CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter deals about type of fabrics used for curative garments, method of extraction of copper enriched medicinal herbal and their application methods, characterization techniques for microcapsules, performance evaluation of finished fabric materials and clinical trials of curative garments.

3.2 MATERIALS

Various materials used in this research work are described in this section. The materials include fabrics, copper enriched medicinal herbal herbs and chemicals used for preparation of microencapsulation and testing of antimicrobial activity of the present study. The research methodology flow chart of this research work is given in Figure 3.1.

3.2.1 Fabrics Used in the Present Research Work

Hundred percent bleached, mercerized cotton plain woven and single jersey knitted fabric was used in this study of curative finish. The construction details of curative garments fabrics specification details are listed in Table 3.1.
Figure 3.1  Research methodology flow chart
Table 3.1 Specification details of fabric construction

<table>
<thead>
<tr>
<th>SI.No</th>
<th>Material</th>
<th>Particulars</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cotton woven Fabric material</td>
<td>Count (both warp and weft)</td>
<td>40Ne</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Ends per Inch</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Picks per Inch</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>GSM</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Weave</td>
<td>Plain</td>
</tr>
<tr>
<td>6</td>
<td>Cotton knitted Fabric material</td>
<td>Count</td>
<td>40Ne</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Wales per Inch</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Course per Inch</td>
<td>56</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>GSM</td>
<td>150</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Structure</td>
<td>Single jersey</td>
</tr>
</tbody>
</table>

3.2.2 Medicinal Herbs

For this research work, five herbs were selected based on their suitability of curative purposes and their successful traditional usages. The selected herbs and their parts used are listed in Table 3.2

Table 3.2 Medicinal herbs and their parts used for curative finish

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Common name of the medicinal herbs</th>
<th>Botanical name of herbs</th>
<th>Family</th>
<th>Plant parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aerva lanata</em></td>
<td><em>Aerva lanata</em></td>
<td>Amaranthaceae</td>
<td>Stem (with bark), leaves</td>
</tr>
<tr>
<td>2</td>
<td>Aloe vera</td>
<td><em>Aloe barbadensis</em></td>
<td>Xanthorrhoeaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td>Cumin seed</td>
<td><em>Cumminum cuminum</em></td>
<td>Apiaceous</td>
<td>Seeds</td>
</tr>
<tr>
<td>4</td>
<td>Marigold</td>
<td><em>Tagetes erecta</em></td>
<td>Compositae</td>
<td>Flower</td>
</tr>
<tr>
<td>5</td>
<td>Mint</td>
<td><em>Mentha piperita</em></td>
<td>Lamiaceae</td>
<td>Whole plant</td>
</tr>
</tbody>
</table>
3.3 HERBS EXTRACTION TECHNIQUES

The methods followed by the traditional medicine practitioners for extracting the medicinal substances from the herbs were chosen for this research work to extract copper enriched medicinal herbs active ingredients.

3.3.1 Preparatory Process for Herbal Extraction

The collected herbs were washed and shadow dried within a temperature range of 30-40ºC. Natural drying was carried out to preserve the important compounds. After drying, the plant parts were ground to break them down into very small units ranging from coarse fragments to fine powder.

3.3.2 Aqueous Extraction

Ten grams of herbs powder was added to 100 ml of distilled water and mixed well. After 24 hrs, the supernatant was collected and stored at 4ºC (Harbone 1973).

3.3.3 Methanol Extraction

Solvent Extraction refers to separating the active substances by physical or chemical means with the aid of a solvent. Ten gram of herb powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hrs, the supernatant was collected and the solvent was evaporated and the crude extract was stored at 4ºC (Harbone 1973).

3.3.4 Identification of Copper Content

One gram of dried ground plant tissue was ashed in a muffle furnace at 500ºC overnight. The ash was digested using conc.HNO₃. The
digested sample was made up to 50 ml in a volumetric flask and used for assay of trace elements using Atomic Absorption Spectrometer (AAS) by suitable hollow cathode lamps of Perkin Elmer A Analyst 100. Five replicates were prepared for each sample.

3.4 MICROENCAPSULATION OF COPPER ENRICHED MEDICINAL HERBS EXTRACTS

Microencapsulation was done using copper enriched medicinal herbs extract as core material and gum acacia as wall material. 10 gms of wall material was allowed to swell for half an hour by mixing with 100 ml of hot water at 60 – 70°C. To this mixture, 50 ml of hot water was added, stirred for 15 min maintaining the temperature between 40°C - 50°C. 10 ml of core material was added and stirred at 300 – 500 rpm for further 15 min followed by drop wise addition of 20% sodium sulphate solution (10 ml) for 5 – 10 minutes. The stirrer speed was reduced and then 5 ml of 17% glycerol was added. The stirrer was stopped and mixture was freeze dried (Lazkom et al 2004; Thilagavathi et al 2007).

3.5 CHARACTERISATION OF MICROCAPSULES

The various parameters were used to evaluate and characterize the microcapsules, these include production yield, microcapsules morphology, average particle size, cytotoxicity analysis and Stability against temperature.

3.5.1 Production Yield

The production yield of the microencapsulation system is the quantity of microcapsules obtained as an outcome of chemical reaction during the microencapsulation processes. It is a necessary factor indicating the quality of synthesis and subsequent handling processes. It is considered to be
better or more efficient when a larger fraction of the microcapsules is produced. During the test, the microcapsules were dried at 100°C right after rinsing with hexane solution, and the dry weight of microcapsules was measured (Yan 2011).

The production yield of the microencapsulation system was calculated by the following Equation (3.1):

\[
\text{PRODUCTION YIELD} \% = \frac{W_0 - W_1}{W_0} \times 100\% \tag{3.1}
\]

where:  
\( W_0 \) (gms) = Weight of Input Materials,  
\( W_1 \) (gms) = Dry Weight of Microcapsules

### 3.5.2 Microcapsules Morphology

Microcapsules were examined under the 100X, 500X and 1000X objective of light microscope to analyze the morphology of capsules.

### 3.5.3 Average Particle Size

The mean particle size of microcapsules was evaluated by means of a zetasizer (Malvern Instruments, Zetasizer 3000 HSA). The microcapsules were first diluted with deionised water in the ratio of 1:20 and 4 ml of the solution was then taken for evaluation. The reduction in average particle size was studied over a period of 240 hours. The particle size was measured using optical microscope.

### 3.5.4 Cytotoxicity Analysis

Cytotoxicity test was used to evaluate the toxicity effect of microcapsules. The human skin keratinocytes HaCaT were removed from
75 mL sterile cell culture flasks with trypsin and neutralized with fetal bovine serum. After washing with phosphate buffered with saline and centrifugation, cells were re-suspended in complete cell culture medium at a concentration of approximately $1 \times 10^5$ cells per mL and counted manually using a haematocytometer under an inverted microscope. Human cells seeded in the 96 wells microtitre plates for 24 hours were prepared for the screening of the microcapsules. The microcapsules were mixed in complete cell culture medium at a stock concentration of 1 mg/mL. Cis-platin used as the positive reference compound was added at a starting concentration of 100 μg/mL. Microcapsules were also added at a starting concentration of 100 μg/mL followed by a serial two-fold dilution and incubated with cells for a further period of 48 hours. Afterwards, keratinocytes were fixed with trichloro acetic acid, washed with distilled water and stained with sulforhodamine B. Afterwards, cells were washed again with acetic acid and the stained cells were resuspended in 100 μL of unbuffered Tris-base. The cellular morphology changes of the cells were observed under optical microscope (Chui et al 2009, Cheng et al 2009, Cheng 2011).

### 3.5.5 Stability Against Temperature

The microcapsules of known weight were immersed into deionised water and then heated at different temperatures for the analysis of heat stability. The released core material was collected after centrifugation and then weighed. The release of core material from the microcapsules at different temperatures, i.e. 40°C, 60°C, 80°C and 100°C was investigated. The heat stability was studied as it was expected that skin caring fabric materials would undergo standard laundering procedures at different temperatures, i.e. 40°C – 60°C, during their normal use and also at high curing temperature upto 100°C – 120°C during the pad – dry – cure coating process.
3.6 METHOD OF APPLICATION OF HERBAL EXTRACT AND MICROCAPSULES ON FABRIC

The cotton fabrics sample were treated with herbal extracts both aqueous and methanol extracts using 8% citric acid as a cross linking agent by pad – dry – cure method. After padding the samples were taken and dried at 80°C for 10 min and cured at 120°C for 3 min.

Cotton fabric were also immersed in the dispersion of microcapsules, padded through pneumatic padding mangle, with an expression of 80% wet pickup, dried at 80°C and cured at 100°C for 3 min. The application of microcapsules onto the fabric was done with and without crosslinking.

3.7 CHARACTERISATION OF COPPER ENRICHED HERBAL TREATED FABRICS

This part discusses the various physical and chemical characterisation methods used to evaluate the surface morphology and antimicrobial activity of copper enriched herbal treated fabric.

3.7.1 Scanning Electron Microscopy

The surface morphology of fabric treated with micro-encapsulated extract was analysed using scanning electron microscope, SEM (Model JEOL-JSM-6396) with a high-energy beam of electrons in a raster scan pattern. The observation was performed in high vacuum mode with secondary electron detector and accelerating voltage of 3-10 kV. The scanning electron microscope was used for confirming the binding, alignment and availability of microcapsules into the fabric sample.
3.7.2 Fourier Transform Infrared Spectroscopy

The Fourier Transform Infrared spectrometer is most useful for identifying active chemical components whether organic or inorganic. The model of Fourier Transform Infrared spectrometer used was SHIMADZU – FTIR – 8400S. The Fourier Transform Infrared spectrum was obtained at a spectral range of 400 – 4000 cm\(^{-1}\) and has a resolution of 0.9 – 1 cm.

3.7.3 Antibacterial Activity Assessment

3.7.3.1 Microorganisms

Bacterial cultures used in the present studies, *Staphylococcus aureus* (MTCC 737) and *Escherichia coli* (MTCC 1687) were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh.

3.7.3.2 Agar diffusion method – qualitative method (SN 195920)

Agar diffusion method is used to determine the effectiveness of antimicrobial agents appropriate bacteriostatic agar media were inoculated with one day old culiuies of fca organisms and poured on Petri dishes and allowed to solidify. Non-sterile test specimen fabric (both copper enriched herbal extracts and microcapsules treated and untreated) were cut into pieces of 15mm diameter and placed on the solidified agar media. test dish was initially stored at 5°C for 24 hours for conditioning and then placed in an incubator the fabric was kept in intimate contact with the media, of there was curling of the fabric, small sterile glass plates were placed on the fabric top hold it in place incubation was done at 37°C for 18-24 hours.

At the end of the incubation time, the test dishes were observed. The agar under the sample was also evaluated. This was important if no zones of inhibition existed. This assessment was made by visual examination as well
as under a microscope with 40X magnification. The evaluation was made on the basis of absence or presence of an effect on bacteria in the contact zone, under the specimen and the possible formation of a zone of inhibition around the test specimen.

3.7.3.3 Quantitative analysis of antibacterial activity (AATCC-100)

Treated and control samples were inoculated with test organisms. After incubation, the bacteria were eluted from the swatches by shaking in known amounts of neutralizing solution. The number of bacteria present in this liquid was determined and the percentage reduction by the treated specimen was calculated. The bacterial counts were reported as the number of bacteria per sample (swatches in jar) not as the number of bacteria per ml of neutralizing solution. ‘0’ counts at 100 dilution was reported as “less than 100”. The percentage reduction (R) of bacteria by the specimen treatments was calculated using the following formula:

\[ R = 100 \left( \frac{B - A}{B} \right) \]

where, A is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period; and B, the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at ‘0’ contact time).

3.8 PHYSICAL PROPERTY ASSESMENT

The antimicrobial treated cotton fabric samples were tested to assess the following physical properties as per the given standard methods. Twenty samples were tested for each test and the average value is given for the discussion.
3.8.1 Air Permeability (ASTM D 737-99)

The specimen were preconditioned as per the method described in practice D 1779 for preconditioning textiles by bringing them to approximate moisture equilibrium at standard atmosphere.

If it is known that the material to be tested is not affected by heat or moisture, preconditioning and conditioning is not required when agreed upon in a material specification or contract order.

The conditioned specimens were tested in the standard atmosphere, that is 21 ± 1°C (70 ± 2°F) and 65 ± 2 % relative humidity, unless otherwise specified in a material specification or contract order. The test specimen was handled carefully to avoid altering the natural state of the material and tested by placing it on the test head of the air permeability tester and analysed as per manufacture’s operating instructions. The coated surface of test specimen was placed down to minimise the edge leakage.

The tests were carried out at the water pressure differential specified in material specification or contract order. In the absence of a material specification or contract order, water pressure differential of 125 Pa (12.7 mm or 0.5 in. of water) was used.

Individual test results were recorded in SI units as cm³/s/cm² and in inch-pound units as ft³/min/ft² rounded to three significant digits.

For special applications, the total edge leakage underneath and through the test specimen was measured in a separate test, with the test specimen covered by an airtight cover, and subtracted from the original test result to obtain the effective air permeability.
3.8.2 Water Vapour Permeability (BS 7209)

In the British Standard version of this method the specimen under test is sealed over the open mouth of a dish containing water and placed in the standard testing atmosphere. After a period of time to establish equilibrium, successive weighings of the dish are made and the rate of water vapour transfer through the specimen is calculated. The water vapour permeability index is calculated by expressing the Water Vapour Permeability (WVP) of the fabric as a percentage of the WVP of a reference fabric which is tested alongside the test specimen. Each dish is filled with sufficient distilled water to give a 10 mm air gap between the water surface and the fabric. A wire sample support is placed on each dish to keep the fabric level. Contact adhesive is applied to the rim of the dish and the specimen, which is 96 mm in diameter, is carefully placed on top with its outside surface uppermost. The cover ring is then placed over the dish and the gap between cover ring and dish sealed with PVC tape. A dish which is covered with the reference fabric is also set up in the same way. All the dishes are then placed in the standard atmosphere and allowed to stand for at least 1 h to establish equilibrium. Each dish is then weighed to the nearest 0.001 g and the time noted. After a suitable time for example overnight the dishes are reweighed and the time noted again.

**Calculation:** \[ WVP = \frac{24M}{At} \text{ g/m}^2/\text{day} \]

where \( M = \) loss in mass (g),
\( t = \) time between weightings’ (h),
\( A = \) internal area of dish (m2).

\[ A = \frac{\pi d^2 \times 10^{-6}}{4} \]

where \( d = \) internal diameter of dish (mm).
3.9 EVALUATION OF DURABILITY OF HERBAL TREATED FABRICS

3.9.1 Wash Durability of Finished Fabrics (AATCC 124 1996)

This was performed to evaluate the performance of the textile material. Flat fabric specimens were subjected to standard home laundering practices. They were subjected to hand or machine washing, alternative machine wash cycles and temperatures and alternative drying procedures. Evaluation was performed in comparison with appropriate reference standards.

Test specimens: Representative specimen fabrics of 92 X 92 cm in size cut parallel to the fabric length and width were prepared. Possibly each specimen contained different groups of lengthwise and width wise yarns. The specimens were marked to indicate the length wise if fraying is expected in laundering.

Procedure: This method had a material to liquor ratio of 1:30 with 0.2% detergent using 1993 AATCC standard reference detergent. The washing machine had conditions were water level was 18 ± 1 gal, agitator speed: 179 ± 2 spm, wash time: 12 min, spin speed: 645 ± 15 rpm, final spin cycle: 5-6 min. One laundering cycle included subsequent steps of 5 minutes of laundering and 2 minutes of rinsing, followed by another 2 minutes of rinsing and tumble drying. For tumble-drying, the washed and rinsed specimens were placed in the tumble dryer and the temperature was set below 60°C for 10 minutes. The dryer was operated until the total load was dry. The load was removed immediately after the machine stops to avoid over drying. The selected washing and drying cycles were repeated upto 50. One wash cycle was considered equivalent to 5 washes in domestic practice.
**Evaluation:** The residual antimicrobial activity was measured by shake flask method (AATCC 100 1993) as described earlier after 5 wash cycles and efficiency was measured in terms of percent bacterial reduction.

### 3.9.2 Perspiration Fastness of Herbal Treated Fabrics

A fastness property of copper enriched herbal treated samples to perspiration was tested using AATCC TM 15-2002. The perspiration solution was prepared using urea (0.75 g) and sodium chloride (2.56 gms) in 1000ml of water to a pH 6.0 with acetic acid and alkaline pH 7.5 was obtained using sodium carbonate (3 g/l). Specimen (10X4 cm) soaked in the perspiration solution were placed in between the glass plates under load of 4.5 kg, maintained for 6 hour and change in colour was assessed using grey scale.

### 3.10 PERFORMANCE EVALUATION OF CURATIVE GARMENTS

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.

**Table 3.3 Grading system for subjective evaluation of skin diseases**

<table>
<thead>
<tr>
<th>SI.No</th>
<th>% of Improvement</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 50%</td>
<td>Poor</td>
</tr>
<tr>
<td>2</td>
<td>50 – 70%</td>
<td>Average</td>
</tr>
<tr>
<td>3</td>
<td>70 – 90%</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>90%</td>
<td>Excellent</td>
</tr>
</tbody>
</table>
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 suggestions from the doctor and patients the grade system formulated for subjective evaluation of selected skin diseases, the grade system as show in Table 3.3.