5.0 SUMMARY AND CONCLUSION

Infectious diseases are one of the leading causes of illness and death in humans worldwide. Infectious diseases are newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic ranges. Developing countries such as India suffer disproportionately from the burden of infectious diseases given the confluence of existing environmental, socio-economic, and demographic factors.

Most of the emerging infectious diseases are caused by microorganisms. Bacterial infections cause a global health problem and giving a negative impact on human welfare and the economy. A threat of emerging infectious disease is drug-resistance pathogens because of the high burden of bacterial disease for the emergence and spread due to the misuse of antibiotics, poor quality drugs, combined with substandard hygiene and living conditions.

Antimicrobial agents have saved the human race from a lot of suffering due to infectious disease burden. Antimicrobial agents are used to kill or inhibit the growth of the microorganisms. These are huge benefits in human health. They have their ability to interact with the negatively charged lipids of bacterial membranes which leads to a destabilization and permeabilization of the cell membrane, multiple stresses on the membrane and leakage of the cell content due to the increasing resistance of pathogenic bacteria.

Antimicrobial agents may be either bactericidal (killing the target organisms) or bacteriostatic (inhibiting its growth). Most of the bactericidal agents are effective, but bacteriostatic agents can be beneficial when they act defences of the host to destroy the organisms. The mechanism of action of antimicrobials include inhibition of cell wall synthesis, , disruption of cell membrane function, inhibition of nucleic acid synthesis, inhibition of protein synthesis and inhibition of folic acid synthesis.

However, the increase in multidrug resistance pathogenic organism seems to be a global issue. It creates a therapeutic problem which leads to serious health issues particularly in the developing countries.

Medicinal plants play an important role in the human health care system. Nowadays, they are important due to the increasing number of antibiotic-resistant strains. It has shown promising microbiostatic and microbiocidal activities against some pathogenic microbionta.

Plants produce secondary metabolites and play an important role in protecting plants against predators and microbial pathogens due to the biocidal properties against microbes or repellence to herbivores and to protect human beings. Plants derived medicines are safer than synthetic medicines.

Flavonoids are basic structure of polyphenols with C6-C3-C6 skeleton. It exhibit pharmacological properities and biochemical effects. Flavonoids possess anticarcinogenic, oestrogenic and antiallergic activities.

One of the traditional medicinal plant is *Trianthema portulacastrum* which belongs to the family Aizoceae. It is the weed plant in the field crops. It has bitter and possesses analgesic, stomachic and laxative, blood anemia and inflammation. Likewise, various parts of the plant have also been reported for their biological activities.

From the earlier study in our laboratory, *Trianthema portulacastrum* has been subjected to preliminary antimicrobial screening and it has been found that *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most susceptible microorganisms.

The present study was carried out to find out the mechanism of action of the leaves of methanolic extract and flavonoid fractions of *Trianthema portulacastrum* on *Staphylococcus aureus* and *Klebsiella pneumoniae.* The results obtained from the present study analysed the bacterial time kill kinetics of the methanolic extract and flavonoid fractions on the selected bacterial pathogens. The hemolytic assay was also performed to know about the cytotoxicity of the methanolic extract and flavonoid fractions. The post antibiotic effect was also performed to characterize the phytoconstituents. The surface morphology of the damaged bacterial cells was evaluated by Scanning Electron Microscopy.

From the results of bacterial time kill kinetics, it has been revealed that the flavonoid fractions at a concentration of 125mg at an incubation time of 120 minutes are more effective on

Staphylococcus aureus than *Klebsiella pneumoniae*. This shows that flavonoid fractions have more bactericidal activity when compared with methanolic fractions.

The hemolytic activity of *Trianthema portulacastrum* shows the effect of cytotoxicity on goat red blood cells. Similarly, among these two fractions, flavonoid fractions were found to lyse the red blood cells to 7% while methanolic extract revealed a 15% hemolysis. This result proved that the flavonoid fraction show a non cytotoxicity property to the human cells.

The leakage of intracellular substances such as nucleic acid and protein from the bacterial cell membrane was analyzed by UV absorption method. The methanolic extract and flavonoid fractions of *Trianthema portulacastrum* were added with the bacterial suspension of *Staphylococcus aureus* and *Klebsiella pneumoniae,* in which the flavonoid fractions were found to increase the leakage of protein and nucleic acids with the increase in incubation time from the bacterial cell membrane of both the tested pathogens. Thus the results revealed that both the methanolic extract and flavonoid fractions can penetrate the cell membrane causing the leakage of intracellular materials. Among these two tested pathogens, *Staphylococcus aureus* was found to show the maximum leakage of nucleic acids and proteins at 260nm and 280nm.

The membrane permeability potential of *Trianthema portulacasturm* was performed by estimating the level of protein and sugar released from the bacterial cell membrane. From the observation, it has been revealed that among the methanolic extract and flavonoid fractions, the amount of sugar released from the bacterial cell membrane was higher in both methanolic extract and flavonoid fractions. Hence both the phytochemical fractions can enter the cell envelope of bacterial cell causing the leakage of reducing sugar. Among the two tested pathogens, both were found to be susceptible to the activity of methanolic extract and flavonoid fractions.

The DNA binding ability of methanolic extract and flavonoid fractions of *Trianthema portulacastrum* was performed by gel retardation assay. The results of electrophoretic mobility indicated that there is a significant damage in flavonoid fractions treated DNA of *Staphylococcus aureus* than *Klebsiella pneumoniae* when compared with the untreated control DNA. From the results and the Integral Density Value, it was clearly proved that the flavonoid fractions have the

ability to bind DNA. This revealed that the antibacterial compounds act as inhibitors of nucleic acid synthesis.

From the results of post antibiotic effect, it has been proved that the methanolic extract and flavonoid fraction have shown a significant effect on *Staphylococcus aureus.*

The membrane damage on the *Staphylococcus aureus* cells treated methanolic extract and flavonoid fractions was analyzed by Scanning electron microscopy. It found to be more distortion of the shape of the cells with depression on the surface of the cell. From these results, both the methanolic extract and flavonoid fractions would seem to alter the morphological changes in the surface of the bacterial cell membrane.

Thus the results of the present study proved the mode of antibacterial action of *Trianthema portulacastrum* on *Staphylococcus aureus* and *Klebsiella pneumoniae.* Therefore, the leaves of *Trianthema portulacastrum* has been proved as a safer drug indicating its non-cytotoxic property and found to kill the bacterial cells by permeating the membrane and damage the DNA of the bacterial cells.

Suggestions for the future study

- The antibacterial activity of the leaves of *Trianthema portulacastrum* plant can be later tested on animal models and human cell lines.
- The mechanism of action of flavonoid fractions of *Trianthema portulacastrum* on fungal pathogens can be checked.
- Individual flavonoids can be purified by column chromatography and checked for the antibacterial mechanism