ABSTRACT

The continuing increase in the incidence of multi drug resistant pathogenic bacteria and shortage of new antimicrobial agents are the prime driver in efforts to identify novel antibacterial classes and it is a serious problem in treatment with infectious diseases. Therefore it is essential to synthesis novel compounds and evaluate the antimicrobial properties of the newly synthesis compounds. Pyridinium salts are used as an effective antimicrobial agent and various antimicrobial applications in industrially and domestically for a long time.

This work mainly focused on synthesis of pyridinium based salt and study of the *in vitro* interaction of aqueous solution of this compound with bacterial cells and to determine the cytotoxic effect. In this experiment *Staphylococcus aureus*. *Streptococcus* species. *Bacillus subtilis*, *Klebsiella* species and *Escherichia coli* bacterial cells were used as models to screen for antibacterial activity.

The convergence synthesis of 4-phenylpyridine and α-(bromo)methylstyrene were carried out separately and finally combined to form 4-phenyl-1-(2-phenyl-allyl)-pyridinium bromide. This synthesis was carried out according to the previously published method. As a first step phenylmagnesium bromide and N-methylformylpyridinium chloride were prepared and then combined to yield 4-phenyl-N-methylformyldihydropyridine. Then the compound was hydrolyzed using a 10% ammonium chloride solution. Then it was rearomatized to 4-phenylpyridine by potassium permanganate. Then it was precipitated as the hydrochloride salt in ethanol medium. Then the isolated product was basified and the resulting phenylpyridine was later recrystallized using ethanol/diethylether solvent system. As a second step allylic bromination of α-methylstyrene was achieved and later the product (α-(bromo)methylstyrene) was isolated by column chromatography and TLC (Thin layer chromatography). Finally the combination reaction of phenylpyridine and α-(bromo)methylstyrene was carried out to obtain 4-phenyl-1-(2-phenyl-allyl)-pyridinium bromide. Then the isolated product was recrstallised using the ethanol/diethylether solvent system.

Then the investigation was undertaken to assess antimicrobial properties of this pyridinium based salt in order to establish the possibility of this compound as an antimicrobial agent. Initially evaluate the susceptibility of this compound against above mentioned bacterial strains *in vitro* using disk diffusion method. According to the antimicrobial test results strong inhibitory effect was observed on *Staphylococcus aureus* (larger inhibition zone was formed) and *Escherichia coli* showed very small sensitivity (very small inhibition zone) towards the 4-phenyl-1-(2-phenyl-allyl)-pyridinium bromide while all the other tested bacterial strains (*Streptococcus* sp, *Bacillus subtilis*, *Klebsiella* sp) revealed resistant against the compound.

Staphylococcus aureus was selected to determine the MIC (Minimum Inhibitory Concentration) by the disk diffusion method because it was exhibited larger inhibition zone for the pyridinium salt. It was done by as preparing five fold dilutions of 4-phenyl-1-(2-phenyl-allyl)-pyridinium bromide salt in water and introduced this series to the petriplates containing equal amount of bacterial lawns using 6 mm paper disk. The MIC of this salt for *Staphylococcus aureus* is ≤ 20 µg/ml.

Mode of interaction and the exact mechanism of binding of this compound to the bacterial cells are still not understood, according to the previous studies it can be suggested that quaternary pyridinium type compounds and compounds having pyridinium moiety mainly effects on bacterial cell walls. And also it can be supposed that different sensitivities of bacterial strains towards the compound mainly due to their cell wall differences.

Finally comparison of antibacterial activity of 4-phenyl-1-(2-phenyl-allyl)-pyridinium bromide with four other antibiotics (penicillin, Cloxacillin, Erythromycin and Vancomycin) which are given to cure *staphylococcus aureus* infections was carried out using disk diffusion method.