CHAPTER 9

SUMMARY AND CONCLUSIONS

9.1 SUMMARY

The objective of the present study is to investigate the various copper enriched medicinal herbs suitable for the development of curative fabrics and garments. The 12 copper enriched medicinal herbs were collected and their copper content was evaluated. Out of the 12 herbs, five copper enriched medicinal herbs namely *Aerva lanata*, *Aloe barbadensis* Mill, *Cumminum cyminum* Linn, *Tagetes erecta* and *Mentha piperita* have been found suitable for the development of curative garments. Herbal extraction techniques were studied and analysed. The active compounds were extracted from the copper enriched selected medicinal herbs were obtained the aqueous extraction techniques and solvent extraction technique. Based on the observation of the antimicrobial activity of solvent extracts i.e., methanolic extracts (polar solvent), have higher range of bacterial inhibition, when compared to petroleum ether extracts (non – polar solvent) and hence methanolic extracts (polar solvent) have been chosen for the further research work.

The coacervation technique is used to develop the microcapsules by chemical microencapsulation system. Microencapsulation was done using copper enriched medicinal herbs such as *Aerva lanata*, *Aloe barbadensis* Mill, *Cumminum cyminum* Linn, *Tagetes erecta* and *Mentha piperita* extract as core material and gum acacia as wall material. Analysis and characterisation
of the microcapsules in terms of production yield, structure of microcapsules, average micro particle size, cytotoxicity and stability of microcapsules at various temperatures were studied.

The bleached cotton fabric was used to develop curative fabrics by applying the copper enriched medicinal herbs extracts using aqueous and methanolic medium with pad – dry – cure method. In another application method, the microcapsules were developed using selected herbs’ methanolic extract and applied on the cotton fabrics using pad – dry – cure method under optimized process parameters by the experimental design of Box-Behnken method. In total 75 microcapsules applied samples of five selected herbs namely, *Aerva lanata*, *Aloe barbadensis* Mill, *Cumminum cymimum* Linn, *Tagetes erecta* and *Mentha piperita* and three different process parameters like concentration of extract, concentration of cross linking agent and curing temperature have been developed and their antimicrobial activity characteristics have been critically analysed. Based on the antimicrobial test results, the process parameters such as concentration of extract, concentration of cross linking agent and curing temperature were finalized. Under optimized process parameter, five samples were produced using five selected medicinal herbs and their characteristics critically analysed.

Antimicrobial activity of treated curative fabrics was done by measuring qualitatively (zone of inhibition, SN195920) and quantitatively (% of bacterial reduction, AATCC 100). FTIR analysis of treated fabric confirmed the presence of functional groups of herbal extracts on the curative fabrics. SEM analysis confirmed the presence and availability of microcapsules on the surface of treated fabric. The curative fabrics were assessed for their physical and chemical properties such as air permeability, water vapour permeability and wash durability, and perspiration fastness as per the standard methods. Development of curative garments such as shirt,
polo T-shirt, mask and gloves was carried out. The performance analysis of developed curative garments has been done field trail at “Sri Gayathri Nature Cure Hospital” Coimbatore.

9.2 CONCLUSIONS

In this research work, study has been carried out to identify various copper enriched medicinal herbs to cure selective skin diseases. 12 herbs were identified based on their copper content and successful traditional usage. Trials conducted for 12 herbs and five herbs *Aerva lanata*, *Aloe barbadensis* Mill, *Cumminum cyminum* Linn, *Tagetes erecta* and *Mentha piperita* were selected for this research work based on the possession of required characteristics such as copper content, antimicrobial activity, curative activity and suitability for applying on the cotton fabric.

The selected herbal extractions were carried out using aqueous and solvent extraction techniques. Five samples from selected five herbs have been produced using aqueous extraction techniques. In the case of solvent extraction techniques, the methanolic extracts (polar solvent) and petroleum ether extracts (non – polar solvent) have been used and the antimicrobial activity assessment has been carried out. It concludes that methanolic extracts (polar solvent) gives better results than petroleum ether extracts (non – polar solvent), hence polar solvent of methanolic has been chosen for the further research work.

Microcapsules have been produced using the methanolic extracts of the five selected medicinal herbs. The coacervation technique was used to develop the microcapsules by chemical microencapsulation system. Microencapsulation was done using copper enriched medicinal herbs methanolic extracts of *Aerva lanata*, *Aloe barbadensis* Mill, *Cumminum cyminum* Linn, *Tagetes erecta* and *Mentha piperita* as core material and gum
acacia as wall material. The microcapsules, production yield percentage for the selected medicinal herbs shows a maximum of 92.8% in *Tagetes erecta* and minimum of 82.7% in *Mentha piperita*. The structures of the microcapsules were examined under the magnification level of 100X, 500X and 1000X it shows that the formation of core and wall of microcapsules, and it is confirmed that the produced microcapsules are of small spherical shape with fairly uniform distribution. The particle sizes of microcapsules were evaluated by mean of relationship between the means particle size of microcapsules and the duration of dispersion time. The average particle size of non-dispersed microcapsules was 10.8 – 11.6 µm. The large particle size was probably attributed to the aggregation of copper enriched herbal encapsulated microcapsules. After 168 - 240 hours of dispersion with surfactants, the average particle size for all the copper enriched herbal encapsulated microcapsules reached 3.7 - 3.8 µm, the reduction in particle size was about 70 - 75% when compared with the non-dispersed microcapsules. The particle size of microcapsules did not reduce further when the dispersion duration was prolonged to 240 hours. The particle size was found to stabilize at 3.8 µm, the reduction was a linear one and the data shows significant reduction in particle size of the microcapsules. Cellular morphology changes were analysed using HaCaT human keratinocytes. It was clear that the herbs encapsulated microcapsules did not create any wrinkle and cytotoxicity on the HaCaT cells up to a maximum concentration of 100 µg/mL after incubation of 48 hrs. The stability of microcapsules were evaluated at various temperatures like 40, 60, 80 and 100°C based on the release rate percentage of core material, during the temperatures of 40°C and 60°C the release rate percentage of core material 0.34% – 0.38% and 0.39% – 0.44% for all five herbal encapsulated microcapsules.

Aqueous extract of copper enriched medicinal herbal (*Aerva lanata, Aloe barbadensis* Mill, *Cumminum cyminum* Linn, *Tagetes erecta* and
Mentha piperita) treated on the fabric was done based on Box and Behnken experimental design by varying the process parameters like extract concentration, dipping time and concentration of cross linking agent. The extracts were applied by the pad-dry-cure method and the process parameters were optimized using response surface methodology. With the optimum values of concentration of the herbal extract, concentration of cross linking agent and dipping time, trial was performed using the aqueous extract of copper enriched medicinal herbs. The effect of these parameters on the antimicrobial activity of herbal treated cotton fabrics was analysed. The zone of inhibition of treated sample increases with the concentration of the herbal extract. The influence of dipping and cross linking agent percentage on the zone of inhibition was significant for both Staphylococcus aureus and Escherichia coli. The agar diffusion results show high and clear zone of inhibition in mm against wide spectrum human pathogenic bacterial strains of Staphylococcus aureus and Escherichia coli. The maximum zone of inhibition was shown Aerva lanata at 49 mm and 31mm, and the minimum zone of inhibition of 45 mm and 30 mm shown by Aloe barbadensis Mill microcapsules treated fabric.

These results were also supported by obtaining an excellent correlation between the experimental and modeled results by formulating the linear relationship between crosslinking agents, extract concentration and duration of dipping time. The percentage contributions of the process parameters including their interaction effects have also been computed to determine their influence on the antimicrobial activity of copper enriched medicinal herbal aqueous extract treated fabric.

Methanolic extracts of copper enriched medicinal herb extracts were treated on the cotton fabric using citric acid as a cross linking agent, at the same time direct application of extracts were applied without crosslinking
agent using pad-dry-cure method and the antimicrobial activity of copper enriched medicinal herbal treated cotton fabrics with and without crosslinking agent and microcapsules were tested according to the standard methods for analysis of both qualitative and quantitative parameters and the results were compared with that of control samples. Based on the observation from this study, the effects of crosslinking also influence to create bonding between fibre morphology and methanolic extracts and give a better penetration of medicinal herbs into fabric at higher level when compared to without crosslinking agent.

Microencapsulated extract of copper enriched medicinal herbal (Aerva lanata, Aloe barbadensis Mill, Cumminum cyminum Linn, Tagetes erecta and Mentha piperita) applied of fabric were done based on Box and Behnken experimental design by varying the process parameters like concentration of extract, concentration of cross linking agent and curing temperature. The microencapsulated extracts were applied by the pad-dry-cure method, and microencapsulated extracts process parameters were optimized using response surface methodology. The effects of these process parameters on the antimicrobial activity of microcapsules treated cotton fabrics were investigated. Based on the observation, the optimized process parameters were used to apply them onto the cotton fabric surface and assess the antimicrobial activity of the microcapsules treated fabrics. The zone of inhibition of microcapsules treated fabrics increases with the concentration of the herbal extract. The influence of curing temperature and cross linking agent concentration on the zone of inhibition was significant for both the bacterial strains. The agar diffusion results show high and clear zone of inhibition in mm against wide spectrum human pathogenic bacterial strains of Staphylococcus aureus and Escherichia coli. The maximum zone of inhibition was shown by Aloe barbadensis Mill at 33 mm and 27 mm used for the tests, and the minimum zone of inhibition of 29 mm and 25 mm for Staphylococcus
aureus and Escherichia coli respectively was shown Mentha piperita microcapsules treated fabric. These predicted and actual results were also supported by obtaining an excellent correlation between the experimental and modeled results by formulating a linear relationship between crosslinking agents, extract concentration and curing temperature.

The surface morphology of the microencapsulated copper enriched medicinal herbal herbs of Aerva lanata, Aloe barbadensis Mill, Cumminum cyminum Linn, Tagetes erecta and Mentha piperita treated fabric was analyzed by SEM, it shows the binding and presence of the microcapsules in the fibre assembly of the fabrics. The SEM results confirm that the microcapsules are distributed over the fibre surface. The FTIR pattern represents the functional group present in the treated fabric samples. This confirms that copper enriched herbs encapsulated in the microcapsules and also their effective application on the treated cotton fabric samples.

The physical properties such as air permeability and water vapour permeability were assessed for the microcapsules treated fabrics as per standard methods, based on the results, significant differences were observed between treated and control samples, that difference did not affect the basic properties of the cotton material.

The antimicrobial efficacy of copper enriched medicinal herbs of (Aerva lanata, Aloe barbadensis Mill, Cumminum cyminum Linn, Tagetes erecta and Mentha piperita) treated cotton fabric was measured both in terms of zone of inhibition (qualitative analysis (SN195920)) and bacterial reduction % (quantitative analysis(AATCC 100)) against on Staphylococcus aureus and Escherichia coli. It is clear that the aqueous extract, methanolic extract and microencapsulated copper enriched herbal treated fabric showed better antimicrobial activity than control fabric. Based on the observations, microencapsulated treated fabric showed antimicrobial activity in the range of
21 mm – 24 mm against on *Staphylococcus aureus* and 20 mm – 24 mm against on *Escherichia coli*, because the microencapsulated fabrics will release the core material after one or two washes only.

The wash durability, of both aqueous and methanolic extract and microencapsulated herbal treated samples were assessed after 5 washes and 10 washes by using AATCC 124, it is found from this test that the microbes resistant activity of extract treated samples did not shows better activity after 15 washes, this is because that the herbs extracts were applied only on the surface and core of the fibre assembly, on the other hand, microencapsulated samples shows higher bacterial reduction even after 15 washes against *Staphylococcus aureus* and *Escherichia coli*.

The microencapsulated copper enriched medicinal herbs treated fabric samples were used to construct the curative garments. The products are shirt, polo T-shirt, mask and gloves. The developed products were evaluated for their performance based on their curative effects during field trials. The performance of the copper enriched medicinal herbs treated curative garments were evaluated by conducting clinical trial at a nature cure centre by giving it to patients. The patients were advised to wear the garment next to the skin for 10 – 12 hours per day especially during sleeping time to get better result and to carry out the washing procedure with non – ionic detergents and dry them in shadow to retain medicinal property. The performance of the copper enriched herbal treated curative garments was evaluated once in a week and the clinical trial was conducted for 7 weeks duration.

Based on the feedback from patient and the nursing staff, the outcome of the trial as per the medical practitioner was made. The comparison was done for the period of 7 weeks before and after treatments during the wear trial of curative garments.
• The encapsulated *Aerva lanata* treated sample was found to reduce the seasonal skin allergy by 95% and scabies by 90%.

• The encapsulated *Aloe barbadensis* Mill treated sample was found to reduce the inflammatory skin disorder by 90%, vitiligo by 90% and lichen planus by 85%.

• The encapsulated *Cumminum cuminum* Linn treated sample was found to reduce the seasonal skin allergy by 95%, scabies by 90% and urticaria by 60% but results of eczema from the field trial it showed less significant effect at 45%.

• The encapsulated *Tagetes erecta* treated sample was found effective against allergic dermatitis. Based on the surveillance good results were obtained from the field trial a decrease of 85% for allergic dermatitis and 80% for parthenia allergy was observed and the skin recovered its original colour.

• Excellent results were obtained in treating detergent allergy in hands (Chemical Allergy). The encapsulated *Mentha piperita* treated sample was found to reduce the detergent allergy by 95%.

### 9.3 SCOPE FOR FUTURE WORK

The present work paved way for further research on the following aspects:

• There is a scope to try with various wall materials and to study their influence on the curative performance. Gelatin, chitosan, etc., are the various wall materials available that can be used for the microencapsulation of the herbal extracts.
• There is scope to try optimisation with parameters such as core wall material ratio, release factor study.

• The present method of imparting antimicrobial activity to the fabrics could be extended to different fabric material and structures for varied applications and therefore, has wide scope for future research.