

## **2.2. Antimicrobial activity**

### **a. Antibacterial activity**

The antibacterial activity of synthesized compounds (250 µg/mL in DMSO) was preliminarily studied by disc diffusion method. The procedure followed for the disc diffusion method is given below.

A suspension of *Staphylococcus aureus* was added to sterile nutrient agar at 45°C. The mixture was transferred into sterile petri-dishes to a depth of 3 mm and allowed to solidify. Sterile discs of 5 mm in diameter (made of Whatmann Filter paper) were immersed in solutions of synthesized compounds. Sterile discs immersed in DMSO were used as control. Both chemical-treated and DMSO-treated discs were laid down onto bacteria mixed agar plates. The plates were allowed to stand for 1 hour at room temperature followed by incubation at 37°C for 24 hours and observed for antibacterial activity. The diameter of the zone of inhibition was measured in each plate. The average zone of inhibition was calculated. A similar procedure was adopted for the antibacterial activity studies against other organisms.

### **b. Antifungal activity**

The procedure described above was followed for antifungal activity against *Aspergillus niger* NCCS 1196 and *Candida albicans*. Compounds were treated at several different concentrations using DMSO as a solvent.

### **c. Determination of Minimum Inhibitor Concentration**

The procedure followed to find out MIC by Broth Dilution Method is given below.

Standardized inoculum (matched to McFarland BaSO<sub>4</sub> standard) of suspension of organisms was prepared. A series of glass tubes containing different concentrations of test compounds dissolved in DMSO and spillover in nutrient broth were incubated with one drop of inoculum and shaken gently to mix the contents. Two growth control tubes were also prepared by mixing 0.1 mL of control and 0.9 mL of sterile saline and its optical density was determined. The control contained  $1 \times 10^5$  colony forming units /mL which is equivalent to 20 colonies.

Tubes were incubated for 24 hours at 37°C in air. The turbidity developed in each tube was recorded by UV-Visible spectrophotometer. The turbidity produced by the broth (without inoculum) was considered as 100 % transparency. The minimum inhibitory concentration (MIC) was noted as the concentration of the test sample which completely inhibits the growth of the microorganism i.e. 100 % transparency.