3

EXPERIMENTAL PROCEDURE

The experimental procedure adopted for the study on **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound Dressing"** consists of a series of processes, techniques and instruments, which is sub-divided into four phases.

- The first phase consisted of literature survey, collection of information for the properties of wound dressing band aids, selection of herbs, preparation and processing of herbs, selection and testing the physical properties of yarn, weaving and assessing the physical properties of woven fabrics.
- The second phase included the extraction process of herbs with three different solvents namely Hexane, Ethyl acetate and Methanol. Qualitative Phytochemical Analysis of Herbal Extracts was also done to select best suited solvent extraction for final study.
- The third phase comprised of the selection of microbial cultures, determination of Minimum Inhibitory Concentration (MIC) against selected microorganisms, polyherbal formulation and assessing antimicrobial activity, wound scratch assay in fibroblast cell line method analysis.
- The fourth phase involved the preparation of Micro and Nano-encapsules and fabric finishing with the selected polyherbal extract by Dip and Dry and Exhaust method. The finished fabrics were tested for its physical properties and were finally subjected to product development and evaluation.

Detailed subheadings under each phase is presented as follows;

PHASE I

3.1 Literature Survey

3.1.1 Collection of Information to study the Properties of Wound Dressing Bandaids

3.1.1.1 Selection of Method for Data Collection

3.1.1.2 Preparation of Interview Schedule

3.1.1.3 Pilot Study

3.1.1.4 Actual Interview

3.2 Selection of Yarn and Testing of Physical Properties of Cotton Yarn

3.2.1 Yarn Count cv%

3.2.2 Evenness Percentage of Yarn

3.2.3 YarnTenacity

3.2.4 Yarn Hairiness

3.2.5 Moisture Content

3.2.6 Yarn Thickness

3.2.7 Yarn Twist per Inch

3.2.8 Physical properties of Cotton Yarn

3.3 Fabric Formation and Pretreatment of Fabric

3.3.1 Warp Winding

3.3.2 Weaving

3.3.3 Pretreatment

3.3.3.1 Desizing

3.3.3.2 Scouring

3.3.3.3 Bleaching

3.4 Selection of Herbs

3.4.1 Taxonomy of Plants

3.4.2 Plant Authentication

3.5 Processing of Herbs

3.5.1 Drying

3.5.2 Garbling

3.5.3 Grinding

PHASE II

3.6 Herbal Extraction

3.6.1 Extraction by Soxhlet

3.6.2 Optimisationof Herbal Extraction

3.6.3 Qualitative Phytochemical Analysis of Herbal Extracts

3.6.3.1 Test for Carbohydrates

3.6.3.2 Test for Tannins

3.6.3.3 Test for Saponins

3.6.3.4 Test for Flavonoids

3.6.3.5 Test for Alkaloids

3.6.3.6 Test for Quinones

3.6.3.7 Test for Glycosides

3.6.3.8 Test for Cardiac Glycosides

3.6.3.9 Test for Terpenoids

3.6.3.10 Test for Phenols

3.6.3.11 Test for Coumarins

3.6.3.12 Test for Steroids and Phytosteroids

3.6.3.13 Test for Phlobatannins

3.6.3.14 Test for Anthraquinones

PHASE III

3.7 AntimicrobialTesting

3.7.1 Selection of Microbial Cultures

3.8 Determination of Minimum Inhibitory Concentration (MIC)

3.8.1 Antimicrobial Activity of Herbal Extract by Agar Well Diffusion Method

3.8.2 Polyherbal formulation

3.8.3 Antimicrobial Activity of Polyherbal Extract byAgar Well Diffusion Method

3.8.4 Wound Scratch Assay of Polyherbal Extract

PHASE IV

3.9 Application of Polyherbal Extracts on Cotton Fabrics

3.9.1 Dip and Dry Method

3.9.2 Exhaust Method

3.9.2.1 Preparation of Polyherbal Microencapsules by Ionic Gelation Process

3.9.2.2 Preparation of polyherbal Nanoencapsules

3.10 Antimicrobial Activity by AATCC 147

3.11 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

3.12 Scanning Electron Microscopic (SEM) analysis

3.13 Testing of Physical Properties of Polyherbal Pretreated Woven Fabric

3.13.1 Fabric Weight

3.13.2 Tensile Strength and Elongation

3.13.3 Air Permeability

3.13.4 Water Absorbency

3.13.5 Vertical Wicking

3.13.6 Sinking

3.13.7 Water Holding Capacity

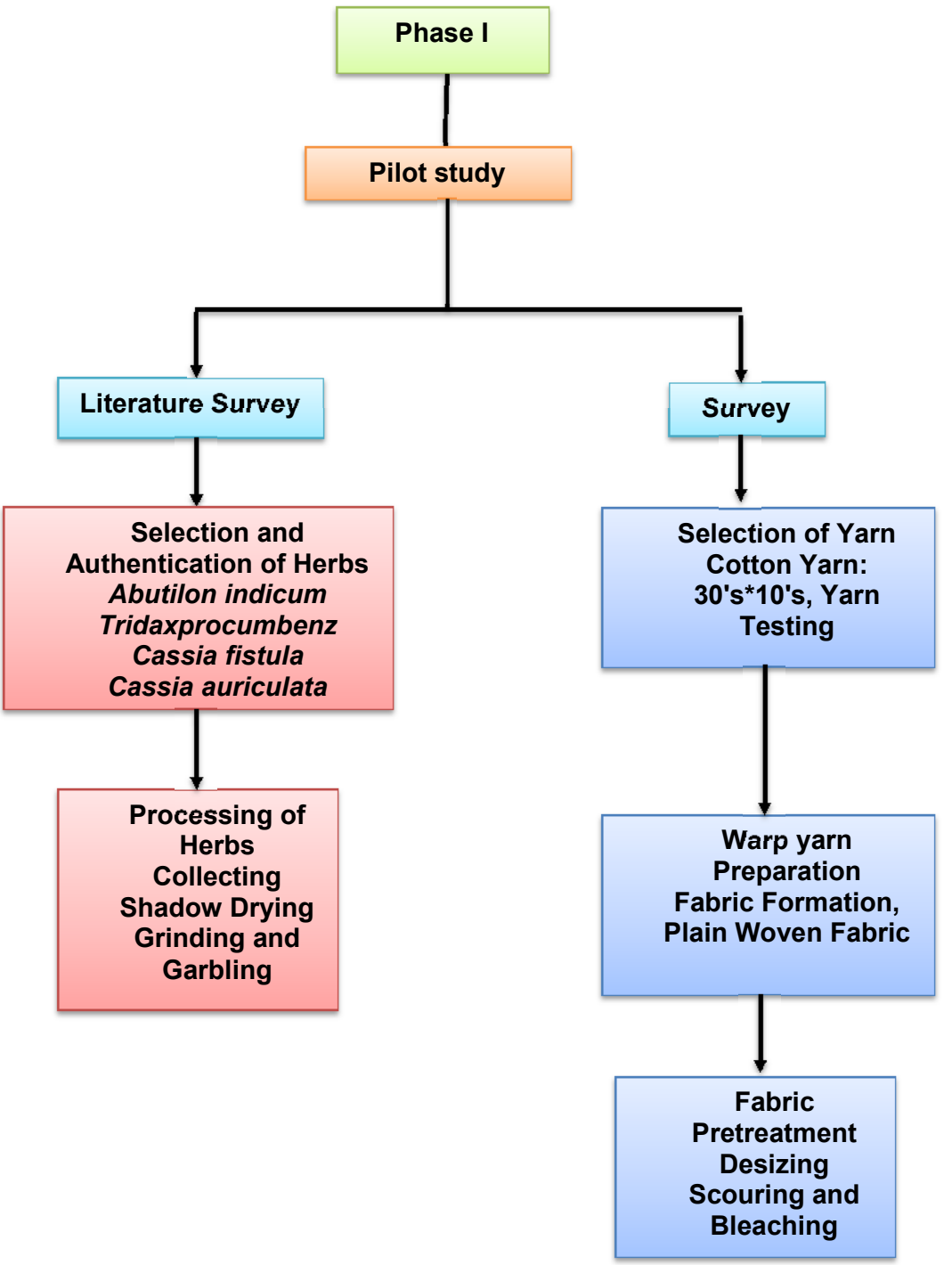
3.14 Product Development and Evaluation

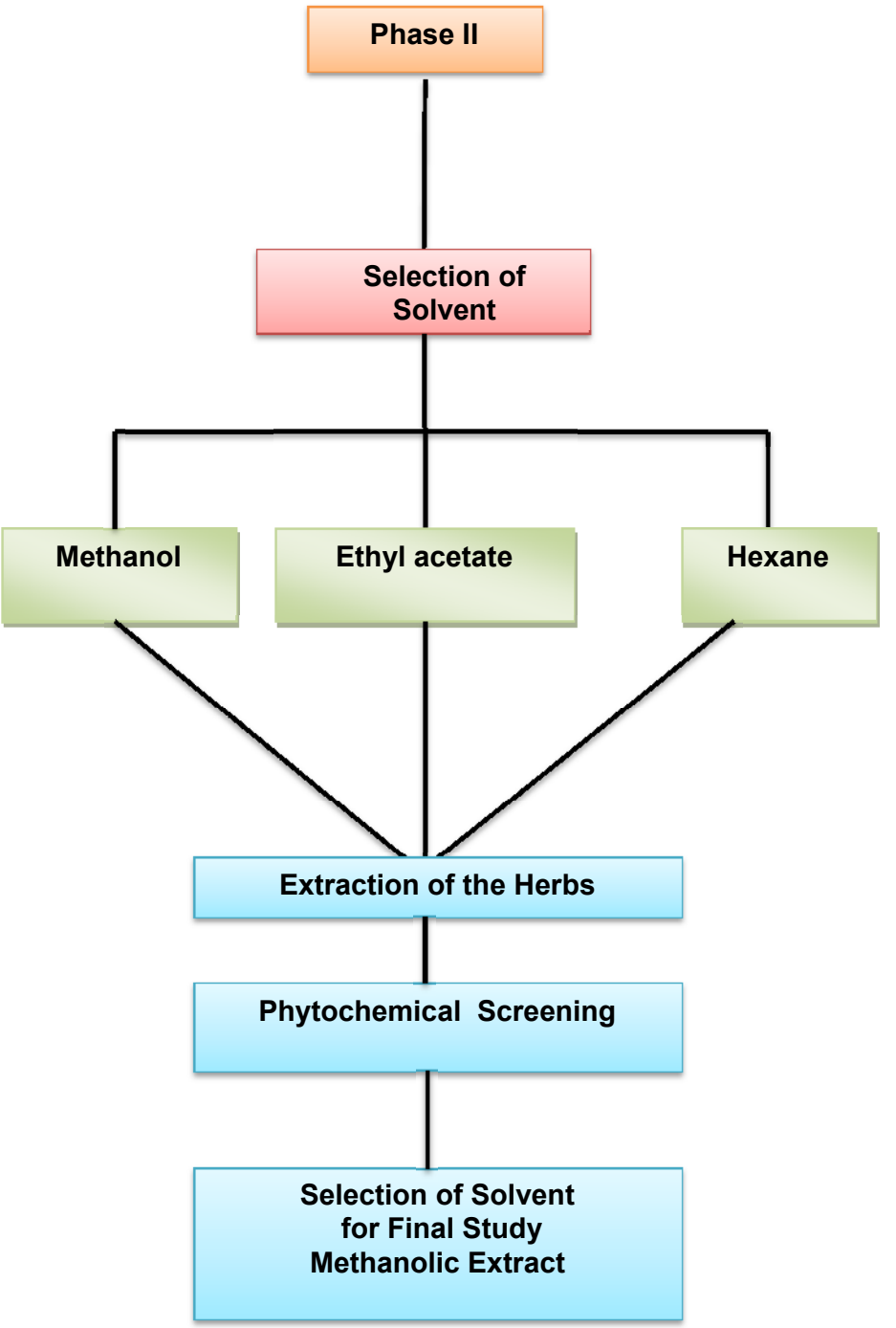
3.14.1 Microbial Filteration Test

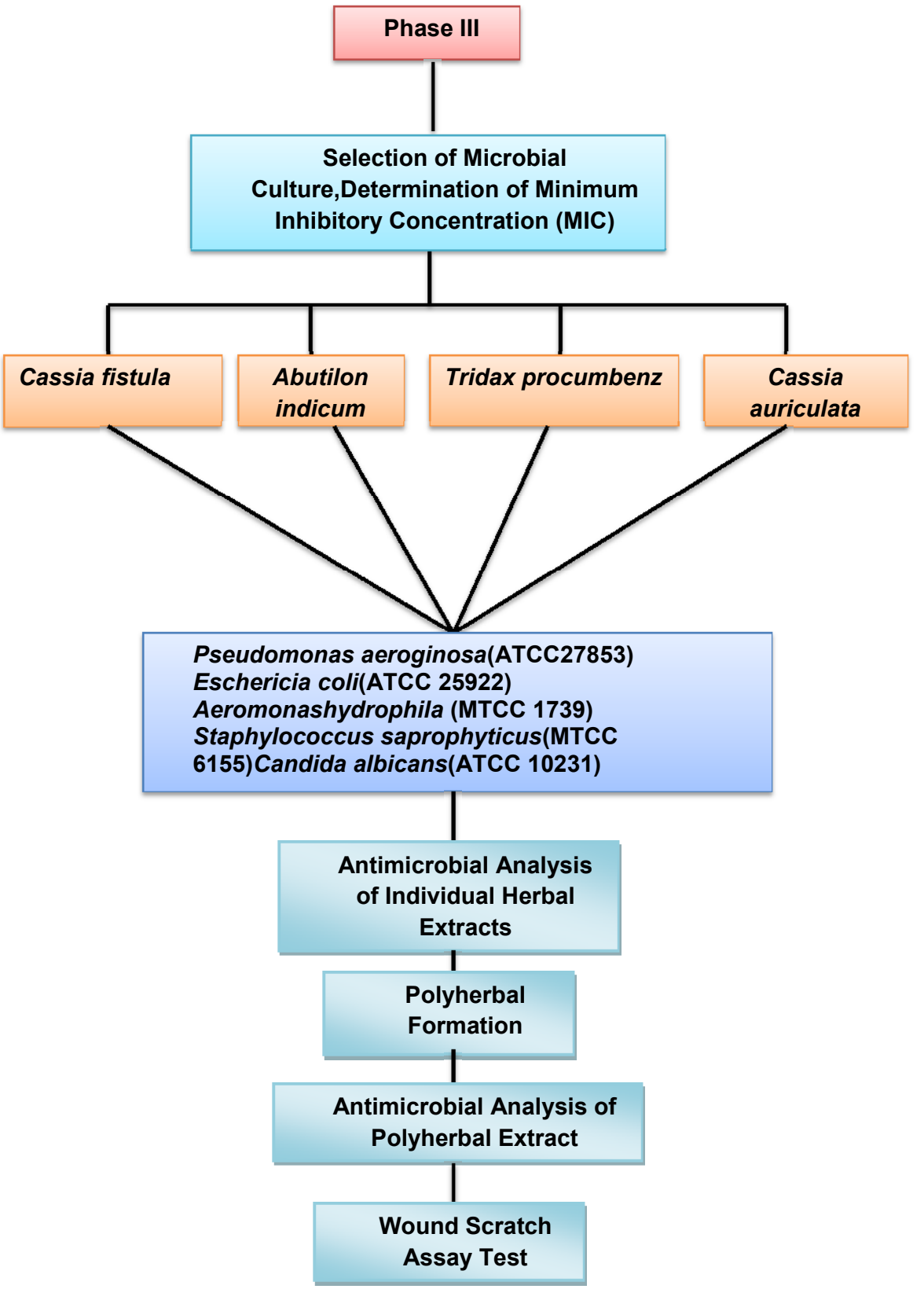
3.14.2 Bandaids Toxicity Test

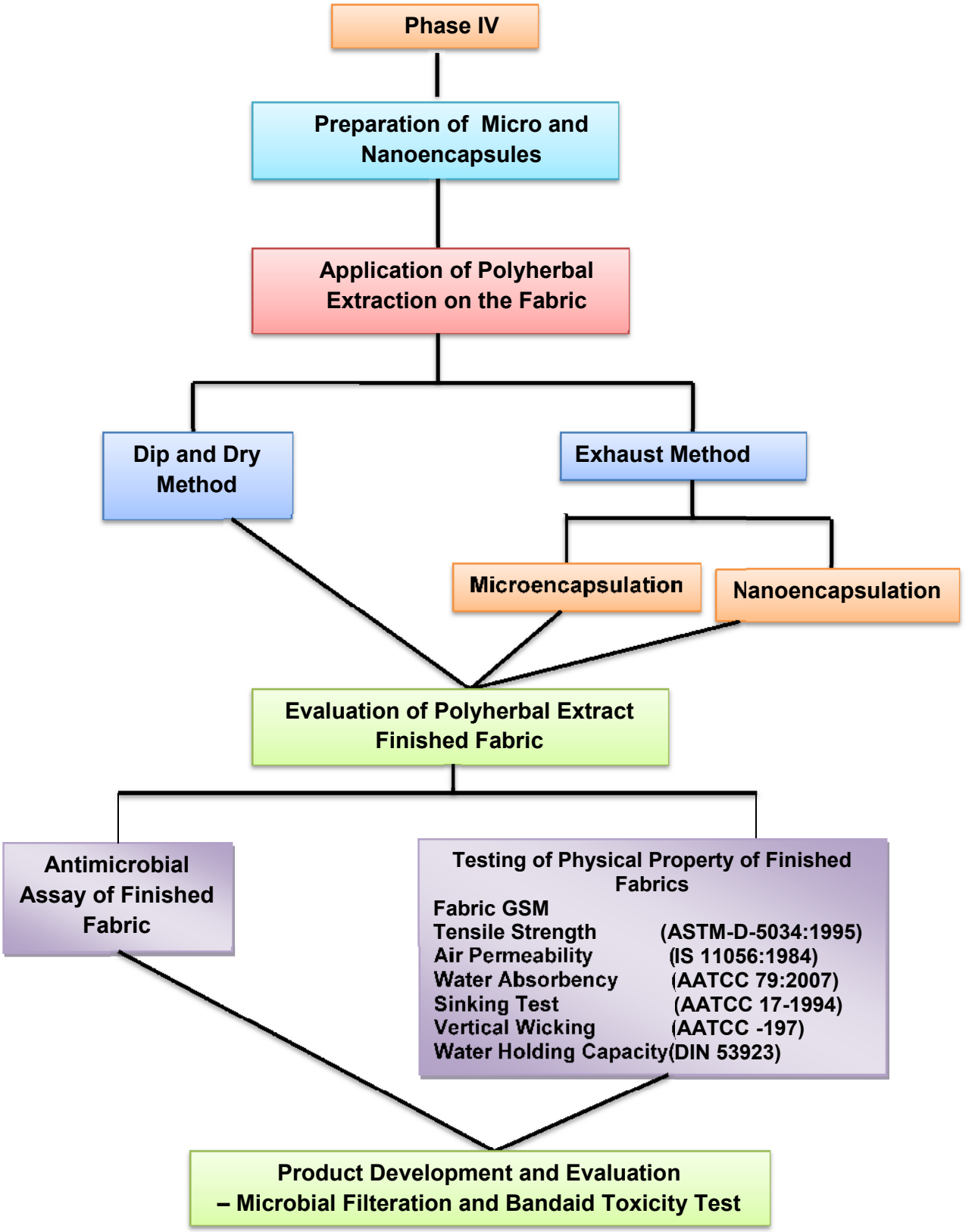
3.15 Statistical Analysis

The schema chart for the different phase of the study are presented as follows.




Phase II
Selection of Solvent
Methanol
Ethyl acetate
Hexane
Extraction of the Herbs
Phytochemical Screening
Selection of Solvent for Final Study Methanolic Extract


Phase III
Selection of Microbial Culture,Determination of Minimum Inhibitory Concentration (MIC)
Cassia fistula
Abutilon indicum
Tridax procumbenz
Cassia auriculata
Pseudomonas aeroginosa(ATCC27853)
Eschericia coli(ATCC 25922)
Aeromonashydrophila (MTCC 1739)
Staphylococcus saprophyticus(MTCC 6155)Candida albicans(ATCC 10231)
Antimicrobial Analysis of Individual Herbal Extracts
Polyherbal Formation
Antimicrobial Analysis of Polyherbal Extract
Wound Scratch Assay Test


Phase IV
Preparation of Micro and Nanoencapsules
Application of Polyherbal Extraction on the Fabric
Dip and Dry Method
Exhaust Method
Microencapsulation
Nanoencapsulation
Evaluation of Polyherbal Extract Finished Fabric
Antimicrobial Assay of Finished Fabric
Testing of Physical Property of Finished Fabrics
Fabric GSM
Tensile Strength (ASTM-D-5034:1995)
Air Permeability (IS 11056:1984)
Water Absorbency (AATCC 79:2007)
Sinking Test (AATCC 17-1994)
Vertical Wicking (AATCC -197)
Water Holding Capacity (DIN 53923)
Product Development and Evaluation – Microbial Filteration and Bandaid Toxicity Test

PHASE I

3.1 Literature Survey

Literature survey for the study was carried out in the library of Avianashilingam Institution of Home Science and Higher Education for Women, Coimbatore, South Indian Textile Research Association, South Indian Mill Association, Coimbatore and Kamaraj University, Madurai.

3.1.1 Collection of Information for the Properties of Wound Dressing Bandaids

Based on the survey of literature, it is clear that the wounds are based on healing, exposure to environment, visibility, hygiene, texture, tissue loss and appearance. They are also classified into acute, chronical, open, closed, internal, external, clean, infected, penetrating, non-penetrating, miscellaneous, superficial, partial thickness, full thickness, necrotic, sloughy, granulating and epithelializing. Each of these wounds need different care, method of treatment and even the use of bandaid vary. Physicians have a clear idea about all type of wound,its depth and care needed. In order to get a clear idea of the common wounds the types of bandaids, the fabrics to be used and their properties the investigator decide to collect the information from the physicians.

For framing interview schedule, the investigator collected the information about the commercially available wound dressing and band aids with respect to their size, price, type and properties. The details of commercially avilable wound dressing are given in Appendix(I)*.

3.1.1.1 Selection of Method for Data Collection

Interview is first hand information and it is a face to face interview method of data collection. It helps the individual to collect the maximum information within a short duration. An interview schedule is basically a list containing a set of structured questions that have been prepared and serve as a guide for interview. Investigator used this method to collect information or data about a specific topic or issues. Therefore, based on the above facts the investigator selected interview as the best method to collect data from doctors. An interview schedule was prepared.

3.1.1.2 Preparation of Interview Schedule

In research interview a list of questions are formulated and given to the respondent to answers for the purpose of testing hypothesis and assumption.

There are two types of interview schedule.

1. In depth interview schedule
2. Structured interview schedule

In depth interview schedule is used for open end interviews to obtain in depth information, usually they are used on serious topics or sensitive issues. The questions are open ended and provided to the interviewer to ask for clarification or further informations where as, the structured interview schedule is often compared with the survey forms, or questionnaires because of their similarities. The interview schedule contains the structured question that are used during the interview and the responses are recorded. Considering this fact the investigator framed the structured interview schedule.

3.1.1.3 Pilot Study

Pilot study was conducted among the surgeons, senior doctors and physiscians to test the questionnaire and based on the responses the interview schedule was restructured as given in the Appendixes (II).

3.1.1.4 Actual Interview

The investigator got the permission from The Dean, PSG Institute of Medical Science and Research, Coimbatore and meet the surgeons, senior doctors and physiscians, a total of fifty members responded and the required data was collected. The data was recorded systematically and consolidated. The results of the interview schedule is presented in the Table VII and VIII.

3.2 Selection of Yarn and Testing of Physical Properties of Cotton Yarn

Based on the recommendation of Hampton,(1980) (Patent No:4,207,885), the warp must be strong to be held under high tension during the weaving process, unlike the weft which carries almost no tension.

Hence, 30s Ne count yarn of high count was used for the warp and 10s Ne was used for the weft.Yarn testing was performed prior to weaving to identify the stability and efficacy of the yarn for fabric construction (Plate I). Testing of physical properties of cotton yarn such as Yarn count CV%, Eveness percentage of yarn, Tenacity of yarn, Yarn hariness test, Moisture content, Yarn thickness and Twist Pre Inch were carried out as per the procedure explained below;

3.2.1 Yarn Count CV% (ASTM D 1907-01)

The lea count of 30s Ne and 10s Ne count yarn was measured by Wrap Reel (Plate II)120 yards of yarn sample was taken from 30's and 10's count yarn and was conditioned under the standard atmospheric condition of relative humidity of 65±2% and temperature at 27±2ºc. The lea of the yarn was taken and fixed to the lea testers hook carefully.The Lea strength analyser (Plate III)was switched on and the rupture of the yarn lea had been recorded. The process was repeated and the readings were taken finally.The average breaking load and the yarn strength had been determined following ASTM D1907-01 standards.

3.2.2 Evenness Percentage of Yarn (ASTM- D-1425-96)

For the study, the Uster Evenness Tester (Plate IV)was used and the U% of the yarn had been calculated. The Uster evenness tester measures the thickness variation of a yarn by measuring capacitance . The yarn is passed through two parallel plates of a capacitor whose value is continuously measured electronically. The unevenness is always expressed between successive lengths and over a total length of yarn. If the successive lengths are short, the value is sometimes referred to as the short-term unevenness. The measurements made by the Uster instrument are equivalent to weighing successive 1 cm lengths of the yarn. *U* value gives an overall number for yarn irregularity and hence it is the most widely used measurements to study yarn evenness.

Using the procedure Uster evenness testing method, the U% of selected yarn of 30's and 10's count had been calculated for 120 yards. The tests were done according to ASTM-D-1425-96 standards.

Yarn Testing

Plate I 30's and 10's yarn cones

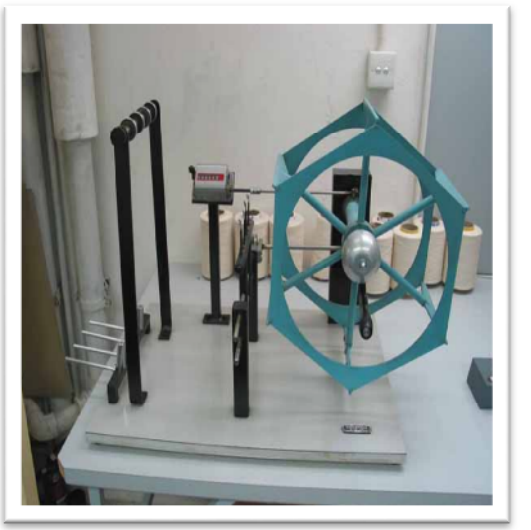


Plate II Wrap reel

Plate III Lea strength analyser

Plate IV Yarn evenness tester

Yarn Testing

Plate V Yarn Tenacity Tester

Plate VI Yarn Hairiness Tester

Plate VII Moisture Content Test

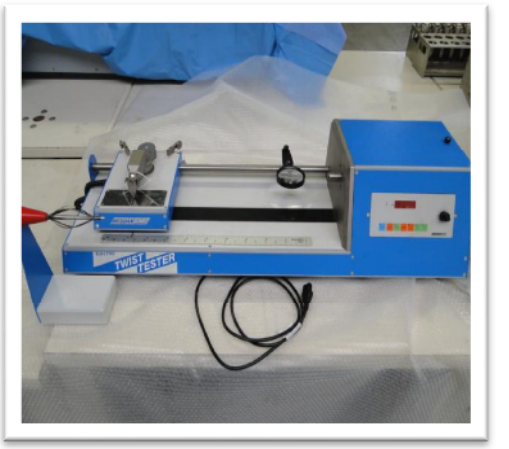


Plate VIII Yarn twist tester

3.2.3 Yarn Tenacity (ASTM –D-2256-97)

Tenacity of yarn is one of the most important testing operation.The conventional strength and elongation of the yarn has been tested for tenacity in accordance with the Constant Rate of Extension(CRE). The measuring principle is suitable for the testing of textile yarns (staple and filament yarns), technical yarns, woven fabrics and skeins. The tenacity of the 30's and 10's count yarn had been tested in Yarn Tenacity Tester (Plate V) using the constant rate of elongation

method in the standard atmospheric condition with a relative humidity of 65±2% and temperature at 27±2ºc. The yarn was fixed between the clamps and elongated at the constant rate. As the extension continues, the tension in the sample reached to its maximum value and broken at weakest point.

Care was taken during yarn with drawal without obstruction in yarn path. Testing speed used was 5000 mm per minute and the values were recorded.

3.2.4 Yarn Hairiness (ASTM-D-5647-01)

For the study, the yarn hairiness was measured using Zweigle Yarn Hairiness Tester (Plate VI). The hairiness of a yarn characterizes the number of projecting and freely moving fibre ends or fibre loops. The number of projecting fibres per unit length was assessed. The measurement technique used by this instrument was based on the Photoelectric principle. Each yarn sample of 100mm was taken for assessing the hairiness of yarns. This apparatus counts the number of hair from the edge of the yarn to 25mm. The hairs were counted simultaneously by a set of photo cells which were arranged at 1, 2, 3, 4, 6, 8,10,12,15,18,21 and 25mm from the edge of yarn. The yarn was illuminated from the opposite side by the photocells and as the yarn runs past the measuring station, the hairs cut the light off momentarily from the photocells, which causes the electrical circuits to count. The instrument measures the total number of hairs in each length category for the set test length. The yarn speed is fixed at 50m/min but the length of yarn tested may be varied. The instrument calculates the total number of hairs above three mm in length which can be used as a comparison with the Shirley instrument. It also computes a hairiness index which has been especially devised for this instrument and which is intended to combine all of the information measured by the instrument. All the tests were done according to ASTM-D-5647-01 standard and the values were calculated for 30's and 10's count yarns in mm.

3.2.5 Moisture Content (ASTM D 2495-01)

To measure the moisture content,specimens are weighed, dried in an oven, and reweighed.The difference between the original mass and the oven-

dry mass is calculated in percentage either as moisture content or moisture regain (ASTM,2001). For the study, the moisture content in yarn was determined by Oven Drying Method. The standard atmospheric condition with a relative humidity of 65±2% and temperature at 27±2ºcwas maintained.

Ten yarn samples were separately weighed in the weighing machine, dried and then the mass of the yarn was calculated using ASTM D 2495-01 standard testing method. The amount of moisture in cotton yarn was determined under prescribed conditions and expressed in percentage of the mass of the moist material (Plate VII).

3.2.6 Yarn Thickness (ASTM-D-1425-96)

For the study, the thickness or the diametre of the yarn had been identified using Usters Evenness Tester. The Usters evenness tester measure the thickness of the yarn by passing the yarn through the two parallel plate of capacitors whose value is continuously measured electronically. The evenness tester therefore have a module for determining the thickness variability. Thickness variability is hover even caused by the weight variability of the yarn. The thickness of the yarn is measured in mm.

Using the procedure Uster evenness testing method, the yarn thickness of 30's and 10's count had been calculated for 120 yards. The tests were done according to ASTM-D-1425-96 standards.

3.2.7 Yarn Twist Per Inch (ASTM D1442-2007)

For the study, the twist of the 30's and 10's count yarn had been calculated in Automatic Electronic Twist Tester (Plate VIII) which worked on the principle of untwist-twist testing method. 10 samples of 30's and 10's count with the gauge length of 125 mm were taken and Twist per Inch (TPI) was calculated according to ASTM D1442-2007 standard. It is based on the fact that yarns contract in length as the levelof twist is increased. Therefore if the twist is subsequently removed, the yarn will increase in length reaching a maximum under a suitable tension. The

test procedure is to untwist the yarn until all its twist has been removed and then to continue twisting the yarn in the same direction, until it returns to its original length. The basis of the method is the assumption that the amount of twist inserted is equal to the twist that has been removed

3.2.1.8 Physical properties of Cotton Yarn

The results of the physical properties such Yarn count CV%, Eveness percentage of yarn, Tenacity of yarn, Yarn hariness test, Moisture content, Yarn thickness and Twist pre inch with specific count of 30's and 10's are presented in Table I

Table I

Physical Testing of Cotton Yarn

S. No	Name of test	Name of test method	30s Ne yarn	10s Ne yarn
1	Count (CV%)	ASTM D 1907 - 01	3.3%	2.8%
2	Evenness of yarn (U %)	ASTM D 1425 - 96	12.2	12.1
3	Yarn tenacity (cN/tex)	ASTM D 2256 - 97	18	12
4	Hairiness (mm)	ASTM D 5647 – 01	2.3	3
6	Moisture content (%)	ASTM D 2495 - 01	8.5	7.7
7	Yarn thickness (mm)	ASTM-D-1425-96	0.25	0.71
8	Twist per inch including direction of twist per sample			
8a	Twist in single yarn (TPI)	(ASTMD1422/D1422 M)	32.6	22.7
8b	Balance of twist (TPI)		14.44	8.2

From the Table I, it is evident that the combed yarn with specific count of 30's and 10's were evaluated for physical property testing such Yarn count CV%, Eveness percentage of yarn, Yarn tenacity, Yarn hariness, Moisture content, Yarn thickness and Twist Pre inch The testing methods were done according to ASTM standards. For the 30's count yarn the count CV% was found to be 3.3% and the eveness as 12.2 U%. The yarn tenacity of the cotton yarn was 18 cN/Tex and

Hariness of the yarn was noted to be as 2.3 mm. The moisture content of the yarn was 8.5%, and the yarn thickness was measured as 0.25mm. as far as the yarn twist is concerned, twist in the single yarn and balance twist was noted to be 32.6 TPI and 14.44 TPI respectively.

As far as the 10's yarn count is concerned, it was noted that the yarn count CV% was 2.8%, eveness of the yarn 12.1 U%,yarn tenacity as 12cN/Tex,hairiness as 3mm,evenness as 12.1%, moisture content as 7.7% and the yarn thickness as 0.71 mm. Regarding the twist per inch of the single yarn and the balance of the twist,the values were found to be 22.7 TPI and 8.2 TPI respectively. The yarns were taken for the weaving processes. The yarns thus used for weaving were prepared as described further.

3.3 Fabric Formation and Pretreatment of Fabric

3.3.1 Warp Winding

Drum winding method was followed for warp winding. This was a simple method used for plain weaving designs. The yarn package is frictionally driven by using a driving drum to make the yarn in the traverse motion.Warping or beaming was done to arrange the yarn in length wise or parallel to one another to arrange the thread. The primary operation of warp-making in which ends with drawn from a warping creel, evenly spaced in sheet form,were wounded onto a beam (known as warper's beam) to substantial length of 18" width.This was winding of total number of warp ends in full width in a single operation from creeled bobbin. Direct beaming or warping was used for long runs of greige fabric and simple patterns Figure (I).

Sizing or Slashing operation was carried out for warp yarns to strengthen, smoothen and lubricate them. In machine sizing warp was transferred from a warp beam to a loom beam. Warp in sheet form was with drawn form a warp beam was passed through a sow-box and the squeezing rollers of a sizing machine. Size solution was applied by immersion or by contact with a partially immersed roller.The warp was dried by hot air or by contact with steam-heated

cylinders.During slashing, the exact number of warp yarns required in fabric was wound onto the loom (or weaver's) beam. The warp ends were then passed through the drop wires of the warp stop motion, the heddles of the harness frames and the dents at the reed.

Warping was achieved by drawing -in or tying-in, the choice depending upon whether or not the new warp was different from the warp already on the loom.The process of drawing every warp end through its drop wire, heddle eye and reed dent manually was carried out a length of warp yarn, just enough to reach to the other side of the frame, was unwound. Leasing (i.e. selecting warp) of the warp at this stage simplifies the separation of the yarns. Then they are threaded through drop-wires, heddle eyes and reed dents.

3.3.2 Weaving

For the study 30’s and 10’s count single yarns were used for fabric construction. Weaving was done at Kumaraguru College of Technology, TIFAC Core, Coimbatore. 500 grams of yarn was used for weaving 2metre length and 18” wide fabric. Totally 8 meter fabric was woven for the study. Semi automatic Shuttle loom (Sakamoto) was used. The loom speed was set as 180rpm and the efficiency was determined as 80%. 1^{x} 1 plain weave structure was opted for the study.The ends per inch of the yarn had been calculated as 58 where as, the picks per inch as 30 similarly the cover factor had been calculated as 3.86mm and 6mm respectively and Tappet shedding was used. The plain woven structure was selected since the existing band aids were made ofthis structures. Moreover, this structure is easy to produce and more comfortable when it is used as a wound dressing material.

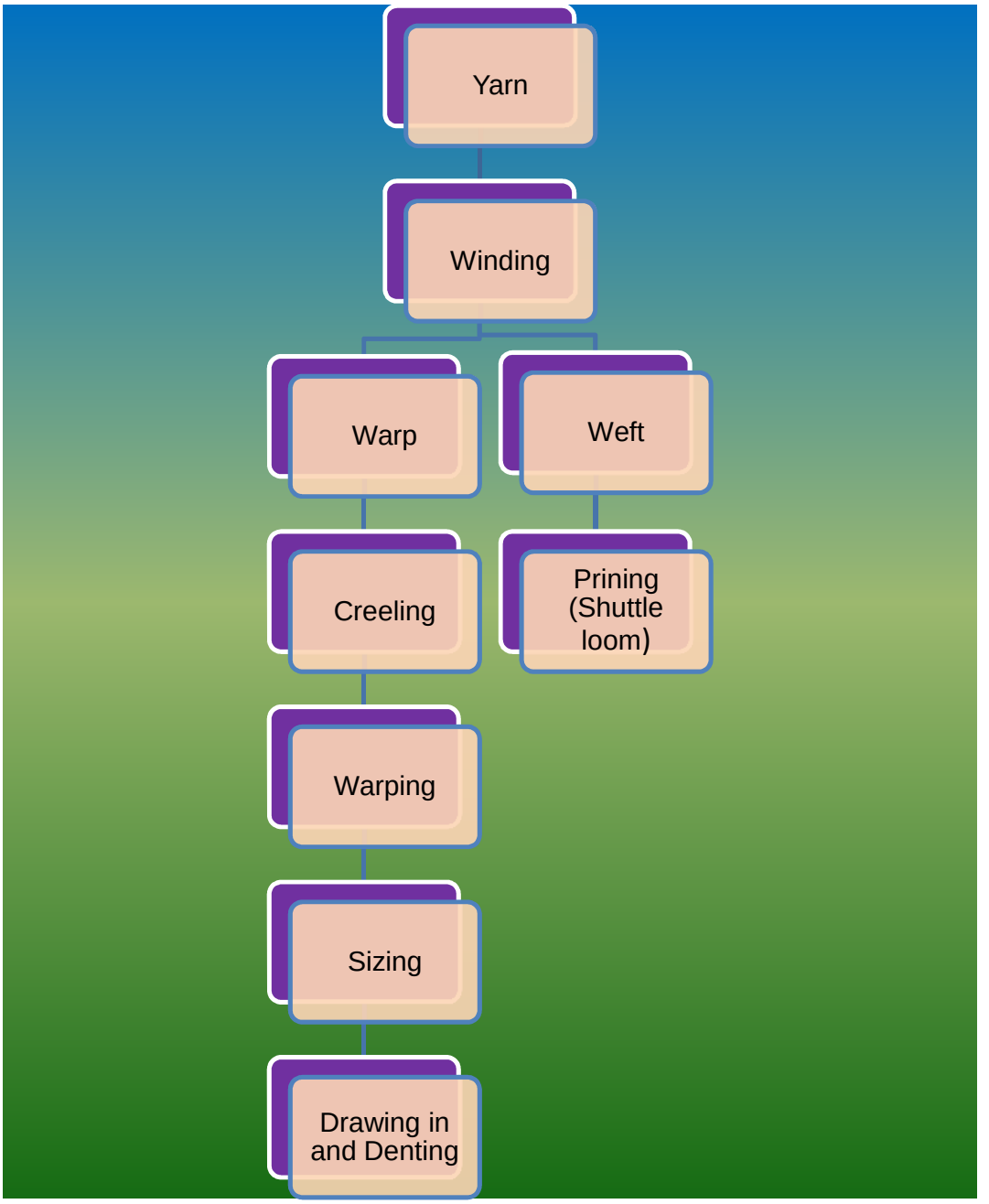


Figure I

Warp Beam Preparation

3.3.3 Pretreatment

Following are the pretreatment processes which were carried out in the wet processing laboratory of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

3.3.3.1 Desizing

The desizing processes for the woven fabric was carried out with the following recepies,

Recipe for desizing	
Material:liquor ratio	1: 10
Time duration	1 hr
Temperature	30^{o}c
Hydrochloric acid	few drops
Fabric weight before desizing	94.544 grams
Fabric weight after desizing	92.544 grams

The woven fabric was dipped in two liters of soft water in which few drops of hydrochloric acid was added and boiled for 60 minutes at 30□c and stirred continuously (Plate IX). The processes was carried out for two meter of woven fabric. The material was removed from the vessel, washed thoroughly in soft water and dried. The weight of fabric before and after desizing was noted. No major difference had been noted in the weight of the fabric.

3.3.3.2 Scouring

The scouring process for the woven fabric was carried out using the following recepie,

Recipe forScouring	
Material:liquor ratio	1: 10
Sodium hydroxide	3.75 gpl
Sodium bi carbonate	1 gpl
Temperature	80^{o}c
Wetting agent	few drops (turkey red oil)

Time	30 min
Fabric weight before scouring	92.544 g
Fabric weight after scouring	92.121 g

The woven fabric was scoured with a Material:liquor ratio of 1:10. The scouring bath was added with 3.7 gpl and 1 gpl of Sodium Hydroxide and Sodium Bicarbonate respectively. Few drops of Turkey Red Oil was added as wetting agent and the scouring bath temperature was maintained at 80 □ c. Two meter of fabric was boiled in the scouring bath for 30 minutes at the ph of 10 (Plate X). The weight of the fabric before and after scouring was noted. No major difference in the weight of the fabric was recorded.

3.3.3.3 Bleaching

The following is the recipe adopted for bleaching process of woven fabric.

Recipe for Bleaching	
Material:liquor ratio	1: 10
Hydrogen peroxide (35 %)	1gpl
Sodium hydroxide (NaOH)	3.75 gpl
Wetting agent	1 gpl
Sodium silicate	3 gpl
Magnesium sulphate (Epsom salt)	1 gpl
Temperature	80ºC
Time	60min
Water	2 liter

Adopting the above recipe bleaching process was carried out Material:liquor ratio was taken as 1:10. Bleaching bath containing two liter of water, one grams per liter of Hydrogen Peroxide, 3.75 gpl of Sodium Hydroxide, one gram per liter of wetting agent, three gram per liter of sodium silicate, onegram per liter of Epson salt was used for bleaching processes. Temperature maintained was 80 □ c and time taken for the bleaching of woven fabric was 60 minutes. The bleached fabric was then taken out, rinsed thoroughly and dried in the shade (Plate XI)

Fabric Pretreatment

Plate IX Desizing

Plate X Scouring

Plate XI Bleaching

3.4 Selection of Herbs

3.4.1 Taxanomy of Plants

The herbal plant extracts were used as antimicrobial finishing agents.The herbs *Abutilon indicum, Tridax procumbenz, Cassia fistula and Cassia auriculata* for the study was selected based on their potentiality of antmicrobial nature as studied through the literature survey (Ali et al, 2003, Ali and Qaiser, 2009, Anyensu et al, 1978, Maneemegalai et al, 2010, Satpute et al, 2015, Christudas et al, 2015 and Thilagavati et al, 2007). The herbs were collected in and around the districts of Coimbatore and Madurai and are shown in Plates XII,XIII,XIV and XV and The Taxanomy of the selected herbs is given in Table II.

Table II

Taxanomy of Selected Herbs

Taxanomy	***Abutilon indicum***	***Tridax procumbenz***	***Cassia fistula***	***Cassia auriculata***
Kingdom	Plantae	Plantae	Plantae	Plantae
Subkingdom	Viridiplantae	Tracheobionta	Viridiplantae	Tracheobionta
Superkingdom	Embryophyta	Spermatophyta	Streptophyta	Spermatophyta
Division	Tracheophyta	Magnoliophyta	Tracheophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Malvales	Asterales	Rosanae	Fabales
Family	Malvaceae	Asteraceae	Fabacea	Fabaceae
Genus	*Abutilon*	*Tridax*	*Cassia*	*Cassia*
Species	*Indicum*	*procumbenz*	*fistula*	*Auriculata*

For the present study, herbs such as *Abutilon indicum,Tridax procumbenz, Cassia fistula* and*Cassia auriculata* were selected based on their antimicrobial potentiality.

3.4.2 Plant Authentication

The authentication of the plants namely *Abutilon indicum,Tridax procumbenz, Cassia fistula* and *Cassia auriculata* was conducted in the

department of Botanical Survey of India (BSI) at The Tamilnadu Agricultural University,Coimbatore. The plants were collected in Theni and the collected plants were pressed together in between two objects to dry. Then the plant samples were pasted on an A4 sheet and submitted to the Department of Botanical Survey of India, The Tamilnadu Agricultural University, Coimbatore for the authentication tests Appendix (III)

Herbal Plants Used for the Study

Plate XII *Abutilon indicum*

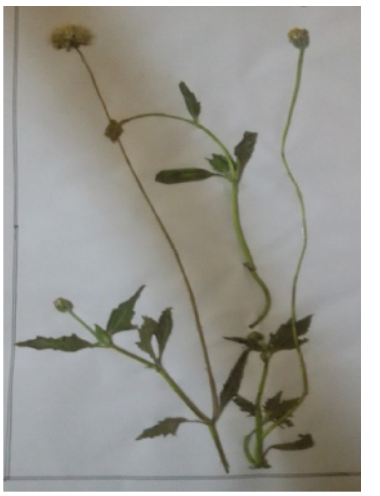


Plate XIII *Tridax procumbenz*

Plate XIV *Cassia auriculata*

Plate XV *Cassia fistula*

3.5 Processing of Herbs

3.5.1 Drying

The plants such as *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* were collected, washed and dried at room temperature and shadow dried till the moisture was expelled.The moisture content of the plant was reduced to less than 14 % with proper drying (Plate XVI a.b.c and d).

3.5.2 Garbling

The garbling process served as the first step to ensure the purity and cleanness of the medicinal plant materials. After the bulk amount of the selected sources such as *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* part was collected, all extraneous and unwanted matters including dirt like soil, dust, mud and rubbles, impurities such as insects, rotten tissues and residual non-medicinal parts were separated from the plants. The process also involved the removal of foreign substances, damaged parts, and unwanted plant parts besides sieving and trimming process. Although sorting was done by mechanical means, in some cases, the garbling was performed by hand operation (Plate XVII).

3.5.3 Grinding

In the grinding processes, the separated leaves of *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* were mechanically broken down to a very small units ranging from larger coarse fragments to fine powder. Powders were prepared to a suitable particle size by grinding for further processing. Grinding or mincing of the leaves was carried out in a mixer grinder. Finely ground herbs absorb water much more quickly than unground herbs and the ground material was quickly packed in air tight containers.The fine powder obtained after grinding was used for extraction (Plate XVIII).

Dried Herbs

Plate XVI (a) Dried *Abutilon indicum*

Plate XVI (b) Dried *Cassia auriculata*

Plate XVI (c) *Dried Tridax procumbenz*

Plate XVI(d) Dried *Cassia fistula*

Processing of Herbs

Plate XVII Garbling

Plate XVIII Grinding

Plate XIX Soxhlet apparatus

PHASE II

3.6 Herbal Extraction

For the herbal extraction, Soxhlet method was chosen to extract the content from the herbs with the principle of infusion method.The weighed quantity of herb was kept in contact with known quantity of menstruum for a specified period of time and at the end of the period, the supernatant liquid were collected and poured into receiver. Hence menstruum was kept either cold or hot depending on the source used. The extraction process was conducted to separate the soluble plant metabolites, leaving behind the insoluble cellular residue.The initial crude extracts contained complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids.

3.6.1 Extraction by Soxhlet

For the study,finely ground sample of *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* was placed in a porous bag or “thimble” made from a strong filter paper or cellulose, and then placed in thimble chamber of the Soxhlet apparatus. Extraction solvent was heated in the bottom flask, vaporizes into the sample thimble, condensed in the condenser and dripped back. When the liquid content reaches the siphon arm,the liquid contents were emptied into the bottom flask again and the process were continued.

The powdered herbs were filled in the thimble and placed in the Soxhlet extractor. 100 grams of the ground powder of the herbs was loaded in the thimble and 1000ml of solvent was used in the Soxhlet extractor to obtain the needed extract.The extractor was filled with solvent solution of Hexane, Ethyl acetate and Methanol individually and the temperature of 60^{o}c was set and left for 6 hours. Later the extracts of the individual herbs of individual solvents were collected as shown in Plate XIX.

3.6.2 Optimisation of Herbal Extracts

The herbs *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* were treated with the solvents namely Hexane, Ethyl acetate and

Methanol for extraction.To optimise the extraction efficiency of bioactive compounds, as well as antioxidant capacity from the plants part, optimisation was done to identify the most appropriate solvent for further extraction. The isolation of bioactive compounds and antioxidant capacity of the selected source were optimised. Further the extractions were screened for the phytochemical constitution and on the basis of the phytochemical components present in the extract, appropriate solvent had been selected as shown in Plate XX,XXI,XXII,XIII(a,b,c,d) and Table III,IV,V and VI.

3.6.3 Qualitative Phytochemical Analysis of Herbal Extract

Qualitative phytochemical analysis of each of the herbal extract was carried out on the leaf extracts using different solvents such as Hexane, Ethyl acetate and Methanol to identify the major natural chemical groups such as carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides terpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins and anthraquinones as per the procedures given below;

3.6.3.1 Test for Carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated Sulphuric acid were added. Presence of purple or reddish colour indicated the presence of carbohydrates.

3.6.3.2 Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicated the presence of tannins.

3.6.3.3 Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes length wise. Formation of 1cm layer of foam indicated the presence of saponins.

3.6.3.4 Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow colour indicated the presence of flavanoids.

3.6.3.5 Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicated the presence of alkaloids.

3.6.3.6 Test for Quinones

To 1ml of extract, 1ml of concentrated Sulphuric acid was added. Formation of red colour indicated the presence of quinones.

3.6.3.7 Test for Glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicated the presence of glycosides.

3.6.3.8 Test for Cardiac Glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated the presence of cardiac glycosides.

3.6.3.9 Test for Terpenoids(Keller-kilani test),

To 0.5ml of extract, 2ml of chloroformand concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicated the presence of terpenoids.

3.6.3.10 Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicated the presence of phenols.

3.6.3.11 Test for Coumarins

To 1 ml of extract, 1ml of 10% sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarins.

3.6.3.12 Test for Steroids and Phytosteroids

To 1ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuricacid. The appearance of brown ring indicated the presence of steroids and appearance of bluish brown ring indicate the presence of phytosteroids.

3.6.3.13 Test for Phlobatannins

To 1ml of plant extract few drops of 2% hydrochloric acid was added.The appearance of red colour precipitate indicated the presence of phlobatannins.

3.6.3.14 Test for Anthraquinones

To 1ml of plant extract, few drops of 10% ammonia solution was added and appearance of pink colour precipitate indicated the presence of Anthraquinones.From the results of phytochemical analysis as given in the Plate XX,XXI,XXII,XXIII(a,b,c) and Tables II,III,IV and Vit was noted that the presence of phytochemical components in the methanolic extract was better when compared to the Hexane and Ethyl acetate mediated extracts. Considering these facts Methanolic mediated plant extract was opted for the final study.

Table III

Phytochemical Screening of *Abutilon indicum*

Sample *Abutilon indicum*			
Phytochemical test	**Inference**		
	Hexane	**Ethyl acetate**	**Methanol**
Carbohydrates	-	-	-
Tannins test	-	-	+
Saponin test	-	-	-
Flavonoid test	-	-	+
Alkaloid test	+	+	+
Quinones	-	-	-
Glycosides test	-	-	-
Cardiac glycosides test	-	-	-
Terpenoids test	-	+	+
Triterpenoids	-	-	-
Phenols	-	-	+
Coumarins	-	-	+
Proteins	-	-	-
Steroids and Phytosteroids	-	-	-
Phlobatannins	-	-	-
Anthraquinones	-	-	-

+ Present,- Absent

From the Table III, the presence of tannins, alkaloids, flavanoids, terpenoids, phenols and coumarins in the methanol extract were observed. Presence of alkaloids and terpenoids was seen in extract of ethyl acetate, and alkaloids in hexane extracts.

Phytochemical Screening of *Abutilon indicum*

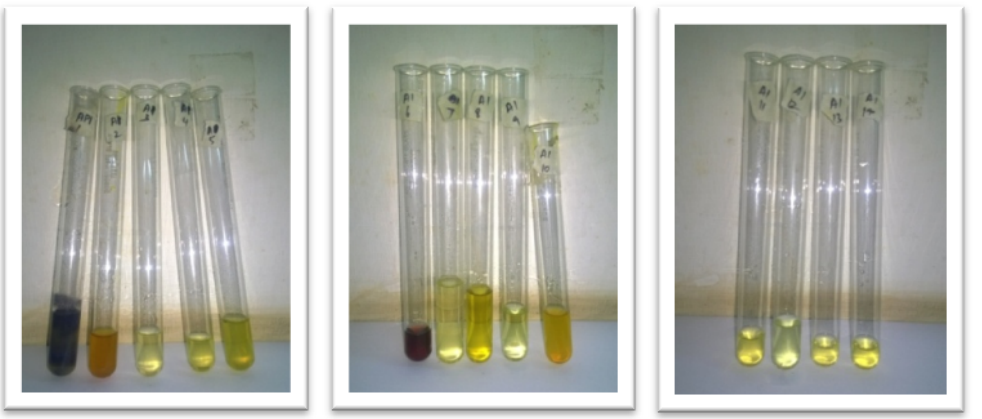


Plate XX (a) Hexane Extract of *Abutilon indicum*

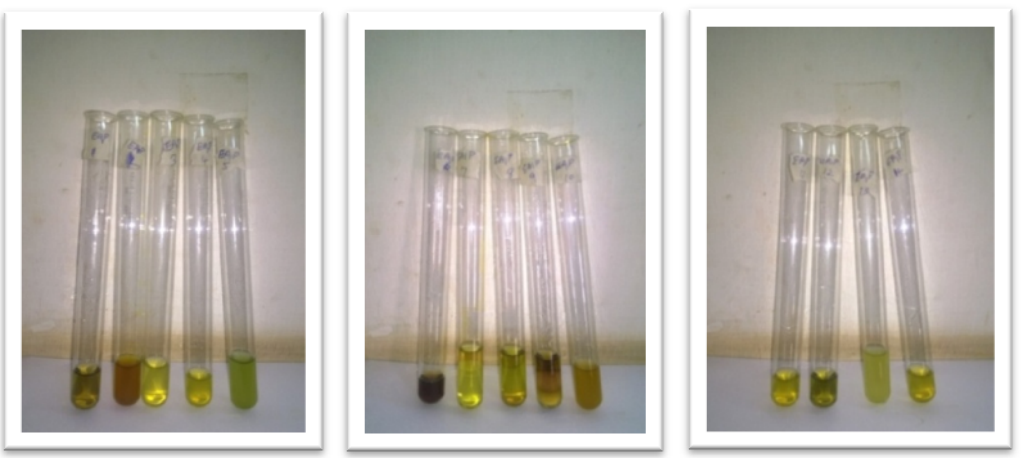


Plate XX (b) Ethyl acetate Extract of *Abutilon indicum*

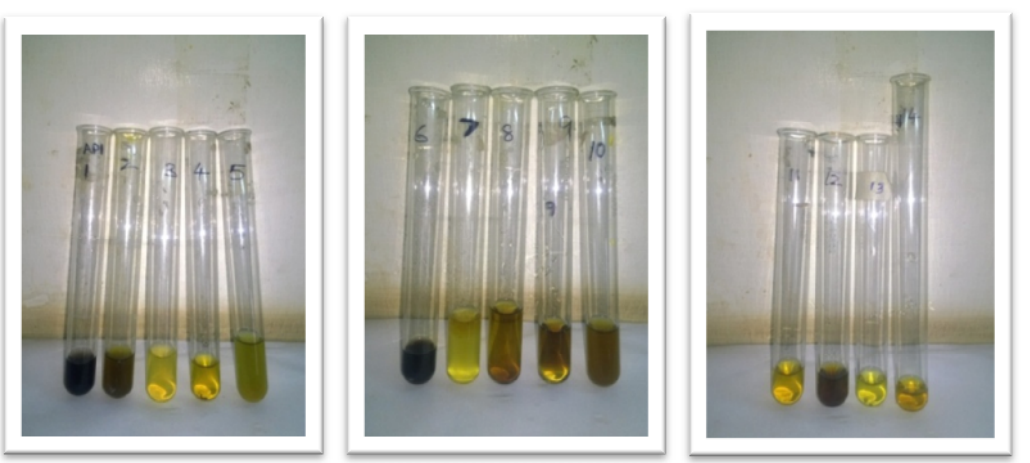


Plate XX (c) Methanol Extract of *Abutilon indicum*

Table IV

Phytochemical Screening of *Cassia auriculata*

Sample *Cassia auriculata*			
Phytochemical test	**Inference**		
	Hexane	**Ethyl acetate**	**Methanol**
Carbohydrates	-	+	+
Tannins test	-	-	+
Saponin test	-	-	-
Flavonoid test	+	+	-
Alkaloid test	-	-	-
Quinones	+	+	+
Glycosides test	-	-	-
Cardiac glycosides test	+	-	-
Terpenoids test	+	+	+
Triterpenoids	-	-	-
Phenols	-	-	+
Coumarins	+	-	-
Proteins	-	-	-
Steroids and Phytosteroids	-	-	-
Phlobatannins	-	-	-
Anthraquinones	-	-	-

+ Present,- Absent

The Table IV shows the presence of carbohydrates, tannins, quinones, terpenoidsand phenols coumarins in the methanol medicated extract of *Cassia auriculata*where as, ethyl acetate medicated extract showed the presence of carbohydrates, flavanoids, quinones and terpenoids. Similarly, when the hexane was used as solvent, presence of flavanoid, quinones,cardiac glycosides, terpenoids and coumarins were noticed.

Phytochemical Screening of *Cassia auriculata*

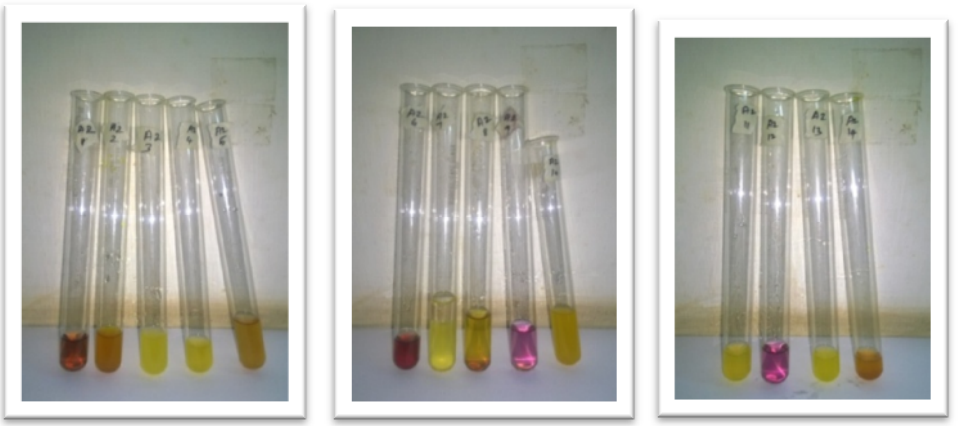


Plate XXI (a) Hexane Extract of *Cassia auriculata*

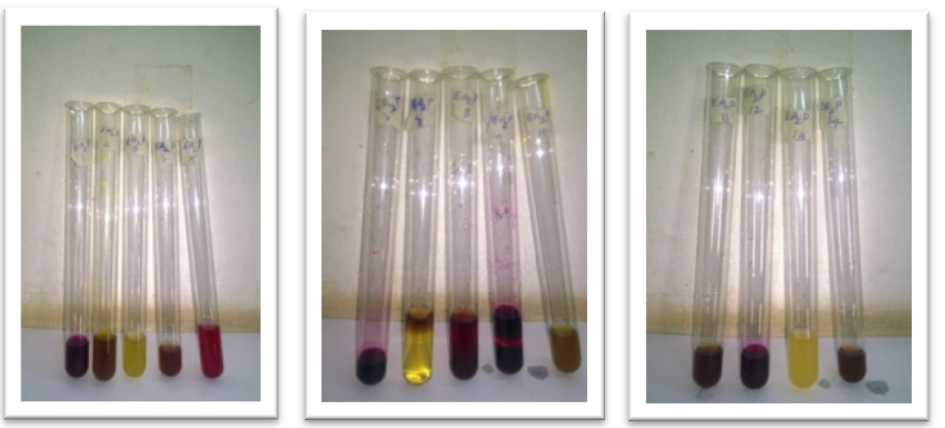


Plate XXI (b) Ethyl acetate Extract of *Cassia auriculata*

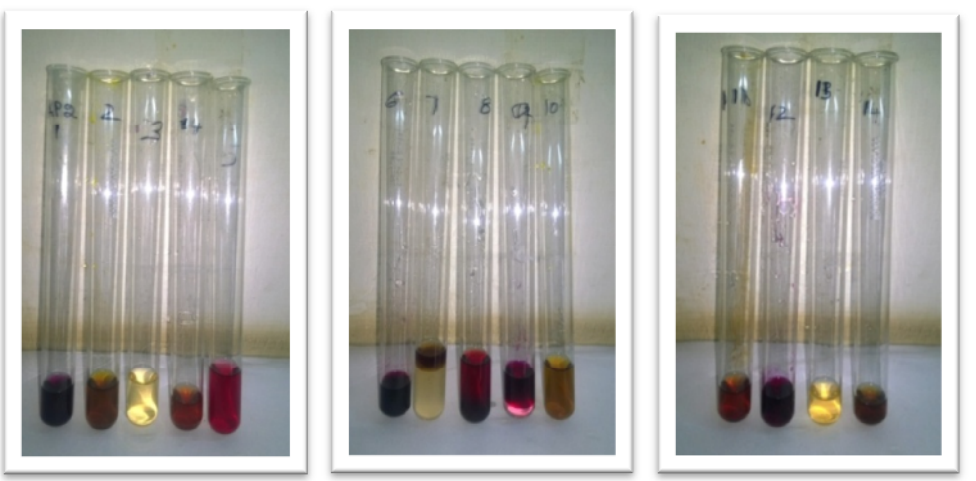


Plate XXI (c) Methanol Extract of *Cassia auriculata*

Table V

Phytochemical Screening of *Cassia fistula*

Sample *Cassia fistula*			
Phytochemical test	**Inference**		
	Hexane	**Ethyl acetate**	**Methanol**
Carbohydrates	-	-	-
Tannins test	-	-	+
Saponin test	-	-	-
Flavonoid test	-	+	-
Alkaloid test	-	+	-
Quinones	-	+	-
Glycosides test	-	-	-
Cardiac glycosides test	+	-	+
Terpenoids test	-	+	+
Triterpenoids	-	-	-
Phenols	-	-	+
Coumarins	-	+	-
Proteins	-	-	-
Steroids and Phytosteroids	-	-	-
Phlobatannins	-	-	-
Anthraquinones	-	-	-

+ Present,- Absent

The Table V shows the presence of tannins, flavanoids, cardiac glycosides, terpenoids and triterpenoids for the Methanolic extract of the plant source.Similarly, flavanoids, alkaloids, quinones terpenoidsand coumarins were identified in the extract of ethyl acetate.The hexane extract of *Cassia fistula* showed the presence of cardiacglycosides.

Phytochemical Screening of *Cassia fistula*

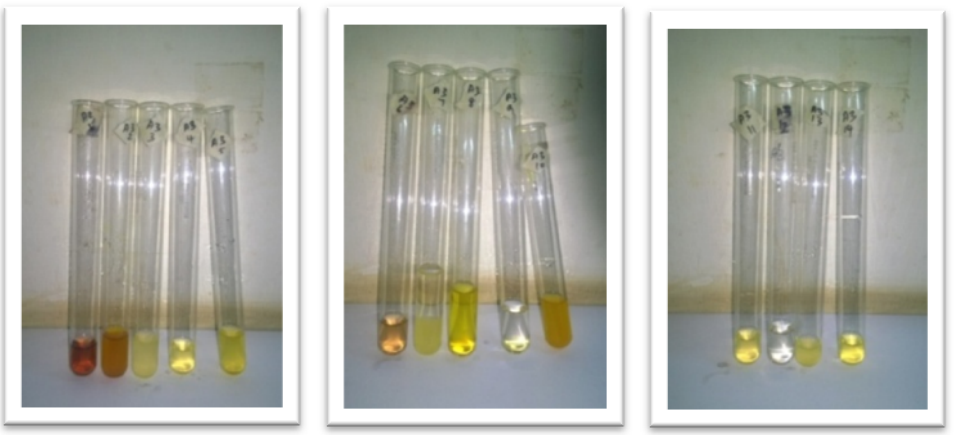


Plate XXII (a) Hexan Extract of *Cassia fistula*

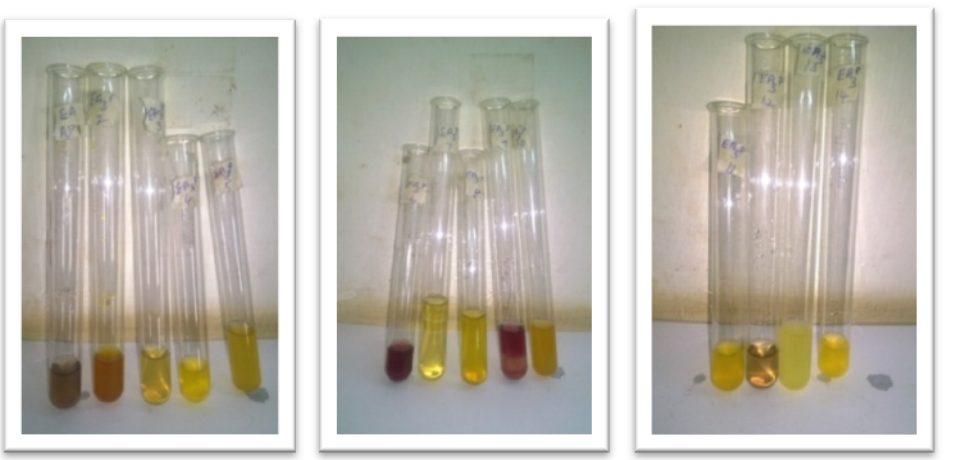


Plate XXII (b) Ethyl Acetate extract of *Cassia fistula*

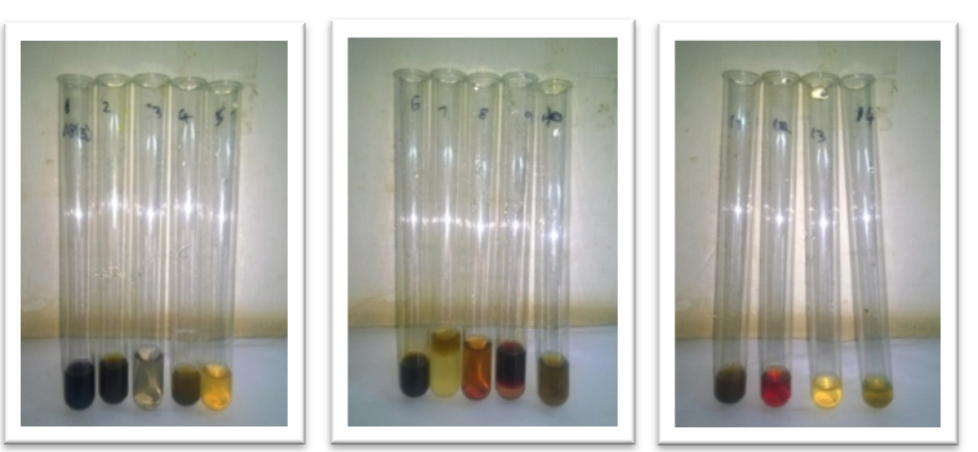


Plate XXII (c) Methanol Extract of *Cassia fistula*

Table VI

Phytochemical Screening of *Tridax procumbenz*

Sample *Tridaxprocumbenz*			
Phytochemicl test	**Inference**		
	Hexane	**Ethyl acetate**	**Methanol**
Carbohydrates	-	-	+
Tannins test	-	-	+
Saponin test	+	-	-
Flavonoid test	-	-	+
Alkaloid test	+	+	-
Quinones	-	-	-
Glycosides test	-	-	-
Cardiac glycosides test	+	+	+
Terpenoids test	-	+	+
Triterpenoids	-	-	-
Phenols	-	-	+
Coumarins	-	-	-
Proteins	-	-	-
Steroids and Phytosteroids	-	-	-
Phlobatannins	-	-	-
Anthraquinones	-	-	-

+ Present,- Absent

From the aboveTable VI,the Methanolic extract showed the presence of carbohydrates, tannins, flavanoids, cardiac glycosides, terpenoids and phenols. The ethyle acetate extract shows the presence of alkaloids, cardiac glycosides and terpenoids. Where as, saponins, alkaloid and cardiac glycosides were present in hexane extract.

Considering the results of phytochemical screening with three different solvents Methanolic extract was opted for the study

Phytochemical Screening of *Tridax procumbenz*

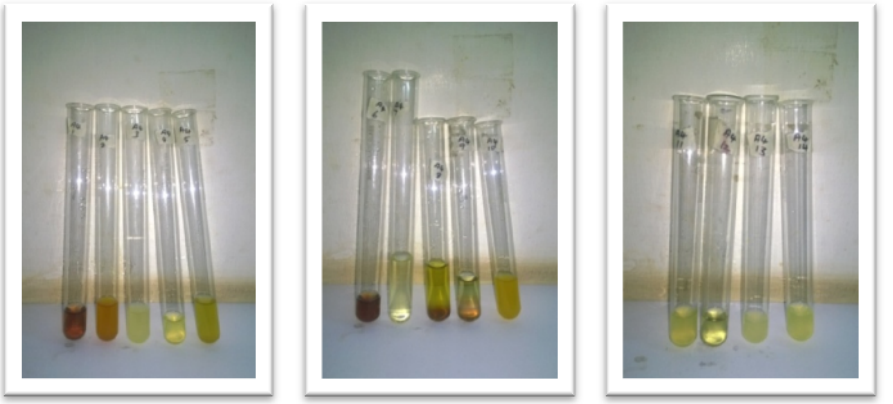


Plate XXIII (a) Hexane Extract of *Tridax procumbenz*

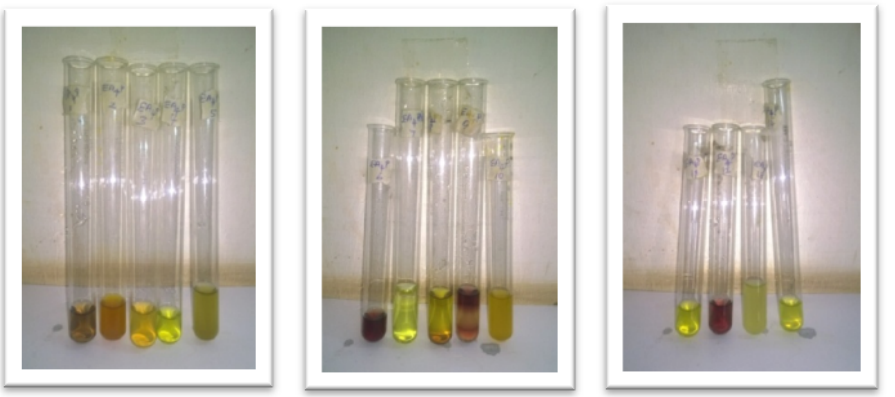


Plate XXIII (b) Ethyl acetate Extract of *Tridax procumbenz*

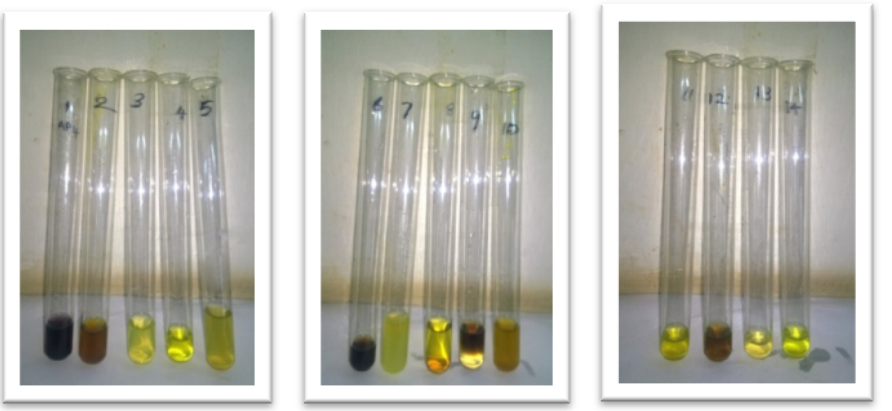


Plate XXIII (c) Methanol Extract of *Tridax procumbenz*

PHASE III

3.7 Antimicrobial Testing

The Agar well diffusion method was adopted for the study. The microbes such as *Pseudomonas aeuroginosa, Staphylococcus saprophyticus, Aeromonas hydrophila, Escherichia coli* and *Candida albicans* were selected for the study. The selected microbes were tested with the individual herbal extracts,polyherbal extract and the zone of inhibition was calculated.

In the agar well diffusion method, a suitable agar medium was prepared, once the agar was solidified, the medium was inoculated and swabbed with bacterial suspension of approximately 1-2 X 108 CFU/mL using cotton swab. The wells were prepared by punching with a six millimeters diameter standard sterile corkborer.These wells were filled up with 25 – 50 µL of the antimicrobial solutions for the testing. Well diffusion test had been used for susceptibility testing of antifungals like fluconazole and itraconazole.The plates were incubated at 35 ± 2°C for 18 – 24 h. The antimicrobial activity was calculated in millimeter by using the expression: ZOI = Total Diameter of growth inhibited zone minus diameter of the well, where, ZOI was Zone of inhibition.

For the study, the well diffusion method had been done using the above procedure. The agar well had been prepared and the medium was inoculated. The inoculated medium was swabbed with the microbial suspention of *Staphylococcus saprophyticus, Pseudomonas aeroginosa, Escherichia coli, Aeromonas hydrophila* and *Candida albicans* which were suspended of approximately 1-2°10^8 CFU/ml using the cotton swab. The swabs were prepared on the medium by punching six millimeter in diameter cork bore. The wells were filled up with 25µL of each herbal extracts. The plates were incubated at 35 ± 2°C for 24 hours. Later the plates were taken out and the zone of inhibition was recorded by the scale in the plates.

3.7.1 Selection of Microbial Cultures

The selected microbial strains were *Pseudomonas aeroginosa* (ATCC27853), *Escherichia coli*(ATCC 25922) *Candida albicans*(ATCC 10231)

and the strains were procured from ATCC (USA) purchased through HiMedia, Mumbai, India. The microorganisms such as *Aeromonas hydrophila* (MTCC 1739) and *Staphylococcus saprophyticus* (MTCC 6155) were procured directly from MTCC, IMTECH, Chandigarh. The selected microbes were most frequently encountered as pathogenic microbial species. They can live in wide variety of environment and were reason for various skin diseases and can show rapid growth on the broken skin which promotes the minor wound into a chronical wound.The effect of the selected plants poly herbal extract against these microbes were tested.The efficiency of the herbal extracts on the microbes were identified for development of fabric for wound dressing.

3.8 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the *Abutilon indicum, Tridax procumbenz, Cassia fistula*, *Cassia auriculata* was tested by two-fold serial dilution method. The extract was dissolved in 5% dimethyl sulfoxide to obtain 2000 µg/ml stock solutions. The samples were diluted to give the final concentrations of 1000, 500, 250, 125, 62.5, 31.25 µg/ml. About 100 µl of 10^5 CFU/ml of the test culture was inoculated in tubes with equal volume of nutrient broth and herbal extract samples,whereas control tube contained only organisms and not the plant extract. The tubes were incubated aerobically at 37°C for 24hours. The lowest concentration produces no visible turbidity.The total incubation period was regarded as the optimized minimum inhibitory concentration. MICs are used by diagnostic laboratories, mainly to confirm resistance, but most often as a research tool to determine the in-vitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. A breakpoint is a chosen concentration (mg/L) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic. If the MIC is less than or equal to the susceptibility breakpoint the bacteria is considered susceptible to the antibiotic. The minimal inhibitory concentrations of a series of antimicrobial agents for human oral organisms were determined under anaerobic growth conditions by an agar dilution assay.

MIC of *Abutilon indicum*

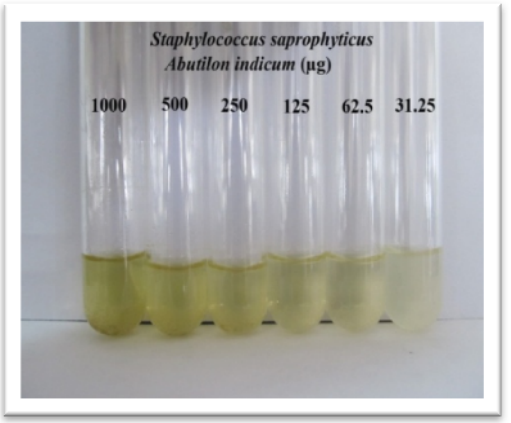


Plate XXIV(a) *S. sapropyticus*

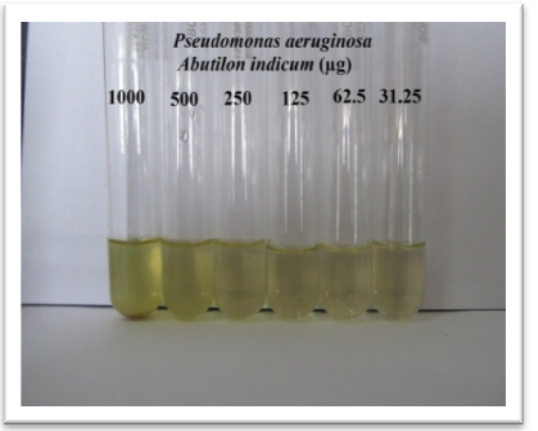


Plate XXIV(b) *P. aeroginosa*

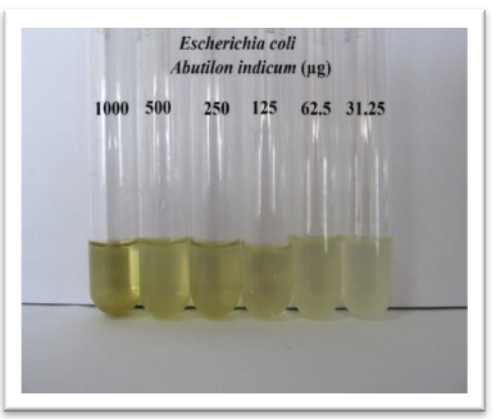


Plate XXIV(c) *E. coli*

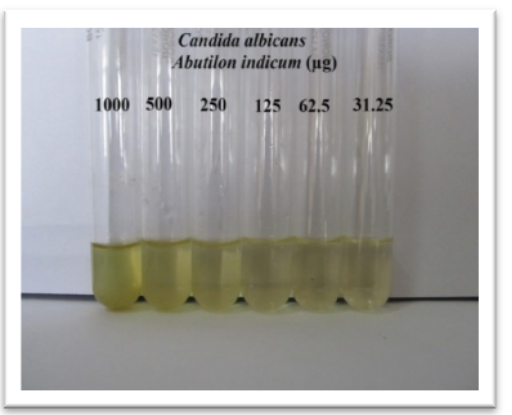


Plate XXIV (d) *C. albicans*

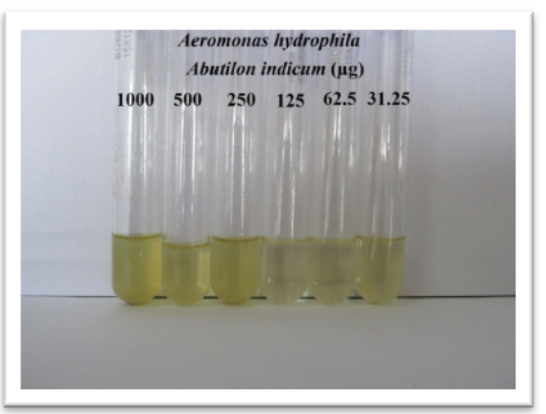


Plate XXIV (e) *A. hydrophila*

MIC of *Cassia auriculata*

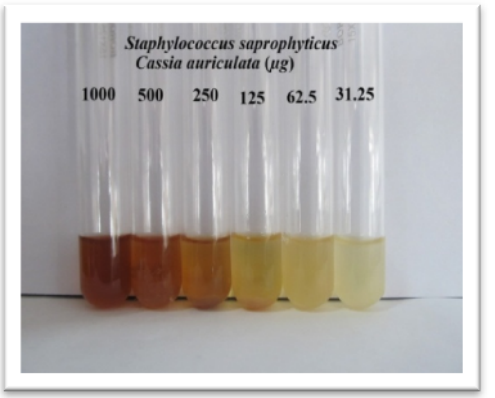


Plate XXV(a) *S.Saprophyticus*

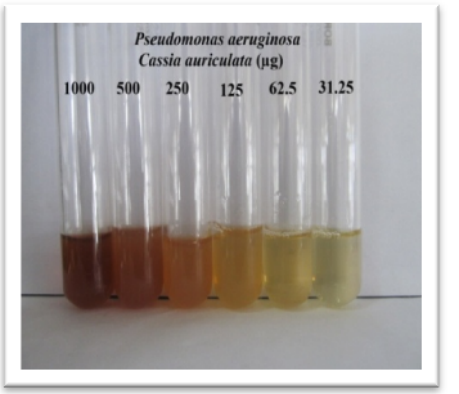


Plate XXV(b) *P. aeroginosa*

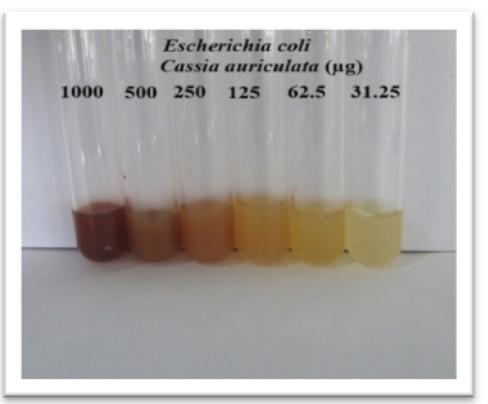


Plate XXV(c)*E. Coli*

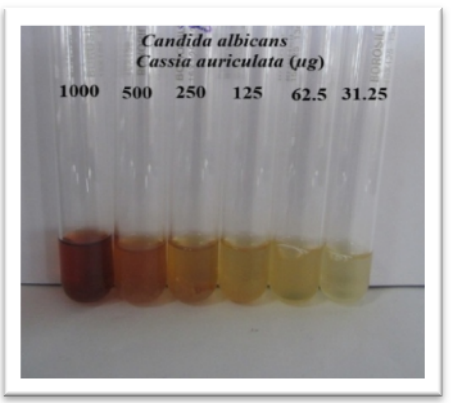


Plate XXV(d)*C. albicans*

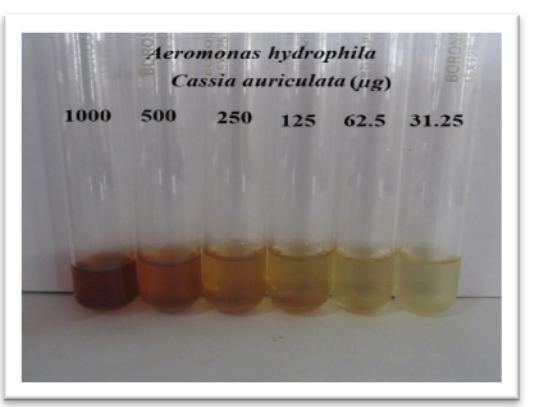


Plate XXV(e)*A. hydrophila*

MIC of *Cassia fistula*

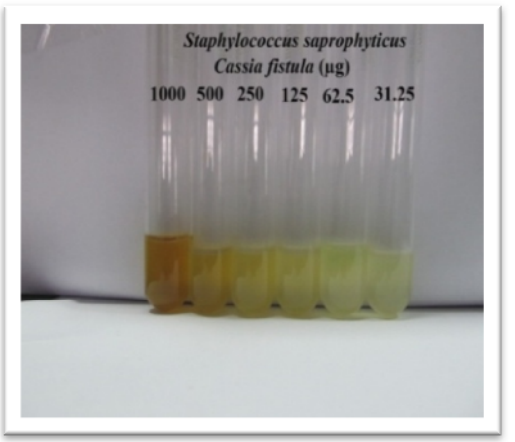


Plate XXVI(a)*S.saprophyticus*

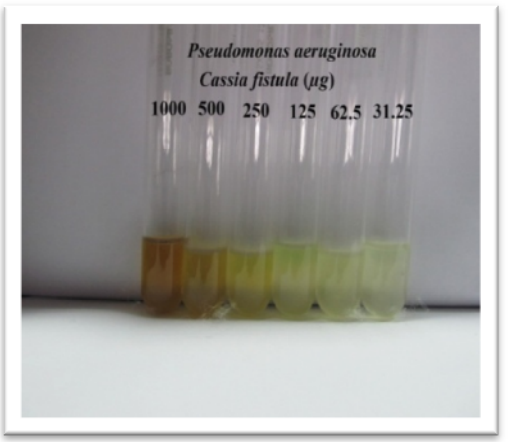


Plate XXVI(b)*P. aeroginosa*

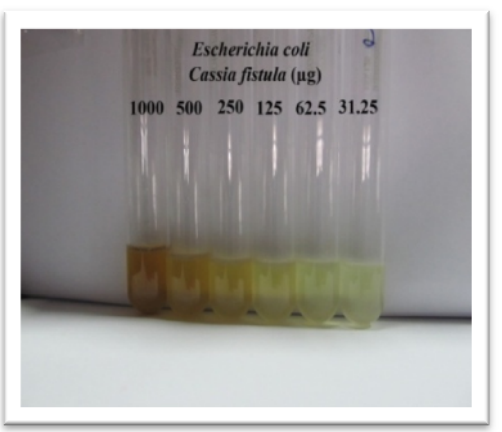
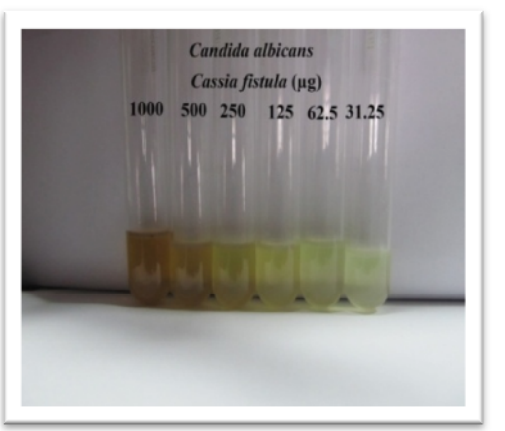


Plate XXVI(c)*E. coli*



PlateXXVI(d)*C. albicans*

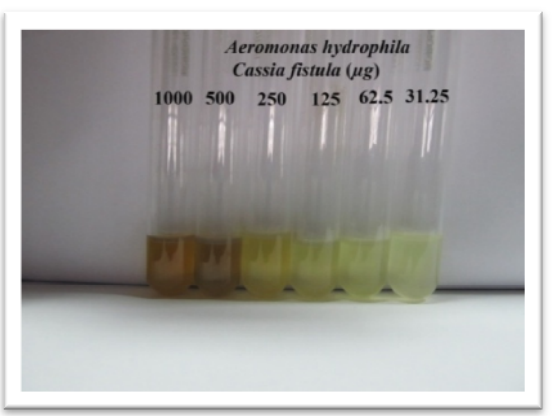


Plate XXVI(e)*A. hydrophila*

MIC of *Tridax procumbenz*

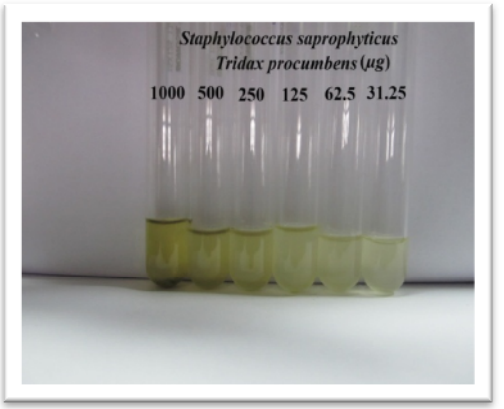


Plate XXVII(a)*S. Saprophyticus*

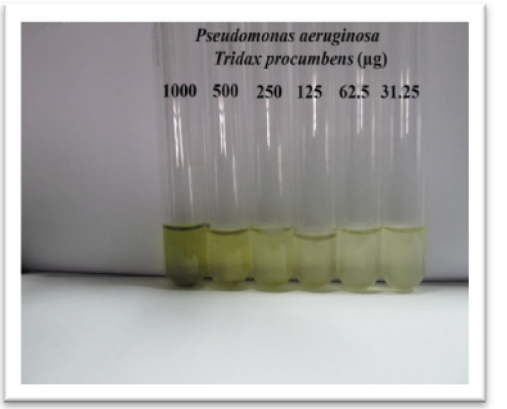


Plate XXVII(b)*P. Aeroginosa*

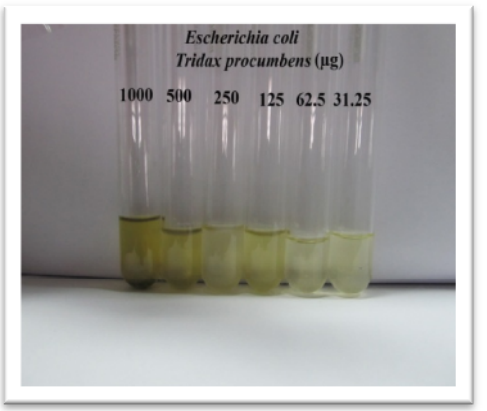


Plate XXVII (c)*E. coli*

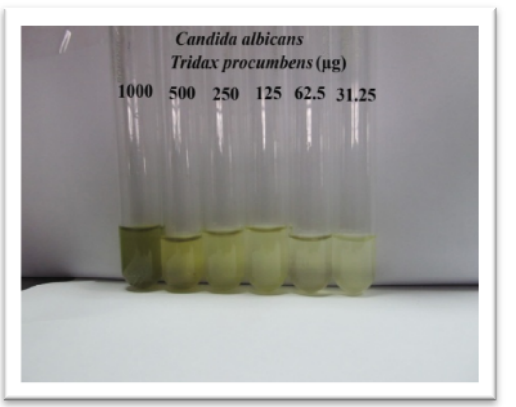


Plate XXVII(d) *C. Albicans*

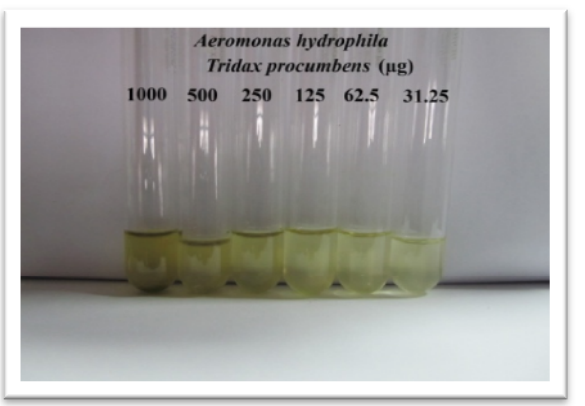


Plate XXVII (e)*A. hydrophila*

The bacteria *Staphylococcus sapropyticus, Pseudomonas aeroginosa, Escherichia coli, Aeromonas hydrophila* and Fungi *Candida albicans* were well known human pathogen which causes skin infection and occur in small cut, burn,minor wound and skin infections that may be lead to cronical injuries and skin diseases. If the wounds were not cared properly, skin infection would be worser, therefore it is ideal to minimize the infection Plate XXIV,XXV,XXVI,XXVII (a,b,c,d,e).

3.8.1 Antimicrobial Activity of Herbal Extract by Agar Well Diffusion Method

The antimicrobial activity of the *Tridax procumbenz,Cassia fistula, Cassia auriculata* and *Abutilon indicum* were calculated and minimum inhibitory concentration is shown in plates XXVIII (a,b,c,d and e) Sterile nutrient agar plates were prepared. The plates were allowed to solidify for 5 minutes and wells of 6 mm were punctured using a well borer. 0.1% inoculum suspension of test bacterium *Staphylococcus saprophyticus* (MTCC 6155)*,Escherichia coli* (ATCC 25922)*, Aeromonas hydrophila* (MTCC 1739)*, Candida albicans* (ATCC 10231) *and Pseudomonas aeruginosa* (ATCC27853)) was swabbed uniformly over the surface of the agar. 100 µl of each herbal extract was loaded into the well and the plates were kept for incubation at 37ºC for 24 hours. The antimicrobial activity was evaluated in terms of zone of inhibition, measured and recorded in millimeters.

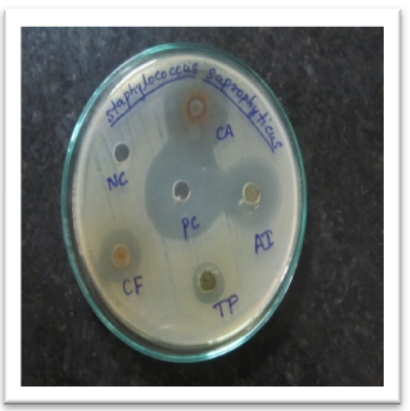
The extract of *Cassia auriculata* showed better antimicrobial activity than *Tridax procumbenz, Cassia fistula* and *Abutilon indicum.*Levofloxacin was used as positive control and distilled water was used as a negative control. Considering the MIC, value the herbal extract was taken for test. The *Tridax procumbenz* has taken double the time of other extracts and the antimicrobial value was calculated Plate XXVIII(a,b,c,d,e) .

3.8.2 Polyherbal Formulation

The determined MICs of each herb were taken for preparing polyherbal formulation. The crude extracts of *Abutilon indicum,Tridaxprocumbenz, Cassia fistula and Cassia auriculata* were taken in defined proportion of (1:2:1:1) based on MICs.

The polyherbal formulation was prepared by mixing the content in a magnetic stirrer and stored in the containers as per the procedure suggested by, "The Ayurvedic Formulary of India, 2003. Even though, *Cassia auriculata* showed better activity than the other herbs it was essential to develop a synergistic polyherbal formulation (or) extract to avoid the resistant characteristic of the microbes. Poly herbal extract can be applied against many different type of microbes (Plate XXX).

Agar Well Diffusion of Individual Herbal Extract



PlateXXVIII (a)
S. saprophyticus

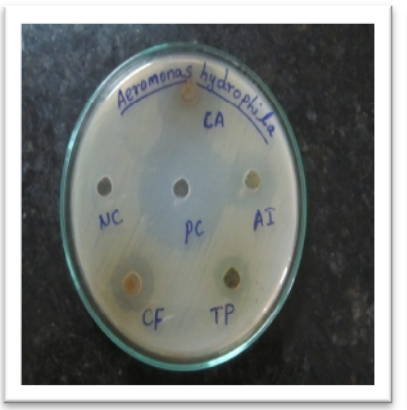


Plate XXVIII (b)
A. hydrophila

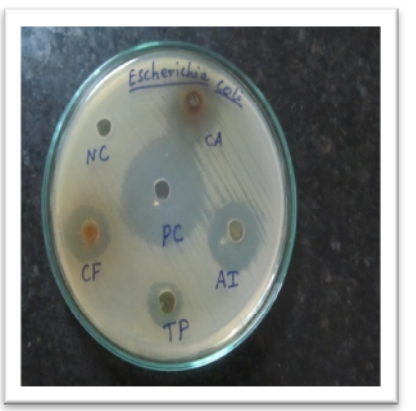
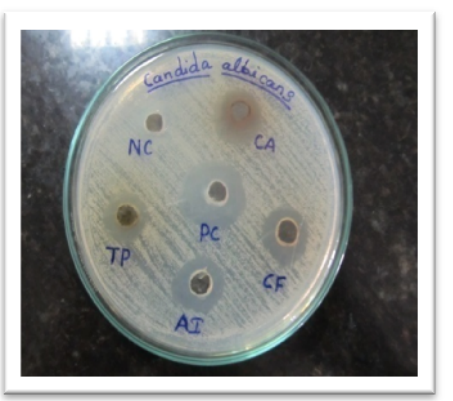


Plate XXVIII (c)
E. coli

Plate XXVIII(d)
P. aeroginosa



PlateXXVIII(e)
C. albicans

3.8.3 Antimicrobial Activity of Polyherbal Extract by Agar Well Diffusion Method

For the study, the sterile nutrient agar plates were prepared. The plates were allowed to solidify for 5 minutes and wells of 6 mm were punctured using a well borer 0.1% inoculum suspension of test bacterium *Staphylococcus saprophyticus* (MTCC 6155), *Escherichia coli* (ATCC 25922), *Aeromonas hydrophila* (MTCC 1739), *Candida albicans*(ATCC 10231) *and Pseudomonas aeroginosa* (ATCC27853) was swabbed uniformly over the surface of the agar. 50 µl, 100 µl, 150 µl and 200 µl of polyherbal extract were loaded into the well and the plates were kept for incubation at 37ºC for 24 hours. The antimicrobial activity was evaluated in terms of zone of inhibition, measured and recorded in millimeters.

Agar Well Diffusion of Poly Herbal Extracts

Plate XXIX(a)
S. Saprophyticus

Plate XXIX (b)
C. albicans

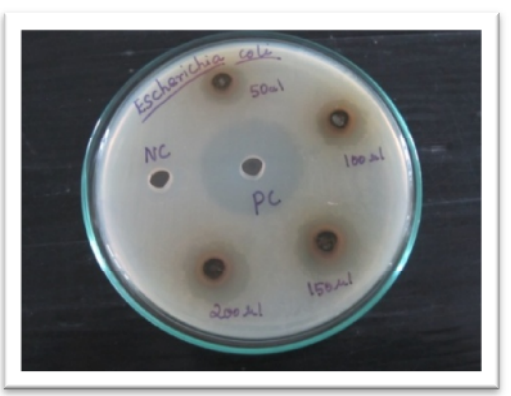


Plate XXIX (c) *E.coli*

Plate XXIX (d)*A.hydrophila*

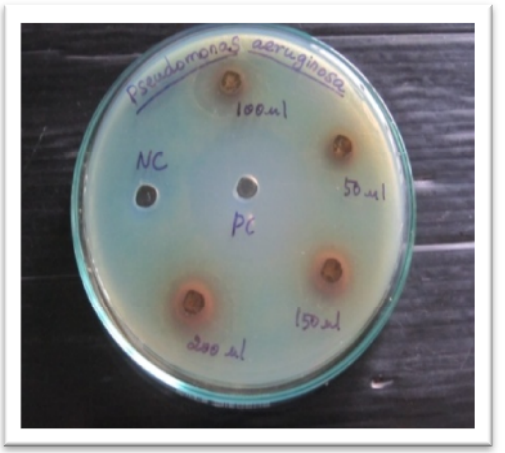


Plate XXIX (e)
P.aeroginosa

Plate XXX
Double cone blender

The procedure of well diffusion method followed in individual herbal extract, the antimicrobial testing for poly herbal extract was also performed. The polyherbal extraction had been done in the ratio of 1:2:1:1 and antimicrobial activity with *Staphylococuss saprophyticus,Escherichia coli,Pseudomonas aeroginosa,Aeromonas hydrophila* and *Candida albicans* was examined. The polyherb antimicrobial analysis were evaluated for zone of inhibition Plate XXIX(a,b,c,d,e).

3.8.4 Wound Scratch Assay of Polyherbal Extract

The *in-vitro* scratch assay is an easy, low-cost and well-developed method to measure cell migration *in-vitro*. The basic steps involved in creating a ‘‘scratch’’ in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and comparing the images to quantify the migration rate of the cells. Compared to other methods, the *in vitro* scratch assay is particularly suitable for studies on the effects of cell–matrix and cell–cell interactions on cell migration, mimic cell migration during wound healing *in-vivo* and are compatible with imaging of live cells during migration to monitor intracellular events if desired. Besides monitoring migration of homogenous cell populations, this method was adopted to measure migration of individual cells in the leading edge of the scratch and this *in-vitro* scratch assay.

The wound healing effect of the polyherbal extract was analyzed by *In-vitro* Wound scratch assay in fibroblast cell lines. Fibroblast cells were grown in 24 well plates at a density of 1.00 $x10^5$ cells/ml and cultured until ~ 80 % confluency. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper as per the method.

Nomenclature of the treated samples are given as below

Nomenclature of the Treated Samples	
Control	C
Dip and Drying	DDF
Mircoencapsulation	MEF
Nanoencapsulation	NEF

PHASE IV

3.9 Application of Polyherbal Extract on Cotton Fabrics

The functional finishing has becoming the most required for textile materials. The polyherbal extract prepared was finished onto the fabrics by two methods namely Dip and dry and Exhaust method.

Application of Polyherbal Extract on Cotton Fabric

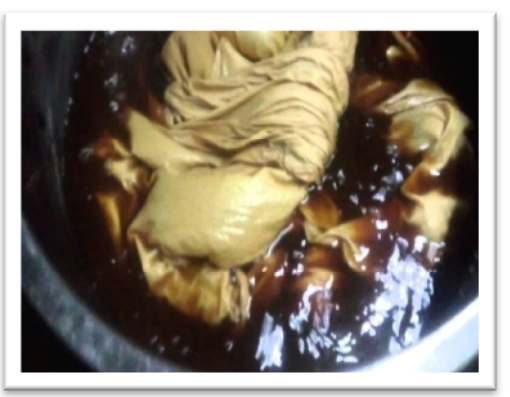


Plate XXXI

Dip and Dry

Plate XXXII

Hot Air Oven

3.9.1 Dip and Dry Method

The desized sterile samples was of two meter.The extracted solvent was added in a beaker .The cotton samples were immersed in the solvent for twenty minutes and then the sample were removed from the solvent and dried in the air without washing. The finished samples were sterilized by UV rays in the laminar air flow chamber to avoid microbial growth on the surface of the fabric. The sterile finished fabric sample was kept in a sterile container (Plate XXXI).

3.9.2 Exhaust Method

Following the above procedure for the study, two meter of the pretreated fabric was weighed and wet. The wet fabric was immersed in the solution containing poly hebal extract with 80% concentrate prepared at the ratio 1:2:1:1 at the material:liquor ratio of (1:10) for 30 minutes at 40□c in water bath with 7% of citric acid as binder.

After treating with herbal finish, the fabric was removed from the bath, squeezed gently and dried at 100□c in the oven for 5 minutes and cured at 120□c for 2 minutes (Plate XXXII)

3.9.2.1 Preparation of Polyherbal Microencapsules by Ionic Gelation Process

For the study, Microcapsules containing extracts of polyherbal extract as core material and sodium alginate as the wall material were prepared. Ten grams of wall material was allowed to swell for half an hour by mixing with 100 ml of hot water. To this mixture 50 ml, of hot water was added and stirred for 15 minutes maintaining the temperature between 40°C and 50 °C. to this mixture.Then 10 ml of core material sodium alginate was added and the mixture is transferred to a centrifuge and rated at 300-500 rpm speed for 15 minutes. This is sprayed into 2% of calcium chloride solution by means of a sprayer. The droplets were retained in calcium chloride collection bath for 15 minutes. In this bath the calcium ions were diffused with the alginate solution, thereby hardening the matrix and forming a solid hydro gel system. The microcapsules were obtained by decantation and repeated washing with isopropyl alcohol followed by drying at 45 °C for 12 hours was done.

The fabric of two meter had been finished with polyherbal extract. By dipping in the prepared microcapsules one litre solution containing 700gram of microcapsules was used to finish one meter of fabric. The fabric samples were immersed in two different binder solution i.e. 8% citric acid and also 8% acidic binder respectively for 30minutes under 50ºC in an oven. After 30min, the fabric was removed and air dried in shade.

3.9.2.2 Preparation of Polyherbal Nanoencapsules

For the study, the herbal extract enclosed with bovine serum albumin was prepared by coacervation process followed by cross-linking with glutaraldehyde. The herbal extract was incubated with the required protein solution (2% W/V) for an hour at room temperature. The pH of the solution was adjusted to 5.5 by 1M HCL using digital pH meter. Then ethanol was added to the solution in the ratio of 2:1 (V/V). The rate of ethanol addition was carefully controlled at 1 ml per minute. The coacervate so formed was hardened with 25% glutaraldehyde for 2 hours to allow cross-linking of protein. Organic solvents were then removed under reduced pressure by rotary vacuum evaporator and the resulting nanocapsulesare purified by centrifugation at (10,000 rpm) at 4 ºC. Pellets of nanocapsules thus obtained were then suspended in phosphate buffer (pH -7.4; 0.1 M) and each sample finally was lyophilized with mannitol (2% W/V).

The nanocapsules obtained are further dried by lyophilisation and they are applied on the cotton fabric by exhaustion method using 8% citric acid as binder. The fabric is finished using the following recipe;

Recepies for Nanoencapsules	
Material:Liquor ratio	1:20
Binder (Citric acid)	8%
Temperature	55 ˚C
Time	30 mins

For the study, the above method was used to prepre the nanocapsules. The poly herbal extract of *Abutilon indium, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* had been used as the core material and bovin serum albumin as wall material which was crosslinked with glutaraldehyde. The herbal extract was incubated with the protein solution at the pH of 5.5 and then ethanol was added to the solution . The prepared nanocapsules were used to finish the fabric by exhaust method. Two meter of the fabric was wetted and immersed in the two liter of water containing herbal nanocapsules and 8% citric acid as binder and set in the temperatureof 55ºc in the hot air oven and let to cure for 30 minutes.

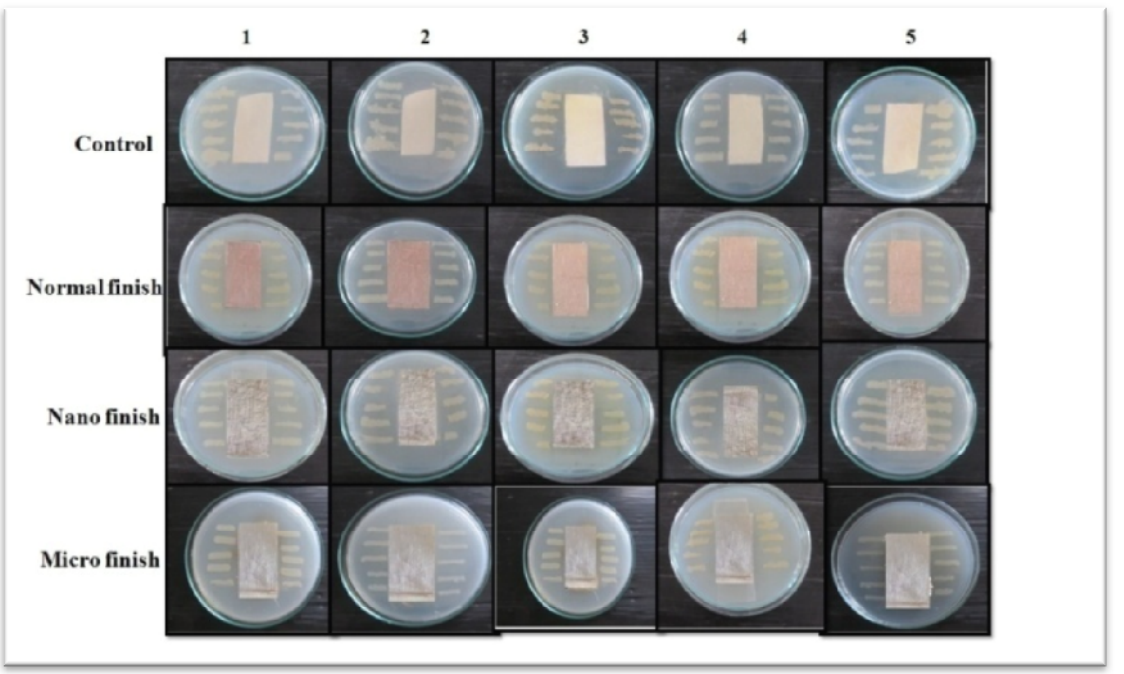
3.10 Antimicrobial Activity by AATCC 147 (AATCC 147 Test Method - 1993)

Using a 4 mm inoculating loop, one loopful of the *Staphylococuss sapropyticus,Escherichia coli,Pseudomonas aeroginosa,Aeromonas hydrophila* and *Candida albicans* were transferred to the surface of agar plates, making five parallel streaks on the central area of a plate without were filling of loop. Test specimens (25×50) were cut with a rectangular die and placed onto inoculated petri plate transversely across the five inoculum streaks. Petri plates were incubated for 18-24 hours at 37ºC. Incubated plates were examined for interruption of growth along the streaks of inoculum beneath the specimen and for a clean zone of inhibition beyond its edge. Zone diameter along a streak on either side of the test specimen was measured using a scale.

The fabric finished by three different methods were subjected to antimicrobial activity by AATCC147 method were shown in Plate XXXIII. The plates were prepared by pouring 15ml of media into sterile petri plates. The plates were allowed to solidify for 5min and the bacterial culture was inoculated as single line followed by the four lines without refilling the inoculation loop. The fabric was cut into 5 X 2.5 size and immersed in three different treatment bath containing crude herbal extract, microencapsulated extract and nanoencapsulated extract for 15 minutes and air dried in at room temperature. The finished fabric with the diameter of 2.5 cm was placed on over the inoculated *Staphylococus ssapropyticus,Escherichia coli,Pseudomonas aeroginosa,Aeromonas hydrophila*

and *Candida albicans* and the plates were kept for incubation at 37ºC for 24 hours. At the end of incubation, zone of incubation formed around the fabric was measured in millimeter and recorded. After incubation, the plates were examined for the zone of bacterial inhibition around the fabric sample. The size of the clear zone was used to evaluate the inhibitory effect of the herbal extract finished fabric (Plate XXXIII).

Agar Well DIffussion of Polyherbal Finished Fabrics



*1 - *Escherichia coli*; 2- *Pseudomonas aeroginosa*; 3 - *Candida albicans*;

4 - *Aeromonas hydrophila* ; 5 - *Staphylococcus saprophyticus*

Plate XXXIII AATCC-147

3.11 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

For the study, the fabrics treated with poly herbal finish were examined for the presence of polyherbal components in the treated fabric. The Dip and Dry , Microencapsulated and Nanoencapsulated fabrics were subjected to FTIR analysis. As per the procedure the fabric samples were placed horizontally on the zincselenide crystal. The infra red light entered on one end and exits on the other

end and the values were recorded automatically. Thus the spectrum of the finished fabric were collected by Attenuated Total Reflectance for FTIR analysis.

3.12 Scanning Electron Microscopic (SEM) Analysis

The surface topography of poly hebal Nanoencapsules finished fabric and Microencapsules finished fabric was observed with a Scanning Electron Microscope (SEM) using Jeol Model - 6390 Scanning Electron Microscopy (SEM). The image mode of the microscopy is secondary electron image and was detected by the E. T detector. The electron gun used in the microscopic analysis accelerates at voltage range of 0.5 – 30 KV and the filament was pre-centered tungsten hairpin filament.

3.13 Testing of Physical Properties of Polyherbal Pretreated Woven Fabric

Textile testing as a whole refers to the vigorous testing done on textile materials which may be inside the laboratory as well as in its natural setting, or using various testing equipments. It plays a crucial role in gauging product quality, ensuring regulatory compliance and assessing the performance of textile materials.

3.13.1 Fabric Weight

For the study,five specimens of control, Dip and dried, Microencapsulated and Nanoencapsulated samples had been cut from the mid of the selected area at the width and breath of one inch and weighed and the results have been mentioned in g/m². GSM cutter was used to cut the fabric and sample was weighed in electronic balance.

3.13.2 Tensile Strength and Elongation (ASTM -D -5034: 1995)

For the study, the ravel strip testing method was adopted. The specimen of ten warp and weft way samples of Control, Dip and dry, Microencapsulated and Nanoencapsulated finished fabrics were cut using the stencil, the specimen was standardised to the atmospheric condition of relative humidity of 65±2% and temperature 27±2ºc prior to Fabric Tensile Strength (Kgf) (ASTM -D -5034: 1995)

The specimen was mounted in the clamp jaws with the drawn parallel line adjacent to the side of the upper and lower front or top, with approximately the same length of fabric extending beyond the jaw at each end. Each specimen either 65 mm (2.5 in.) or 50 mm (2.0 in.) plus 20 yarns, whichever is wider, by at least 150 mm (6 in.) long .The long dimension should be parallel to the direction for which the breaking force is required.Each specimen was ravelled to give a testing width of 50 mm (2.0 in.) by removing an approximately equal number of yarns from each side, or 10 yarns from each side, the samples for a given fabric direction was spaced along a diagonal of the fabric to allow for representation of different warp and weft direction. No fabric had been cut near the selvedge.

The ten samples of the commercial bandaids,control and herbal finished fabric was mounted in the upper clamp of the machine and a uniform pretension applied, not to exceed 0.5% of the full-scale load to the bottom end of the sample, before gripping it in the lower clamp. To achieve uniform and equal tension, the clamps were set at the distance of 200mm for test. The sample at the front inner edge of each jaw was marked to check for sample slippage. When slippage occurred, the mark would moved away from the jaw edge. The machine was operated and the sample was broken. The breaking force and elongation was read in the warp and weft direction and recorded (Plate XXXIV).

3.13.3 Air Permeability (IS 11056: 1984)

For the study, the ten samples were subjected to moisture equilibrium in the standard atmospheric condition of relative humidity of 65±2% and temperature 27±2ºc.A portion of the conditioned specimen of commercial bandaids, Control, Dip and drying, Microencapsulated and Nanoencapsulated were mounted between the circular orifice system with the force of 50 ±5 newton was used to secure the sample. Ten samples were taken for the testing. The area of testing samples were noted as 38.3 cm^2 ± 0.3%.Care was taken to ensure that the fabric was not distorted. The suction fan or other means to force air through the fabric was started; the rate of the flow of air was adjusted till a pressure drop of one centimeter water across the fabric was indicated. The rate of air flow per cm^2 of fabric in cm^3/s was calculated and the rate of air flow was noted in cm^3/s.

The test was repeated at different places. The finished fabric was placed as the finshed side down to prevent the leakage. The test was made with the water pressure difference of 125 pascal (Plate XXXV).

3.13.4 Water Absorbency (AATCC 79:2007)

For the study, the test method for the determination of the water absorbency of fabric was performed in the standard atmosphere.For testing, burette 10±0.05 ml with 0.5 ml graduations that allow a delivery rate of 15 drops per milliliter was used. A location in the wetting conditioned laboratory area that had over head lighting to facilitate the judgment of the test and point was denoted; i.e.. The burette's stop clock position that will deliver the specified number of water drops was also determined. Ten samples of control,polyherbal finished and commercial were taken for testing. Each sample was mounted in an embroidery hoop, so that the side of the specimen to be tested was up, and the surface specimen was taut and free of wrinkles but without stretching or distorting the structure of the fabric. The embroidery hoop with the specimen surface 10±1.0mm was placed below the tip of the burette and one drop of distilled or deionized water was allowed to fall on the cloth. The stopwatch or timer was started immediately. The absorbency was observed without disturbing the setup.The water drop was observed from opposite direction until it finally disapeared. If the water drop disappeared immediately, it was recorded as "zero". If the wetting time exceeded 60s,the time was recorded as "60+s".The same steps were repeated for the additional four test locations(Plate XXXVI).

3.13.5 Vertical Wicking (BS3424)

For the study of vertical wicking BS3424 method had been used. Ten samples were taken for testing.Each sample strip was tested in the standard atmospheric condition of relative humidity of 65±2% and temperature 27±2ºc. A tray was setup and filled with water and dye solution were added for accurate measuring. The fabric samples were suspensed from the holder at angle of 90º perpendicular to the stand. Ten samples of warp and weft were cut from the poly herbal finished fabric, Control and commercial bandaids. The rate (distance per

unit of time) at which liquid travels along, or through a fabric sample was visually observed and manually timed. The length had been set as constant and the increasing in capillary rise was calculated by the time taken to reach the distance of one inch had been noted at one second interval and had been calculated (Plate XXXVII).

3.13.6 Sinking (AATCC 17-1994)

For the study, method of Diaper Service Institute of America had been adopted.This test method was to measure the rate of absorption of a fabric. It was measured in terms of the time required for a folded fabric packet 10cm x 10cm to submerge in distilled water at a temperature of 25°C**.** Absorbency may be assessed in various way, the most popular being the Sinking Time Test (AATCC test method 17-1994)

The ten fabric samples of commercial bandaids, control and polyherbal finished of 25mm X 25mm was cut using the template. A flask was set up and filled with the warm water (25°C) and the fabric had been made to float on the water in the flask and the sinking time of the fabric was noted and recorded (Plate XXXVIII).

3.13.7 Water Holding Capacity (DIN 53923)

For the study, the samples were washed and let to dry. The standard atmospheric condition of relative humidity of 65±2% and temperature 27±2^{o}cwas maintained prior to fabric testing. Ten samples of 0.625 mm length and wide of 3.2mm were cut using the template and wet for 20 minutes in the tray containing water, dried under the same condition and were measured. The water absorbed by the sample was dried again and again for ten samples taken from each of the finished fabric and measured (Plate XXXIX).

Physical Properties of Pretreated Fabric

Plate XXXIV Tensile strength tester

Plate XXXV Air Permiability tester

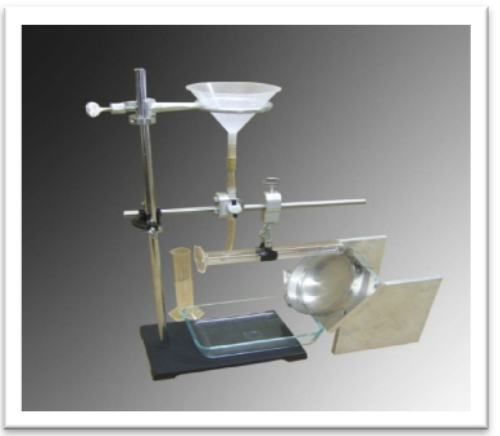


Plate XXXVI Absorbency tester

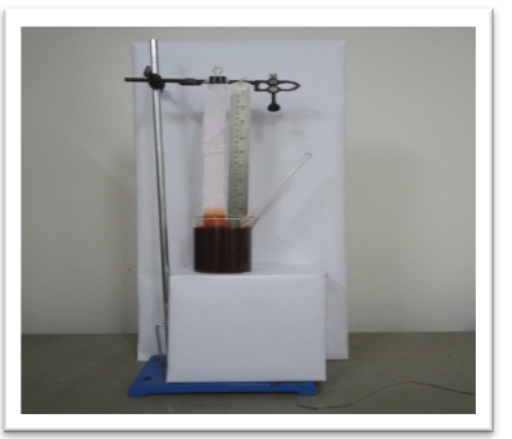


Plate XXXVII Vertival wicking tester

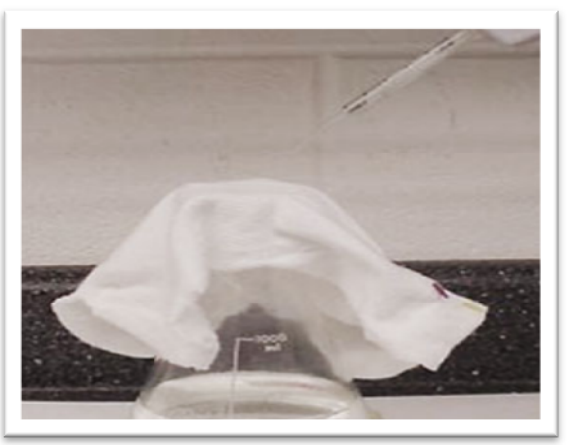


Plate XXXVIII Water Holding capacity tester

3.14 Product Development and Evaluavtion

The polyherbal treated fabric of DDF,MEF and MEF had been developed into a bandaids samples of 2 inch length and 3 inch width at SITRA, Coimbatore. The prepared bandaids were tested for microbial filteration efficiency and bandaid toxicity test at Gram Positive Research and Development Laboratory, Coimbatore. The visual testing for bandaids was not carried out since qualitative tests were done.

3.14.1 Microbial Filteration Test

Antibacterial activity for each medical textile samples (Micro finished, Dip-dry finished, Nano finished and Control) was determined using Bacterial Filteration test method. The method is a modified method of AATCC 100-2004. The antibacterial properties of materials can be studied by quantitative test methods. Quantitative test is the proper indicator of degree of antibacterial activity when the antibacterial agents are fixed on to the textile material or are unable to leach out or filtered.All the test samples (Micro finished, Dip-dry finished, Nano finished and Control) were subjected to antibacterial assay. Briefly, 1.0ml of 12hours challenge bacterial inoculum (*Escherichia coli* ATCC 25922,*Staphylococcus saprophyticus*ATCC 6538, *Aeromonas hydrophila* ATCC 100-2004 and *Pseudomonas aeroginosa* ATCC 100-2004) was dispersed as droplets over the swatches (test samples) using a micropipette. The swatches were inoculated in pre-sterilized 250ml Erlenmeyer flasks. After all the samples were inoculated, the flasks were incubated at 37 ± 2°C for 18h before being assayed for bacterial population density. The bacterial population density was determined by extracting the bacteria from the test sample by adding 100ml of distilled water to each flask and shaken using an orbital shaker for 1min. Then aliquots were serially diluted and spread plated to determine the bacterial density. The difference in number of viable bacteria was evaluated on the basis of the percentage reduction. Percentage reduction was calculated using the following formula.

$$\textit{Bacterial reduction } R\ (\%) = A - B/A \times 100$$

Where, *R* is percentage reduction; *A* is the number of bacteria in the broth inoculated with test sample immediately after inoculation i.e., at zero contact time and *B* is the number of bacteria recovered from the broth inoculated with test sample after the desired contact period of 18 hours.The significant reduction in the fungal reduction as percentage was calculated from the number of Fungal CFU obtained from their respective culture plates. The number of Fungal CFU was calculated based on the number of colonies obtained from the test samples after incubating for 0^{th} hour and 18^{th} hour (Mosmann, 1983). The result are presented in Table XXII and XXIII.

3.14.2 Bandaid Toxicity Test

The mouse fibroblast cell lines (L_{929}) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The monolayer cells were detached with trypsin-Ethylene Diamine Tetra Acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution (extracts from Microcapsule finished swatches, Dip-dry swathces and Nanoparticle finished swatches) was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as

control and triplicate was maintained for all concentrations.3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader (Monks,1991). The results are presented in Table XXIV.

3.15 Statistical Analysis

The result for the subjective and objective evaluation of the untreated and treated fabrics values were statistically analysed. The values were evaluated using the software package known as statistical package for social science (SPSS). The statistical tool adopted for the study was Analysis of Variance.