CHAPTER – II

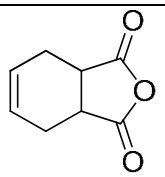
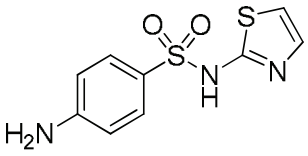
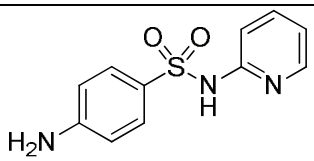
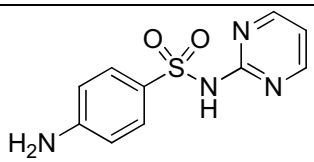
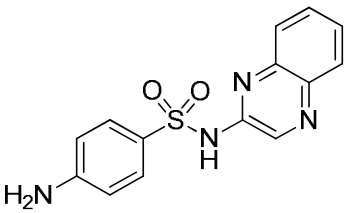
MATERIALS AND METHODS

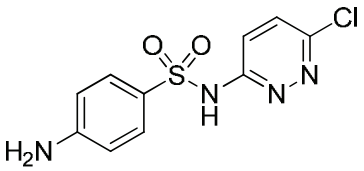
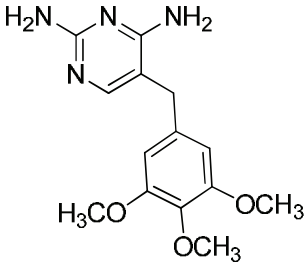
CONTENTS

2.1 MATERIALS USED

All the reagents employed in process investigation were received as analar grade locally. The details of chemicals are in Table 2.1.

Table 2.1: Data about reagents and their supplier

Name and structure	Supplier name
 Tetrahydrophthalic anhydride	Otto Chemie Pvt. Ltd.
 Sulfathiazole	Enomark Healthcare
 Sulfapyridine	Triveni Chemicals
 Sulfadiazine	Macsen Laboratories
 Sulfaquinoxaline	Vea Impex Pvt. Ltd.

 Sulfachloropyradazine	A R Life Sciences
 Trimethoprim	Kavya Pharma
$CuCl_2$ Copper (II) chloride	Real Metal Chem Pvt. Ltd.
$Cu(CH_3COO)_2$ Copper acetate	Lakshita Chemicals
$Co(C_2H_3O_2)_2$ Cobalt (II) acetate	P K Chlorochem
$Ni(CH_3COO)_2$ Nickel (II) acetate	Mahavir Metal Chem
$Zn(CH_3COO)_2$ Zinc acetate	Krishna Chemicals
$Mn(CH_3COO)_2$ Manganese (II) acetate	Kanha Life science

2.2 TECHNIQUES USED

Different techniques were used in present investigation.

2.2.1 Melting point analysis

The melting points of all the compounds were measured on Digital melting point apparatus (Lab India) and were uncorrected.

2.2.2 Elemental analysis

The elemental content in all the prepared oriental organic compounds were determination Thermo- Scientific Flash Smart elemental analyzer.

2.2.3 Infrared spectroscopy

The IR spectra of all the compounds were performed on FT-IR Spectrometer - 6800 – JASCO Europe instrument. The analysis performed using anhydrous KBr in the 4000-400 cm^{-1} range. The transparent pallet was used for analysis.

2.2.4 NMR spectroscopy:

Proton NMR and ^{13}C NMR of ligands was performed on Brucker Avance III HD 300 MHz wide-bore NMR Spectrometer. Tetra methyl silane (TMS) was used as standard and deuterated Dimethyl formamide (DMF) was used as a solvent.

Brucker Avance III HD 300 MHz wide-bore NMR Spectrometer.

2.2.5 LC-Mass spectroscopy:

Liquid chromatography-mass spectroscopy (LC-MS) is the analytical technique used for identification of unknown compounds as well as to elucidate the of different compounds. The LC-Mass spectrometer (Brucker Avance III HD 300 MHz wide-bore NMR) consists of ionization source, mass analyzer, detectors for data interpretation and analysis and report results.

2.2.6 Magnetism:

The magnetism of metal complexes is an important property which can be anticipated based on molecular structure. This property based measured in terms of molar magnetic susceptibility ((χ_m) by considering their molecular weight of test complex magnetism are two types: (i) Diamagnetism exhibited due to changes of orbital and (ii) Paramagnetism exhibited due to orbital electronic spin and angular momentum. It exists only in organic molecules having impurities of electron with parallel spins diamagnetism, a created from the interaction of paired electrons and the magnetic field. Magnetic area compounds having electron creates two Paramagnetism which relate inversely with temperature. In organic compounds two paramagnestism occurs of they are transition metal ions unpaired electrons.

2.2.7 Reflectance / Electronic spectra:

Most of the transition metal complexes have unique UV-visible reflectance spectra, which is useful to study their geometry and electronic structure. This technique is important due to solid state structural chemistry not studied compared to aqueous solution. This diffused electronic spectroscopy is better for study of solid state properties of materials. In present studies the produced metal chelates are amorphous powder, their electronic spectra have been scanned for getting molecular geometry

2.2.8 Thermogravimetric analysis

Thermogravimetric analysis is an analytical tool to analysis of thermal stability of materials. In this method, changes in the weight of compounds are measured with increasing temperature (°C). Percentage (%) moisture content and volatile content of a sample can be measured by Thermogravimetric analysis techniques. Evaluation of the thermogravimetric analysis.

The first stage of decomposition of these complexes was observed around 10% in 250-500°C temperature range and, it may be due to associated water molecules. The second stage of decomposition of all chelates is rapid and about 50% mass loss. This may be due to metal oxide formation during degradation.

2.2.9 Antimicrobial analysis

2.2.9.1 Antibacterial activity:

There are various methods used for the analysis of antibacterial activities,

2.2.9.1.1 Agar well diffuse method: This is most favorable method to monitoring antimicrobial activity of test samples.

2.2.9.1.2 Agar plug diffusion method,

2.2.9.1.3 Cross streak method, and

2.2.9.1.4 Poisoned food method.

In this testing, the antimicrobial materials is transferred to test cultured inoculated thin layer (coated) glass plate. After diffusion on such chromatogram agar plate removed and there incubated from plate the growth inbition zone of microorganism is measured.

The antimicrobial activity is calculated by,

$$= \frac{\text{Number of bacteria on test sample one day}}{\text{Number of bacteria on without test sample in one day}}$$

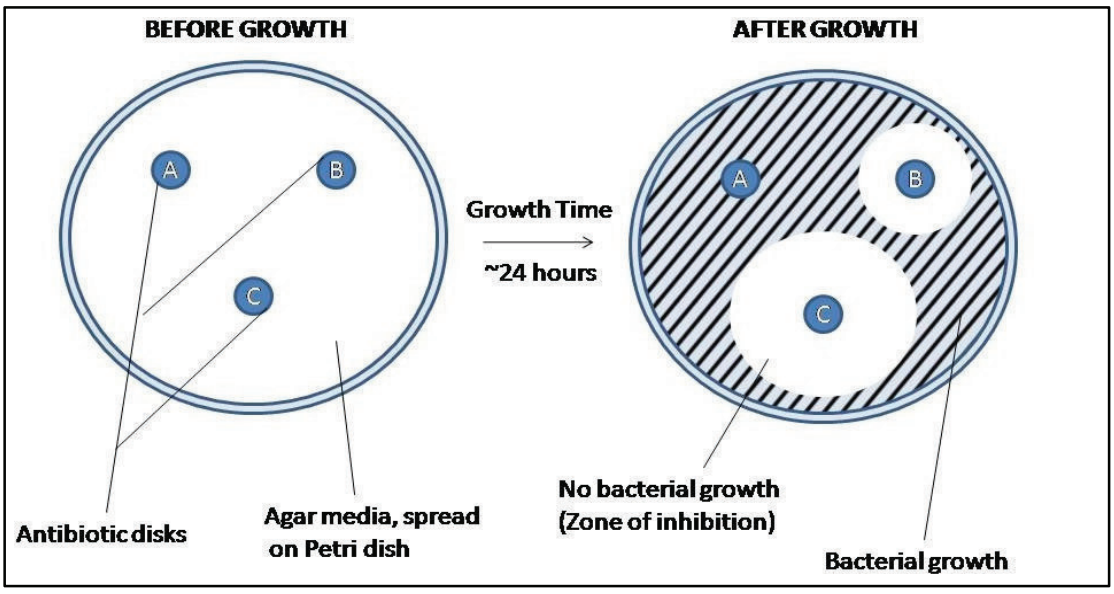


Fig. 2.1: Agar disk diffusion experimental test

(i) Antifungal activity:

The antifungal test methods are divided into main three groups.

- Diffusion method,
- Dilution method,
- Bio-autographic methods and

Many organizations/Labs adopt modified methods for specific samples. In addition to this, there are various test methods used for testing of fungal infections.

- Susceptibility testing (where sample of fungus isolated in culture)
- Antigen testing (where sample are blood, urine, CSF, body fluids)
- Antibody testing [where sample are blood, urine, CSF, body fluids], and
- Molecular tests for DNA, RNA.

Three methods have been standardized for antifungal susceptibility testing of any test sample of compounds.

- Broth dilution method,
- Disk diffusion method,
- Azole agar screening for Aspergillus, other common used methods include gradient diffusion and the use of rapid automated instruments.

Antifungal susceptibility testing it has the become standard method for testing and has a same role of the antibacterial susceptibility testing in microbiology laboratories.

In clinical laboratory Antifungal susceptibility testing (AFST) is pivotal test which give MIC value to assists patient therapy.

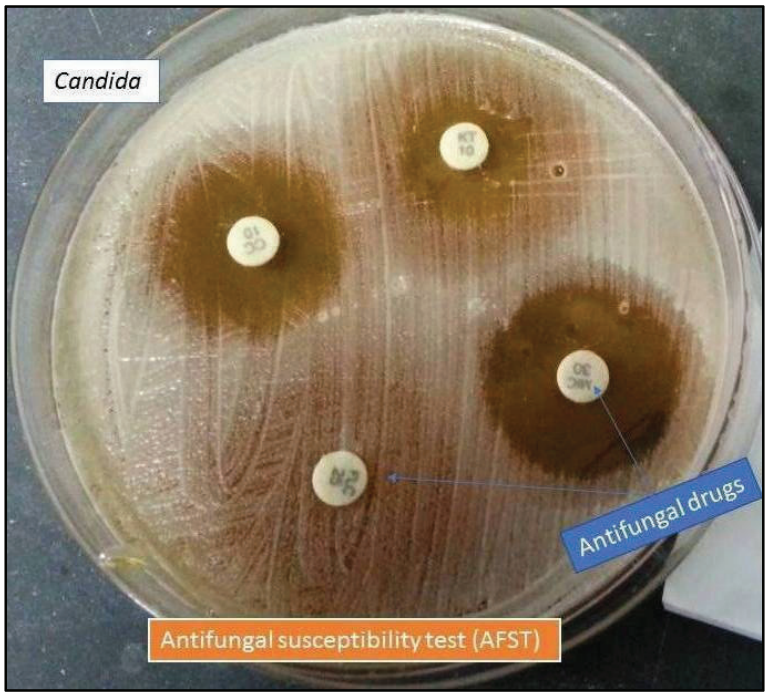


Fig. 2.2: Antifungal susceptibility test (AFST)

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