activities checked against four different bacterial species *E. coli, B. subtills, S. auereus* and *P. aeruginosa*.

Ayaloor S. R., et. al. [32] synthesized the complexes of Cu(II), Ni(II), Co(II), Pd(II) and screened for their biological activity on several pathogenic bacteria. Prakash A., et. al. [33] has been developed bacteria and note growth of bacteria in vitro to evaluate their anti-microbial potential. The complexes of N (1-morpholinosalicylyl)acetamide of Cu(II), Co(II), Ni(II) and Zn(II) showed
good antimicrobial activity against bacteria reported by SathyaD., et. al. [34]. Complexes of 2-hydroxy-1-naphthylidene-2-amino-5-nitrothiazole with Cr(III), Co(II), Ni(II) and Cu(II) and their antibacterial activity was carried out by Jain R. K. et. al. [35]. Prakash A., et. al. [36] reported that complexes of nickel(II) of o-hydroxyacetophenone ethylene diamine showed growth against bacteria in vitro to their anti-microbial potential.

Antimicrobial activity of Cu(II), Ni(II), Co(II), Fe(III), Zn(II) and Mn(II) complexes with their Schiff bases studied by Kulkarni P. A., et. al. [37]. Antimicrobial Properties of Mn(II), Cu(II), Zn(II), and Pd(II) complexes of nitrophenol Schiff Base was studied by Osowole A. A., et. al. [38]. Gulcan M., et. al. [39] has been reported antimicrobial activity of Co(II) and Cu(II) against bacteria. Antimicrobial activity of Ni(II) complexes were derived from 2-substituted benzothiazole ligands studied by Premlata et. al. [40].

The complex, ligand and some of its heterobinuclear complexes showed antibacterial activity against the sensitive organisms Staphylococcus aureus as Gram–positive bacteria, Escherichia coli as Gram–negative bacteria and antifungal activity against the fungi Candida albicans and Aspergillus flavus were studied by Khalil S. M. E. et. al. [41]. The antibacterial activity of the 2'-hydroxyacetophenones and 3, 5 dibromosalicylaldehyde and their metal complexes against E.coli, S. typhi, S. aureus, B. subtilis were studied by Habib S. I. et. al. [42]. Chaudhary R., et al. [43] were synthesized ligands and its complexes with Cu(II), Ni(II) and Co(II) and it was screened for antibacterial activity against S.aureus, B.megaterium, B.cereus and E.coli by cup-plate method. Mapari A. K., et. al. [44] studied the antimicrobial activities of ligands and their mixed ligand complexes were screened by disc diffusion method. Siddiki A. K. M. N. A. et. al [45] reported antibacterial and antifungal activities against some pathogenic bacterial and fungal strains by using disc diffusion method. Sabastiyan A., et. al. [46] reported antimicrobial activity of the ligand and its complexes has been studied by agar-well diffusion method.
5.6 Experimental techniques

For the evaluation of invitro antibacterial activity the following three conditions must be fulfilled. First, the substance to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated. Secondly, favourable conditions (nutritional, environmental etc.) must be provide to offer a maximum opportunity for optimum growth of the organisms in absence of antimicrobial agent and thirdly, there should be a method for measuring antibacterial response obtained by antimicrobial agent.

Various method has been proposed and adopted for measurement of antibacterial activity. These are-

1. Agar diffusion (cup, paper, disc, cylinder) method.
2. Turbidometric method.
3. Special dilution method.

The method used for the present study was Agar well diffusion method. Following organisms were used:

1. *Staphylococcus aureus*
2. *Bacillus subtilis*
3. *Serratia marcescens*
4. *Escherichia coli*
5. *Pseudomonas aeruginosa*

(A) Media

Nutrient Agar medium

Composition:

- Beef Extract 1.5 g
- Peptone 6.0 g
- NaCl 1.5 g
- Agar 20 g
- Distilled water 1000 ml
P^H adjusted to 6.2 – 7.2

Agar was dissolved and 10 ml quantity was distributed in to test tubes.

Above media were found to be suitable for the growth of three the organisms used in present work.

(B) Slant preparation
Nutrient agar media were dissolved in distilled water were and were sterilized by autoclaving. About 5 ml of molten medium was transferred in previously sterilized test tubes. The test tubes were then plugged tightly and were placed in a slanting position to cool and solidify.

(C) Stock culture
Culture was grown on the Nutrient agar slants by incubating them for 24 hours at 37°C.

(D) Culture dilution [Sub-Culturing]
One loopful of stock culture was added to 5 ml of neutrient broth media for inoculated broth was inoculation. This inoculated broth was incubated for 24 hours at 37°C. For all experimental purpose, 24 hours fresh diluted cultures of both the organisms were used.

(E) Preparation of the solution
Antibacterial activity is usually tested by making aqueous solution of the compounds. However, complexes used in the present study were insoluble in water, soluble in DMSO. Therefore, to study antimicrobial activity of the complexes their dilutions were prepared by using DMSO. DMSO may have some antimicrobial activity, therefore blank of DMSO was taken and tested. To check the potency of the compounds, the solution was prepared with 10,000 µg/ml concentration.

(F) Agar well diffusion method
Each time fresh sterile nutrient agar medium was prepared. The proceeding was carried out aseptically. All the apparatus in need were sterilized.
In each sterile Petridis 15-20 ml of molten media was added. Stock cultures of bacteria were prepared in sterile distilled water. 1000µg/mL from each culture tube was inoculated into top agar tube and mixed properly. The top agar then overlayed on N-agar plates. The plates were allowed to solidify at room temperature. Then the cups or agar wells were made with the help of sterilized stainless steel borer. Solutions of test compounds were added to the agar cups/wells. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24 hours. The diameter of the zones of inhibition was measured.

All the testing was done in replicate.

5.7 Results and discussion

Metal complexes exhibit higher biocidal activity as compared to the free ligands, control (DMSO) and good activity compare to standard drugs antibacterial standard Streptomycin. All the ligands exhibit a little antimicrobial activity. The antimicrobial activity of the ligands against all the microorganisms viz., Staphylococcus aureus, Bacillus subtilis, Serratia marcescens, Escherichia coli, Pseudomonas aeruginosa are much lower than standard drugs i.e., Streptomycin. From comparative analysis it is observed that all the metal complexes are more potent antimicrobial activity than the ligands. This improvement in activity of complexes is also be rationalized on the basis of their structure activity relationship. The zone of inhibition was measured (in mm) around the disc and the results are represented in Table I to XI (Series A to Series K).
Series-A

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde) ethylenediamine (ONap-en) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

$$\text{Fe(II)} < \text{Cu(II)} < \text{Ni(II)} < \text{Mn(II)} < \text{Co(II)} = \text{Zn(II)}$$

In the case of bacteria *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde) ethylenediamine (ONap-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

$$\text{Ni(II)} < \text{Cu(II)} < \text{Mn(II)} < \text{Fe(II)} < \text{Zn(II)} < \text{Co(II)}$$

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ethylenediamine (ONap-en) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

$$\text{Mn(II)} = \text{Fe(II)} < \text{Ni(II)} < \text{Co(II)} < \text{Zn(II)} < \text{Cu(II)}$$

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ethylenediamine (ONap-en) against bacteria - *P. aeruginosa*.

$$\text{Cu(II)} < \text{Fe(II)} = \text{Co(II)} < \text{Zn(II)} < \text{Mn(II)} < \text{Ni(II)}$$

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Subtilis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

Series A: Table -I Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>Bacillus Subtilis</td>
<td>S. marcescens</td>
<td>P. aeruginosa</td>
<td>E. Coli</td>
</tr>
<tr>
<td>ONap-en</td>
<td>14</td>
<td>15</td>
<td>07</td>
<td>08</td>
<td></td>
</tr>
<tr>
<td>MeBen-en</td>
<td>12</td>
<td>11</td>
<td>07</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>[Mn(ONap-en)(MeBen-en)]·H2O</td>
<td>20</td>
<td>18</td>
<td>09</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>[Fe(ONap-en)(MeBen-en)]·H2O</td>
<td>17</td>
<td>20</td>
<td>09</td>
<td>09</td>
<td></td>
</tr>
<tr>
<td>[Co(ONap-en)(MeBen-en)]·H2O</td>
<td>24</td>
<td>23</td>
<td>11</td>
<td>09</td>
<td></td>
</tr>
<tr>
<td>[Ni(ONap-en)(MeBen-en)]·H2O</td>
<td>19</td>
<td>14</td>
<td>10</td>
<td>12</td>
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</tr>
<tr>
<td>[Cu(ONap-en)(MeBen-en)]·H2O</td>
<td>18</td>
<td>16</td>
<td>14</td>
<td>08</td>
<td></td>
</tr>
<tr>
<td>[Zn(ONap-en)(MeBen-en)]·H2O</td>
<td>24</td>
<td>21</td>
<td>12</td>
<td>10</td>
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</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-B

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base \(N,N'\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{S. aureus}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Cu(II)} < \text{Zn(II)} < \text{Ni(II)} = \text{Mn(II)} < \text{Fe(II)} < \text{Co(II)}
\]

In the case of bacteria -\textit{Bacillus Subtilis}, the antibacterial activity of mixed ligand complexes is greater than Schiff base \(N,N'\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Ni(II)} < \text{Cu(II)} < \text{Mn(II)} < \text{Zn(II)} < \text{Fe(II)} < \text{Co(II)}
\]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base \(N,N'\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{S. marcescens}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Mn(II)} = \text{Ni(II)} < \text{Co(II)} = \text{Cu(II)} < \text{Fe(II)} < \text{Zn(II)}
\]

The metal complexes were more active relative to Schiff base \(N,N'\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{P. aeruginosa}.

\[
\text{Fe(II)} = \text{Cu(II)} = \text{Zn(II)} < \text{Ni(II)} < \text{Co(II)} < \text{Mn(II)}
\]

In case of \textit{E. coli}, all metal complexes do not affect on \textit{E. coli} bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus, Bacillus Subtulis* then gram negative bacteria *S. marcescens, P. aeruginosa* and *E. coli*.

### Series B: Table - II Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>OAcPh-en</td>
<td>12</td>
</tr>
<tr>
<td>MeBen-en</td>
<td>12</td>
</tr>
<tr>
<td>[Mn(OAcPh-en)(MeBen-en)]·H2O</td>
<td>19</td>
</tr>
<tr>
<td>[Fe(OAcPh-en)(MeBen-en)]·H2O</td>
<td>21</td>
</tr>
<tr>
<td>[Co(OAcPh-en)(MeBen-en)]·H2O</td>
<td>23</td>
</tr>
<tr>
<td>[Ni(OAcPh-en)(MeBen-en)]·H2O</td>
<td>19</td>
</tr>
<tr>
<td>[Cu(OAcPh-en)(MeBen-en)]·H2O</td>
<td>16</td>
</tr>
<tr>
<td>[Zn(OAcPh-en)(MeBen-en)]·H2O</td>
<td>17</td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-C

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base \( \text{N,N'}\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{S. aureus}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Ni(II)} < \text{Cu(II)} = \text{Zn(II)} < \text{Fe(II)} < \text{Mn(II)} < \text{Co(II)}
\]

In the case of bacteria - \textit{Bacillus Substlis}, the antibacterial activity of mixed ligand complexes is greater than Schiff base \( \text{N,N'}\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Ni(II)} < \text{Cu(II)} < \text{Mn(II)} < \text{Zn(II)} < \text{Fe(II)} < \text{Co(II)}
\]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base \( \text{N,N'}\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{S. marcescens}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Cu(II)} < \text{Fe(II)} < \text{Co(II)} = \text{Zn(II)} < \text{Mn(II)} = \text{Ni(II)}
\]

The metal complexes were more active relative to Schiff base \( \text{N,N'}\)-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - \textit{P. aeruginosa}.

\[
\text{Ni(II)} < \text{Fe(II)} = \text{Co(II)} < \text{Mn(II)} = \text{Zn(II)} < \text{Cu(II)}
\]

In case of \textit{E. coli}, all metal complexes do not affect \textit{E. coli} bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Subtis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

Series C: Table - III Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>OAcPh-en</td>
<td>12</td>
</tr>
<tr>
<td>MeBen-opd</td>
<td>11</td>
</tr>
<tr>
<td>[Mn(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>22</td>
</tr>
<tr>
<td>[Fe(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>21</td>
</tr>
<tr>
<td>[Co(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>26</td>
</tr>
<tr>
<td>[Ni(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>19</td>
</tr>
<tr>
<td>[Cu(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>20</td>
</tr>
<tr>
<td>[Zn(OAcPh-en)(MeBen-opd)]·H₂O</td>
<td>20</td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Chapter 5

Series-D

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base \(N,N'-\text{bis}(2\text{-hydroxyacetophenone})\text{o-phenylenediamine} \text{(OAcPh-opd)}\) against bacteria - \textit{S. aureus}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Zn(II)} < \text{Cu(II)} < \text{Ni(II)} < \text{Fe(II)} < \text{Co(II)} < \text{Mn(II)}
\]

In the case of bacteria - \textit{Bacillus Substlis}, the antibacterial activity of mixed ligand complexes is greater than Schiff base \(N,N'-\text{bis}(2\text{-hydroxyacetophenone})\text{o-phenylenediamine} \text{(OAcPh-opd)}\).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Fe(II)} < \text{Cu(II)} < \text{Mn(II)} = \text{Zn(II)} < \text{Ni(II)} < \text{Co(II)}
\]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base \(N,N'-\text{bis}(2\text{-hydroxyacetophenone})\text{o-phenylenediamine} \text{(OAcPh-opd)}\) against bacteria - \textit{S. marcescens}.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Cu(II)} < \text{Ni(II)} = \text{Zn(II)} < \text{Co(II)} = \text{Mn(II)} < \text{Fe(II)}
\]

The metal complexes were more active relative to Schiff base \(N,N'-\text{bis}(2\text{-hydroxyacetophenone})\text{o-phenylenediamine} \text{(OAcPh-opd)}\) against bacteria - \textit{P. aeruginosa}.

\[
\text{Mn(II)} = \text{Co(II)} < \text{Cu(II)} < \text{Ni(II)} = \text{Zn(II)} < \text{Fe(II)}
\]

In case of \textit{E. coli}, all metal complexes do not affect \textit{E. coli} bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Subtilis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

**Series D: Table - IV Antimicrobial activity**

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th>S. aureus</th>
<th>Bacillus Subtilis</th>
<th>S. marcescens</th>
<th>P. aeruginosa</th>
<th>E. Coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAcPh-opd</td>
<td></td>
<td>13</td>
<td>12</td>
<td>07</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>Meben-opd</td>
<td></td>
<td>11</td>
<td>12</td>
<td>06</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>[Mn(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>24</td>
<td>22</td>
<td>13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>[Fe(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>20</td>
<td>17</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>[Co(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>22</td>
<td>24</td>
<td>13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>[Ni(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>19</td>
<td>23</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>[Cu(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>18</td>
<td>19</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>[Zn(OAcPh-opd)(Meben-opd)]·H₂O</td>
<td></td>
<td>17</td>
<td>22</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-E

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)-o-phenylenediamine (ONap-opd) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II)} < \text{Co(II)} < \text{Fe(II)} < \text{Cu(II)} = \text{Zn(II)} < \text{Ni(II)} \]

In the case of bacteria *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II)} = \text{Co(II)} < \text{Fe(II)} = \text{Zn(II)} < \text{Ni(II)} < \text{Cu(II)} \]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II)} = \text{Zn(II)} < \text{Cu(II)} < \text{Fe(II)} < \text{Cu(II)} < \text{Ni(II)} \]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd) against bacteria *P. aeruginosa*.

\[ \text{Co(II)} = \text{Zn(II)} < \text{Mn(II)} < \text{Cu(II)} < \text{Ni(II)} < \text{Fe(II)} \]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Substlis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

### Series E: Table - V Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th><em>S. aureus</em></th>
<th><em>Bacillus Substlis</em></th>
<th><em>S. marcescens</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. Coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ONap-opd</td>
<td></td>
<td>10</td>
<td>13</td>
<td>09</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>MeBen-opd</td>
<td></td>
<td>11</td>
<td>12</td>
<td>06</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>[Mn(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>16</td>
<td>20</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>[Fe(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>18</td>
<td>21</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>[Co(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>17</td>
<td>20</td>
<td>13</td>
<td>08</td>
<td></td>
</tr>
<tr>
<td>[Ni(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>20</td>
<td>24</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>[Cu(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>19</td>
<td>25</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>[Zn(ONap-opd)(MeBen-opd)]·H$_2$O</td>
<td></td>
<td>19</td>
<td>21</td>
<td>10</td>
<td>08</td>
<td></td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-F

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenylenediamine (ONap-opd) against bacteria - S. aureus.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Co(II)} < \text{Cu(II)} < \text{Fe(II)} < \text{Mn(II)} < \text{Ni(II)} = \text{Zn(II)}
\]

In the case of bacteria - *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenylenediamine (ONap-opd).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Fe(II)} < \text{Ni(II)} < \text{Co(II)} = \text{Cu(II)} < \text{Mn(II)} < \text{Zn(II)}
\]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenylenediamine (ONap-opd) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Co(II)} = \text{Ni(II)} < \text{Fe(II)} < \text{Zn(II)} < \text{Mn(II)} = \text{Cu(II)}
\]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenylenediamine (ONap-opd) against bacteria - *P. aeruginosa*.

\[
\text{Zn(II)} < \text{Co(II)} < \text{Ni(II)} < \text{Cu(II)} < \text{Mn(II)} = \text{Fe(II)}
\]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Subtilis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

Series F: Table - VI Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>Bacillus Subtilis</em></td>
</tr>
<tr>
<td>ONap-opd</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>MeBen-en</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>[Mn(ONap-opd)(MeBen-en)]·H₂O</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>[Fe(ONap-opd)(MeBen-en)]·H₂O</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>[Co(ONap-opd)(MeBen-en)]·H₂O</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>[Ni(ONap-opd)(MeBen-en)]·H₂O</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>[Cu(ONap-opd)(MeBen-en)]·H₂O</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>[Zn(ONap-opd)(MeBen-en)]·H₂O</td>
<td>23</td>
<td>26</td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ethylenediamine (ONap-en) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Fe(II) < Ni(II) < Cu(II)}
\]

In the case of bacteria - *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde) ethylenediamine (ONap-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Fe(II) < Cu(II) < Ni(II)}
\]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ethylenediamine (ONap-en) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[
\text{Cu(II) < Ni(II) < Fe(II)}
\]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ethylenediamine (ONap-en) against bacteria - *P. aeruginosa*.

\[
\text{Cu(II) < Fe(II) < Ni(II)}
\]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus*, *Bacillus Subtilis* then gram negative bacteria *S. marcescens*, *P. aeruginosa* and *E. coli*.

Series G: Table - VII Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th>S. aureus</th>
<th>Bacillus Subtilis</th>
<th>S. marcescens</th>
<th>P. aeruginosa</th>
<th>E. Coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONap-en</td>
<td></td>
<td>14</td>
<td>15</td>
<td>07</td>
<td>08</td>
<td></td>
</tr>
<tr>
<td>MeBen-opd</td>
<td></td>
<td>11</td>
<td>12</td>
<td>06</td>
<td>07</td>
<td></td>
</tr>
<tr>
<td>[Fe(ONap-en)(MeBen-opd)]·H₂O</td>
<td></td>
<td>20</td>
<td>22</td>
<td>14</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>[Ni(ONap-en)(MeBen-opd)]·H₂O</td>
<td></td>
<td>21</td>
<td>24</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>[Cu(ONap-en)(MeBen-opd)]·H₂O</td>
<td></td>
<td>24</td>
<td>23</td>
<td>10</td>
<td>09</td>
<td></td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-H

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenyldiamine (ONap-opd) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II)} < \text{Cu(II)} < \text{Ni(II)} < \text{Fe(II)} = \text{Zn(II)} < \text{Co(II)} \]

In the case of bacteria *Bacillus Subtilis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenyldiamine (ONap-opd).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Zn(II)} < \text{Fe(II)} < \text{Mn(II)} = \text{Ni(II)} < \text{Co(II)} = \text{Cu(II)} \]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenyldiamine (ONap-opd) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Zn(II)} < \text{Mn(II)} = \text{Fe(II)} = \text{Cu(II)} < \text{Ni(II)} < \text{Co(II)} \]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenyldiamine (ONap-opd) against bacteria - *P. aeruginosa*.

\[ \text{Ni(II)} = \text{Cu(II)} < \text{Co(II)} < \text{Fe(II)} = \text{Zn(II)} < \text{Mn(II)} \]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus, Bacillus Substlis* then gram negative bacteria *S. marcescens, P. aeruginosa* and *E. coli*.

Series H: Table - VIII Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th><em>S. aureus</em></th>
<th><em>Bacillus Substlis</em></th>
<th><em>S. marcescens</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. Coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ONap-opd</td>
<td>10</td>
<td>13</td>
<td>09</td>
<td>07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ben-en</td>
<td>14</td>
<td>16</td>
<td>08</td>
<td>07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Mn(ONap-opd)(Ben-en)]·H₂O</td>
<td>20</td>
<td>26</td>
<td>09</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe(ONap-opd)(Ben-en)]·H₂O</td>
<td>25</td>
<td>23</td>
<td>09</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Co(ONap-opd)(Ben-en)]·H₂O</td>
<td>26</td>
<td>28</td>
<td>12</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ni(ONap-opd)(Ben-en)]·H₂O</td>
<td>24</td>
<td>26</td>
<td>11</td>
<td>09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cu(ONap-opd)(Ben-en)]·H₂O</td>
<td>23</td>
<td>28</td>
<td>09</td>
<td>09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Zn(ONap-opd)(Ben-en)]·H₂O</td>
<td>25</td>
<td>21</td>
<td>08</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-I

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)ophenylenediamine (ONap-opd) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II)} < \text{Co(II)} < \text{Zn(II)} < \text{Cu(II)} \]

In the case of bacteria - *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II)} < \text{Co(II)} < \text{Zn(II)} < \text{Cu(II)} \]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II)} = \text{Cu(II)} < \text{Co(II)} = \text{Zn(II)} \]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxynaphthaldehyde)o-phenylenediamine (ONap-opd) against bacteria - *P. aeruginosa*.

\[ \text{Cu(II)} < \text{Ni(II)} < \text{Zn(II)} < \text{Co(II)} \]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus, Bacillus Substlis* then gram negative bacteria *S. marcescens, P. aeruginosa* and *E. coli.*

**Series I: Table - IX Antimicrobial activity**

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>ONap-opd</td>
<td>10</td>
</tr>
<tr>
<td>Ben-opd</td>
<td>13</td>
</tr>
<tr>
<td>[Co(ONap-opd)(Ben-opd)]·H₂O</td>
<td>21</td>
</tr>
<tr>
<td>[Ni(ONap-opd)(Ben-opd)]·H₂O</td>
<td>20</td>
</tr>
<tr>
<td>[Cu(ONap-opd)(Ben-opd)]·H₂O</td>
<td>25</td>
</tr>
<tr>
<td>[Zn(ONap-opd)(Ben-opd)]·H₂O</td>
<td>23</td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
Series-J

From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II) < Zn(II) < Ni(II) < Fe(II) < Co(II) < Cu(II)} \]

In the case of bacteria - *Bacillus Substlis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II) < Cu(II) < Fe(II) < Mn(II) < Zn(II) < Co(II)} \]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II) < Cu(II) < Co(II) = Ni(II) < Fe(II) = Zn(II)} \]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *P. aeruginosa*.

\[ \text{Mn(II) = Ni(II) < Cu(II) = Zn(II) < Co(II) < Fe(II)} \]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus, Bacillus Substlis* then gram negative bacteria *S. marcescens, P. aeruginosa* and *E. coli*.

**Series J: Table - X Antimicrobial activity**

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>Bacillus Substlis</em></td>
</tr>
<tr>
<td>OAcPh-en</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Ben-en</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>[Mn(OAcPh-en)(Ben-en)]·H₂O</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>[Fe(OAcPh-en)(Ben-en)]·H₂O</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>[Co(OAcPh-en)(Ben-en)]·H₂O</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>[Ni(OAcPh-en)(Ben-en)]·H₂O</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>[Cu(OAcPh-en)(Ben-en)]·H₂O</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>[Zn(OAcPh-en)(Ben-en)]·H₂O</td>
<td>21</td>
<td>26</td>
</tr>
</tbody>
</table>

These metal complexes do not affect on *E. Coli* bacteria so there is no inhibition zone.
From the experimental examination, we observed that the metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *S. aureus*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II)} < \text{Fe(II)} < \text{Mn(II)} = \text{Zn(II)} < \text{Cu(II)} < \text{Co(II)} \]

In the case of bacteria - *Bacillus Subtilis*, the antibacterial activity of mixed ligand complexes is greater than Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en).

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Ni(II)} < \text{Cu(II)} < \text{Fe(II)} < \text{Zn(II)} < \text{Mn(II)} = \text{Co(II)} \]

From the data it can be seen that the mixed-ligand complexes showed greater activity than Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *S. marcescens*.

However the order of antibacterial activity in terms of metal complexes can be given as,

\[ \text{Mn(II)} < \text{Fe(II)} < \text{Ni(II)} < \text{Cu(II)} < \text{Co(II)} = \text{Zn(II)} \]

The metal complexes were more active relative to Schiff base N,N’-bis(2-hydroxyacetophenone) ethylenediamine (OAcPh-en) against bacteria - *P. aeruginosa*.

\[ \text{Mn(II)} < \text{Co(II)} = \text{Ni(II)} < \text{Fe(II)} < \text{Cu(II)} = \text{Zn(II)} \]

In case of *E. coli*, all metal complexes do not affect on *E. coli* bacteria so there is no inhibition zone.
All the compounds screened are more active against gram positive bacteria *S. aureus, Bacillus Substlis* then gram negative bacteria *S. marcescens, P. aeruginosa* and *E. coli*.

Series K: Table - XI Antimicrobial activity

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>OAcPh-en</td>
<td>12</td>
</tr>
<tr>
<td>Ben-opd</td>
<td>13</td>
</tr>
<tr>
<td>[Mn(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>21</td>
</tr>
<tr>
<td>[Fe(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>20</td>
</tr>
<tr>
<td>[Co(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>26</td>
</tr>
<tr>
<td>[Ni(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>19</td>
</tr>
<tr>
<td>[Cu(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>22</td>
</tr>
<tr>
<td>[Zn(OAcPh-en)(Ben-opd)]·H₂O</td>
<td>21</td>
</tr>
</tbody>
</table>
5.8 Reference


17. Sehdev P. S., Donnenberg M. S. "Arcanum: The 19th-century Italian pharmacist pictured here was the first to characterize what are now known to be bacteria of the genus Serratia" Clin Infect Dis 1999; 29(4):770, 925.


