Chapter 6

Summary and Conclusion

6.1 SUMMARY

- Plant essential oil based oil in water nanoemulsion was formulated by spontaneous emulsification and ultrasonic emulsification.

- Bio-based nanoemulsion was developed from plant based oil with use of surfactants Tween 20 and Tween 80. The process parameters like type of oil emulsifiers, oil to surfactant mixing ratio and emulsification time were optimised.

- A decrease in the surfactant concentration (6%) i.e. 1:1 oil to surfactant ratio resulted in a highly stable system and reduced droplet size with use of sonication.

- Both the oil system such as eucalyptus and orange oil showed reduced droplet diameter of 17 nm and 16 nm with polydispersity index below 0.2.

- The microscopic characterization revealed the droplets to be spherical and the size were found to be in the range of 20-30 nm for both the formulation.

- Eucalyptus oil nanoemulsion 80U1 exhibited dose and time dependent bactericidal activity against food borne bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* and the wound clinical isolate *Staphylococcus aureus*.

- Eucalyptus oil nanoemulsion was found to be non-irritant when tested on the skin of wistar rats. The nanoemulsion triggered wound healing process in wistar rats with a complete healing of wound was observed in 16 days, whereas it took 20 days in each case of eucalyptus oil, standard ointment (neomycin); and in untreated wistar rats.
The present findings suggest that antibacterial and larvicidal activity of eucalyptus oil nanoemulsion is due to the active compound eucalyptol present in the formulation which was confirmed by GC-MS analysis.

Eucalyptus oil nanoemulsion (80U1) demonstrated significant larvicidal activity against *Culex quinquefasciatus* larva within 4 h of treatment than the bulk formulation.

Eucalyptus oil nanoemulsion was impregnated or encapsulated with the use of chitosan for its application in wound treatment and mosquito larval control.

The encapsulated beads with eucalyptus oil nanoemulsion showed an increase in the degree of swelling which in turn favors its fast release. Morphology by scanning electron micrograph showed encapsulated spherical beads with smooth surface.

There was no structural modification in the chitosan polymer and it was found to be similar even after encapsulation, which was confirmed by XRD and FTIR analysis.

Larvicidal activity of encapsulated beads and chitosan (without nanoemulsion) was tested against *Cx. tritaeniorhynchus*. The encapsulated beads (1000 mg) showed 100% mortality within 12 h, whereas, chitosan beads exhibited no mortality even after 48 h of treatment.

The eucalyptus oil nanoemulsion impregnated chitosan films exhibited 100% bactericidal activity against the wound isolate *S. aureus*. The toxicity was observed to increase with the increasing concentration of nanoemulsion onto chitosan matrix. The above results suggest that eucalyptus oil nanoemulsion prevents wound infection and has higher wound healing activity in wistar rats.

Orange oil nanoemulsion (80U1) demonstrated effective anti-yeast activity against the food spoilage yeast, *Saccharomyces cerevisiae*, both in vitro (growth medium) and in vivo studies (apple juice).
o Anti-yeast activity of the orange oil nanoemulsion was due to the reduced droplet size and the major active ingredient present in the oil i.e. Limonene which was confirmed by GC-MS analysis.

o Evaluation of in vivo anti-yeast activity in apple juice showed time and concentration dependent fungicidal activity of orange oil nanoemulsion and sodium benzoate (positive control). The results also confirmed that orange oil nanoemulsion is a better fungicidal agent than sodium benzoate.

o Nanoemulsion treated cells resulted in a greater increase in cell surface hydrophobicity when compared to yeast cells by MATH assay. SEM micrographs confirmed the morphological distortion of yeast upon treatment with orange oil nanoemulsion formulation.

o The orange oil nanoemulsion added to chitosan films had a plasticizing effect, as evidenced by increase in physical and mechanical properties when compared to those of nanoemulsion free films.

o Optical microscopy and SEM images confirmed that droplet aggregation and coalescence did not take place during film drying. The essential oil-free film showed a homogeneous surface without any droplets.

o Chitosan films enriched with 3% and 5% orange oil nanoemulsion showed complete loss (100%) of viability against the food spoilage yeast, *Saccharomyces cerevisiae*, whereas the non impregnated chitosan films reduced the viability of yeast only by 30%.
6.2 CONCLUSION

The present study demonstrates stable nanoemulsion formulation of plant oils like eucalyptus and orange oil by ultrasonic emulsification method. Emulsion formulation was optimized with different process parameters like surfactant concentration, oil to surfactant ratio, and emulsification time. Eucalyptus and orange oil nanoemulsion 80U1 was selected for application studies due to its low surfactant concentration, reduced droplet size and kinetic stability. Eucalyptus and orange oil nanoemulsion 80U1 showed significant bactericidal, larvicidal and fungicidal activity even at lower concentrations. Eucalyptus nanoemulsion was tested to be a non-irritant with higher wound healing efficiency. The impregnated nanoemulsion or encapsulated chitosan showed effective antimicrobial activity against the tested pathogens *Staphylococcus aureus* and *Saccharomyces cerevisiae* and as a larvicidal agent against *Culex tritaeniorhynchus*. The direct interactions of nanodroplets and active ingredients present in the emulsion system played a major role in the application studies. Hence, the formulated nanoemulsions can be further used for different applications like prevention of wound infection, control of mosquito larvae in the environment, and also for food preservation against microbial spoilage and other industrial applications. Thus, these nanoemulsions seem to be an effectual alternative to synthetic or semi-synthetic additives and control agents used in food and environment related applications.