DEVELOPING HERBAL ANTIMICROBIAL FINISHED COTTON FABRIC FOR WOUND DRESSING

Thesis Submitted in Partial Fulfillment of the

Degree of Doctor of Philosophy (Ph.D.)

By

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March 2019

CERTIFICATE FROM THE SUPERVISOR

I certify that the thesis entitled, **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound dressing"** submitted for the degree of Doctor of Philosophy (Ph.D.) by **Ms. S. Archanaa Preetha** is the record of research work carried out by her during the period from September 2010 to March 2019 under my guidance and supervision, and that this work has not formed the basis for the award of any Degree, Diploma, Associate ship, Fellowship or other titles in this university or any other university or institution of higher learning.

Signature of the HOD

Signature of the Supervisor
With Designation

Signature of Dean

DECLARATION

I declare that the thesis entitled, **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound dressing"** submitted by me for the degree of Doctor of Philosophy (Ph.D) is the record of work carried out by me during the period from September 2010 to March 2019 under the guidance of **Dr. G. Bagyalakshmi** and has not formed the basis for the award of any Degree, Diploma, Associate ship, Fellowship, titles in the university or any other university or similar institution of higher learning.

Signature of the Candidate

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1
INTRODUCTION

Textile tradition in India is rich and diverse, and has approximately India has 2.4% of world's area with 8% of global bio-diversity. In developing countries like India, textile and clothing play a vital role in the part of manufacturing, production, employment and also trade. A truly competitive sector in the country is textile industry. The earliest usage of textiles, most likely felt, dates back to the late stone age, approximately 100,000 years ago. Use of textiles, for healthcare also goes back a long way to centuries before Christ. Report concerning use of cotton, flax and silk in the form of wound dressing and even as sutures, dates back as far as 5000BC. Since then, reference to use of textile in medical applicationin preceding civilizations, including the Egyptians, Persians, Romans, Chinese and Indian, were plentiful in various literature (Hiller,1927 and King,2001).

Much of the textile use in medical applications remains unchanged till the late nineteenth century. Later depth in technological advancement led to great interest in internal arrangements of natural fibre in order to alter their physical, morphological and medical characteristics.Textile fibres play an important role in health care and medical sector. Textile materials in the medical field have gradually taken up more important roles with varied medical applications. In addition to protective medical apparels, textiles in fibre and fabric form are used for implants, blood filters and surgical dressings (Thomson and McClain, 2001).

The medical textile products are available in woven, knitted and non-woven forms based on the area of application.However, the part played by fibre-based materials has been on the rise in recent years. Medical textile is one of the areas which need massive attention as it is directly associated with human beings (Rajendran and Anand, 2006).

Medical textiles are products used primarily in medical and biological applications for first aid, clinical and hygienic purposes. It consists of all those textile materials in both consumer and medical markets. Since textile has always been a part of the healthcare industry, the amalgamation of fabric technology and medical science has resulted in the term medical textiles (Chinta and Veena, 2012).Textile industries have found their approach into a multiplicity of medical applications after completing numerous researches.Therefore,medical textile has become the fastest expanding sectors of the textile industry.In the field of technical textiles, medical textiles is a constantly growing and rising area.Even in india the growth for medical textiles is growing (Akter et al,2014). The very main objective of medical textiles is to improve the quality of health care sector through disposable products and enhance the standard in health care sector by minimizing the risk of infections(Flanagan et al, 2011).

The clothes worn close to the skin are now considered as infection control and barrier materials. Antimicrobial textiles with enhanced functionality are being utilized in health and hygiene products. For example, Antimicrobial finishes are used for preventing odour problems generated by bacteria. Microbial infestation is an additional factor that has resulted in the development of antimicrobial finish. Moreover, microbial infestation poses danger to both living and non-living matters. Microorganisms can create problems with fabric raw materials, processing chemicals, wet processes in the mills, roll or bulk goods in cargo space, finished goods in storage and transport, and goods during usage. Microbes are the tiniest creatures that cannot be seen with the naked eye. They include a variety of microorganisms like bacteria, fungi, viruses and algae. Although bacteria and fungi are normal components of the natural environment, they cause specific problems such as staining, discolouration, deterioration, and odour of the fabric. The intrinsicproperties of the material fibres offer room for the growth of microorganisms. Besides, the structure of the substrates and the chemical processes may induce the growth of microbes.

In the textile industry, many opportunities are available to add value and improve products by incorporating novelty finishes. The trend now is to protect the

textiles against microbial infestation. Essentially, with a view to protect the wearer, the textile substrate is treated with antimicrobial materials.Additionally,the green minded customers are opting for ecofriendly textile materials or textile materials treated with medicinal herbs.These herbal textiles are not only permanently effective but also skin compatible and eco-friendly(Sathianarayanan et al,2010). According to history, clothing as a means of protection and healing goes back to the Rigveda, a sacred and ancient Hindu text composed of Ayurvedic scriptures. Until about 100 years ago, people in many parts of India were still using various forms of natural dyeing, in which the clothes are repeatedly dipped in an herb-based preparation after each wash. Medicinal properties of plants have been known to human for centuries. Plants and their products play a vital role in curing various ailments of human being and other animals.

In addition to the primary uses as food and fuel, plants were used for disease control and health management. Ayurveda, Siddha, home medicine and indigenous folk medicine system utilize crude preparations of many herbs for therapeutic purposes. In the development of human culture, medicinal plants play an essential role and they act as a source of traditional medicines. Large number of modern medicines are produced indirectly from plants. Medicinal plants are used in wound healing, pain relieving, anti inflammatory, antipyretic agents, vitalizers, aphrodisiac agents and even for birth control. WHO confirmed that about 80% of the world’s population was served by herbal medicines.

Many researches about medicinal properties of plants including antimicrobial properties are going on and many biologically active and significant ingredients were reported from all parts of the world. Tropical countries like India which harbour a greater floral diversity are potent reservoirs of such medicinal plants and a wide variety of experiments are progressing in this field.

The amount of bio-functional textiles with antimicrobial activity has amplified significantly over the last few years. The consciousness of health and hygiene between the people has increased the demand for antimicrobial textiles which have antimicrobial finishes, in particular to shield against fungal diseases.

Antimicrobial treatment for textile materials is necessary to control microorganisms, reduce odour from perspiration, stains and soil on textile material, reduce the risk of cross infection being carried by feet from ward to ward in hospital, control spread of disease and danger of infection following injury, control the deterioration of textiles particularly in fabrics made from natural fibre caused by mildew.

Antimicrobial fabrics have been tested for use in the medical industry for some time. Now, the only antimicrobial fabric being utilized in the field of medicine are nonwoven and disposable. Application of herbals on fabrics opens up new possibilities as herbal treated garments can be utilized in operation theatre fabric and medical gown. Application of herbals used on cotton has a scope in wound healing or wound dressing manufacture.

Among all the natural antimicrobial agents, the plant products comprise the major segment. Healing power of some of the plants have been used since ancient times. Medicinal plants are the gift of nature to cure limitless number of diseases among human beings. The richness of plants on the earth surface has directed to an increasing interest in the study of different traditional medicinal plant extracts as potential sources of new antimicrobial agents. Herbs are abundantly available in nature and are non toxic and inexpensive. Extract from plant parts such as leaves, roots, seeds and flowers display antimicrobial properties. Due to their eco-friendly nature, herbal finishes are gaining significant momentum.

Antimicrobial extracts can be used as textile finishing agents in solvent form or microencapsulation and nanoencapsulation to improve the durability and controlled release of the extracts. This finish is applied in such a way that appearance and feel of the fabric is not altered and no chemical odour remains. These are applied to textile materials for two purposes as to protect the wearer and the fabric itself.

Various methods have been used for antimicrobial finishing of textile materials depending on the particular active agent and fibre type. In general, two different antimicrobial finishing methods can be distinguished. Antimicrobial agents can be either applied in an after-treatment process or incorporated into the polymer solution prior to extrusion or into the spinning bath.Substance embedded within the fibre structure has to migrate to the fabric surface, and should be slowly released during use in order to be active (Heine et al,2007).

Incorporation of antimicrobial substance within a fibre matrix is suitable only for synthetic fibres as after treatment processes for antimicrobial finish of natural, as well as synthetic fibre, conventional, exhaust and pad-dry-cure methods have been used. In addition, methods like padding, spraying, coating and foam finishing have been developed. Many other methods have also been reported, such as the use of nano sized colloidal solutions, nano particles, chemical modification of the biocide for covalent bond formation with the fibre, crosslinking of the active agent onto the fibre using cross-linker and sol gel processes(Coman et al, 2010).

Textile resources and products that have been engineered to meet up particular needs, are appropriate for any medical and surgical application where a mixture of strength, flexibility,moisture and air permeability are required. The medical textile industries have diversified with new materials and innovative designs. In recent times, function of textiles has been started going beyond the typical wound care for example incontinence pads and plasters (Akter et al,2014).

The wound dressing materials are designed to perform a wide variety of specific functions, depending upon the final medical requirement. The most common application for dressings is to cover wounds aseptically to avoid microbial infection and speed up the healing processes. Such dressing material includes light weight knitted or simple open-weave fabrics frequently made from cotton, which are cut into strips and then desized, scoured, bleached and sterilised (Geetu and Sahu,2014).

Wound healing generally requires support at three levels. First, improving general resistance and support mechanisms that could be obtained from rejuvenative, adaptogenic, palliative, antioxidant, cleansing, detoxifying, buffering, and lubricous activities. Second, stimulating the repair and regenerative mechanisms to prolong cell life, cell migration and cell binding, remove skin blemishes, and improve tensile strength or elasticity of the skin, improve moisture-holding capacity of skin. Third, therapeutic and nutritional activities including anti-inflammatory, antiseptic, and antimicrobial, protein and collagen synthesis and increased stability of biomembranes.

The pathophysiology of wound healing in cell lines play an important role to define the basic mechanism of treatment of wound healing. The polyphenols present in the polyherbal extract are capable of promoting rapid epithelialisation of wounds and also the antioxidant and antimicrobial property of the polyherbal extract when compared to the individual herb (Narendhirakannan,2012). Moreover, Nanoencapsulation is a novel technique rapidly evolving and extensively used in chemical, pharmaceutical, food processing and cosmetics industries.In recent years, textile finishing industries also utilize the Nanoencapsulation technique with respect to finishing. Nanoencapsulation is the slow controlled release of the antimicrobial agent to achieve the desired delay until the right stimulus is obtained.

The incorporation of multifunctional values with herbal extracts in textile material has become a special area of interest in recent years. Fibres, yarns, fabric and other structures with added functional value have been created for a variety of applications. Textile resources and techniques have become a significant plat form for high-tech inventions. Increasing global competition in the textile sector has generated many challenges for textile researchers across the globe. The rapid growth in technical textiles and their end-uses have created many opportunities for the application of innovative finishes.

Considering these aspects in mind the present study on **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound Dressing"** has been attempted to finish the cotton fabric with poly herbal extracts and compared to identify the effect of the fabrics against the skin infective pathogens with the following objectives;

- To study the availability of wound dressing in market and their demand
- To select the yarn for weaving fabric for wound dressing
- To select medicinal herbs
- To optimize herbal extract concentration and determination of polyherbal formation
- To treat the woven fabric with polyherbal extract and test the fabric performance.
- To develop a product and evaluation.

2

REVIEW OF LITERATURE

The literature pertaining to the study on, **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound Dressing"** for the study is presented under the following headings;

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2.1 Healthcare Textiles

Textiles have always played a central role in the evolution of human culture by being at the forefront of both technological and artistic development. The protective aspects of textiles have provided the ground for innovative developments. Textiles have such an important bearing on our daily lives that everyone needs to know something about them. From earlier times, people have used textiles of various types for covering, warmthness, personal adornment, and even to display personal wealth. Today, textiles are still used for these purposes and everyone is an ultimate consumer. The consumers are now increasingly aware of the hygienic life style, and there is necessity and expectation for a wide range of textile products finished with antimicrobial properties (Saranya and Bagyalakshmi, 2015).

“Wellness” is a trend in modern society, which opens up a range of new possibilities i.e textiles with antimicrobials. There are many different types of textiles used today in an attempt to either limit or prevent the transmission of hazardous microorganisms. These materials range from solitary layers of non-woven single use products to composites of woven and knitted multiple use products (Achwal,2003). Hygienic life style has acquired an important place in recent years. Hence there is a need to exhibit high degree of performance in terms of longevity, durability and antimicrobial properties of the fabric (Gopalakrishnan, 2006).

Although bacteria and fungi are normal components of the natural environment, they cause specific problems such as odour, staining, discolouration and deterioration of the fabric. In textile industries, there are many opportunities to improve and add value to products by incorporating novelty finishes (Sakagami, 1995).The key advantage of the antimicrobial textile finish or modification is to protect the wearer against biohazards, control of foul odour and improvement in the performance and aesthetic characteristics of fibre (Mucha et al, 2005).

Combination of textile and its application in medical sciences has resulted into a new field called medical textiles. New areas of application for medical textiles have been identified with the development of new fibres and manufacturing technologies for yarns and fabrics. Development in the field of textiles, either natural or man-made textiles, normally aimed at how they enhance comfort to the users. Development of medical textiles is really meant for converting the painful days of patients and surgeons into the comfortable days (Chinta et al, 2013).

2.1.1. Materials Used for Healthcare Textiles

Healthcare and medical textiles are a major growth area within the scope of technical textiles,which is defined as “Textile materials and products manufactured primarily for their technical performance and functional properties rather than their aesthetic or decoration characteristics”. Technical textiles include, in addition to healthcare and medical textiles, aerospace, industrial, marine, military, safety and transport textiles and geo-textiles. Over the last few decades, there have been significant changes in the textile market, where traditional textile products, or the textile products produced primarily for their aesthetic or decoration properties (e.g.,apparel), account for an increasingly smaller portion, while technical textile products constitute an increasingly larger portion(Zhong, 2013).

The textile material in healthcare and medicine can be used mainly in three forms; Fibre form which is obtained naturally or artificially include fiber and yarn form cotton, wool, silk and artificial fibres include polyester, polypropylene. Yarn form includes staple yarn, twisted yarn and braided yarn.

Fibres used in medical textiles are classified as;

- Degradable fibres: The fibres which gets absorbed by the body within two to three months of implantation. Examples are cotton, viscose, chitin, collagen.

- Non-degradable fibres: The fibres which get degraded by the body in more than six months, generally these fibres are synthetic fibres. Examples are polyester, polypropylene, poly-tetra-fluroethylene.

- Re-absorbable fibres: The textile fibre which is completely biodegradable by the body and produce no harmful degraded product. Examples are polyglycolic acid, polylactic acid and polydioxanone (Junare and Vishwanath, 2017).

2.1.2. Types of Healthcare Textile Products

The variety of natural and man-made fibres available today, offer a wide selection to be used in clothing. Globally natural fibres contribute about 48% to the fibre basket with 38% from cotton (Sharma et al, 2015). Now-a-days, environmental issues are becoming the major factors during the selection of consumer goods. Renewable resources are gaining popularity among the people due to their positive effects on agriculture, environment and economy. Natural fibres being biodegradable are now considered as solemn option to synthetic fibres for use in various fields (Christy and Kavitha, 2014).

Non-implantable materials are used for external applications on the body and these materials may or may not have contact with skin. This includes wound care, bandaids, plasters, pressure garments, orthopaedic belts etc. Implantable materials are used in effecting repair to the body whether it is wound closure such as sutures or replacement surgery such as vascular grafts, artificial ligaments etc. Extra corporeal devices are extra corporally mounted devices used to support the function of vital organs, such as kidney, liver, lung, heart pacer etc. The extracorporeal devices are mechanical organs that are used for blood purification and include the artificial kidney i.e dialyser, the artificial liver,and the mechanical lung. The function and performance of these devices benefit from fibre and textile

technology.Health care and hygiene products made of textile materials are an important area of the healthcare and hygiene sector. The range of products available for healthcare and hygiene is vast, but they are typically used either in the operating theatres or in the hospital wards for hygiene, care and safety of the staff and patients. They could be washable or disposable (Akter et al, 2014).

Wound healing is a dynamic and complex process which requires suitable environment to promote healing process. With the advancement in technology, more than 3000 products have been developed to treat different types of wounds by targeting various aspects of healing process.Local factors which includes hypothermia, pain, infection, radiation and tissue oxygen tension directly influence the characteristics of the wound where as systemic factors are the overall health or disease state of the individual that affect individual's ability to heal. In addition to these factors, poor nutrition, age and protein, vitamins and mineral deficiency canalso prolongs healing times. Based on the wound type, suitable dressing material must be used (Dhivya et al,2015).

Traditional wound dressing products including gauze, lint, plasters,bandaids (natural or synthetic) and cotton wool are dry and used as primary or secondary dressings for protecting the wound from contaminations. Gauze dressings made out of woven and non woven fibres of cotton, rayon, polyesters afford some sort of protection against infection (Boateng et al, 2008). Modern wound dressing have been developed to facilitate the function of the wound rather than just to cover it. These dressings are focused to keep the wound from dehydration and promote healing. Based on the cause and type of wound, numerous products are available in the market (Pandey et al, 2009).

2.1.3. Structure of Textile Materials

Textile is defined as "a general term for fibres, yarn intermediates, yarns, fabrics, and products that retain all the strength, flexibility, and other typical properties of the original fibre or filaments". In other words, textiles are made from the basic elements of fibres. However, to develop healthcare and medical textiles, materials other than textiles have to be included. The medical textile industries

have diversified with new materials and innovative designs. Recently, application of textiles has been utilised beyond the usual wound care, incontinence pads, plasters etc. This makes it necessary, at the very beginning to list related materials and structures that comprise healthcare and medical textiles. Fibres composed of natural or synthetic polymers are spun into yarns, followed by being weaving or knitting into fabrics and further fabricated into specific products, including apparel. Beyond that, there is a variety of structures that are more often used in technical end uses: laminated fabrics can be made by bonding a fabrics with a polymeric films or foams by using an adhesive or by the adhesive properties of one of the layers. By controlling the pore sizes in micro-porous films, waterproof but breathable i.e., water vapour permeable fabrics can be obtained. Such fabrics are desirable in applications like surgical gowns, where both protection and comfort are essential (Jewel,2005).

The development and introduction of new structures of textiles have made revolutions first in our mind and then in the market. Natural fibres are derived from plants, animals, or minerals, to be respectively referred to as cellulosic fibres, protein fibres, and inorganic fibres. They vary in macroscopic size, length and shape (FTC, 1958). Man-made fibres are regenerated from natural resources or synthesized from small, organic molecules. They are generally known as regenerated fibers or synthesized fibres (Zhong, 2013).

Cotton is the backbone of the world’s textile trade. It is also known as “King of fibre” and “White gold”. It is said that cotton is the fibre, which has no season it is equally good for all seasons. Due to its unique fibre structures, it can absorb water up to 2.7 times of its own weight. India is the second largest producer of cotton after china. Cotton is grown on 3% of the total cultivated area in the world (Bhat and Choudhari, 2012).Cotton is the world's most used fibre. It is cool, soft, comfortable and the principal clothing fibre of the world. Its production is one of the major factors in the world's prosperity and economic stability. It forms the background of the world textile (Kesarwanin and Archana, 2009). The unique mechanical properties of cotton make them an ideal textile fibre (Smith and Cothren, 1999).

Cotton cellulose accounts for 88 to 98 percent of the dry weight of raw cotton. Non-cellulosic constituents include wax, protein, salts of inorganic and organic acids, pectic acid and sugars. The presence of these substances affects the properties of the fibre in relation to mechanical processing, as well as the properties of the finished goods. Their removal by finishing operations is usually necessary to obtain the most satisfactory end-use products (Jones, 1962). The quality of the cotton is measured by the length and brightness of the fibre,which depends on the species, the quality of the seeds, nature of soil, the mode of cultivation and climatic conditions (Dantyagi, 2004). All fibre used in medical applications must be non-toxic, non-allergenic, and non-carcinogenic and be able to be sterilized without imparting any change in the physical or chemical characteristics. Cotton is one of the commonly used natural fibres in vast industries (Horrocks and Anand, 1997).

2.1.4. Advantage of Healthcare Textiles

Many textile materials used in traditional applications in healthcare are still found. However,recent development in the advanced healthcare has led to the development of new materials through crosscutting research approaches in the field of textiles, polymer, biomedical, pharmaceutical and medical sciences. (Chinta et al, 2013).

Antimicrobial finishing of textiles has emerged as an important market segment comprising consumer, and technical products for healthcare and hygiene control. Nosocomial infections in hospitals, and surface contamination involving microorganisms indicated the significance of antimicrobial finishing. The antimicrobial finished textiles reduce the growth and transmission of microorganisms (Uddin, 2014).

The primary function of wound dressing is to protect the wound site from contamination and further injuries.The use of standards and quality evaluation techniques to characterise the surgical or wound dressings for product will determine its acceptability and commercial success, Well-designed laboratory tests can provide a useful performance indicator, particularly in comparative terms (Chellamani et al, 2014).

2.2. Microbes

The total mass of all microbes living on earth is approximately 25-fold the mass of all animal, (Gupta and Bhaumik, 2007). Mold, mildew, fungi and bacteria are (microorganism) part of our everyday lives. There are both good and bad types of microorganisms. Thousands of species of microorganisms are found everywhere in the environment and on our body. Understanding microorganisms, with reference to their type, origin and medium of culture provide basis to control the negative effects. This control capability, with the right technology, can provide a valuable feature on a wide range of textiles (Gopalakrishnan, 2006).

2.2.1 Types of Microbes

Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters. Microorganims are wide spread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types: bacteria, archaea, fungi, protozoa, algae, and viruses (Joshi et al, 2000).

Bacteria are unicellular organisms. Most bacteria have a peptidoglycan cell wall. According to the way their cell wall structure stains, bacteria can be classified as either Gram-positive or Gram-negative when using the Gram staining. Bacteria in our body are medically seen as foreign "invaders," and thus would be a concern due to their potential to cause infection and other problems. In line with this, research on microbiology was mainly focused on how to kill bacteria with disinfectants and antibiotics. However, over the last few years, we have begun to appreciate the symbiotic relationship we have with the microorganisms co-habiting our bodies. While some bacteria can cause disease, others play beneficial roles in human health (Daniel et al, 2016).

Bacteria use decaying life forms as a source of energy are called saprophytes. Most fungi are multicellular and their cell wall is composed of chitin. Fungi is reproduced by releasing the spores. Viruses are noncellular entities that consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. Although viruses are classified as microorganisms, they are not considered as living organisms. Viruses cannot reproduce outside a host cell and cannot

metabolize on their own. Viruses often infest prokaryotic and eukaryotic cells causing diseases. Since the parasitic helminths are of clinical importance, they are often discussed along with the other groups of microbes (Badr and Arafat, 2016).

2.2.1.1 *Staphylococcus saprophyticus*

Staphylococcus saprophyticus is the leading Gram-positive aetiological agent of Urinary Tract Infection (UTI). *Staphylococcus saprophyticus*shares many clinical features of urinary tract infection caused by *Escherichia coli*, but differs in pathogenesis, seasonal variation, and geographic distribution. The gastro intestinal tract is the major reservoir of *Staphylococcus saprophyticus*. In an early study (Latham et al,1983), it is noted that rectal, vaginal, and urethral colonization of *Staphylococcus saprophyticus*was associated with UTI caused by this organism. The virulence factors of *Staphylococcus saprophyticus* include adherence to urothelial cells by means of a surface-associated protein, lipoteichoic acid; a hemagglutinin that binds to fibronectin, a hemolysin; and production of extra cellular slime (Gatermann and Heesemann, 1986).

The percentage of antibiotic resistant *staphylococci* in large hospitals through out the world has increased steadily so that now nearly three-fourths of all strains are resistant. These resistant bacteria are found not only in superlative lesions, but also in the noses and on the skin of patients and attendants. The *staphylococcus* was the most common pathogen in surgical sepsis in the pre-antibiotic era, not only in wounds of trauma but in elective surgery as well (Howe, 1957).

2.2.1.2 *Aeromonas hydrophila*

The genus *Aeromonas* belongs to the family *Aeromonadaceae* within the Gamma-positive bacteria and also comprises of Gram-negative, non spore-forming, motile *bacilli* or *coccobacilli* with rounded ends which measure 1-3.5 µm in diameter. They are facultative, anaerobic, oxidase and catalase positive, able to reduce nitrate to nitrite, glucose-fermenting and are generally resistant to the

vibriostatic agent. They grow optimally within a temperature range between 22 and 35°C, but growth occurs in a temperature range from 0 to 45°C for some species (Igbinosa et al, 2012). They tolerate a pH range from 4.5 to 9.0, but the optimum pH range is from 5.5 to 9.0 (Isonhood and Drake, 2002). They are also associated with sepsis and wounds, and with eye, respiratory tract, and other systemic infections and most of them are recognized as human and animal pathogens. (Janda and Duffey, 1988; Janda and Abbott, 1996 and Nichols et al, 1996).

However, there are pathogenic as well as non-pathogenic strains belonging to these groups.Members of the genus *Aeromonas* have been related with a wide variety of illnesses in humans,most common pathogenic manifestation of *Aeromonas* in humans is Bacteraemia which means the presence of bacteria in the blood displaying symptoms such as fever and chills. The bacteria are distributed universally in the surface water and soil and humans may obtain infections through open wounds resulting in serious or fatal debilitating outcomes, such as amputations. *Aeromonas* wounds fall into three categories, listed in order of increasing severity of damage caused: cellulitis, myonecrosis, and ecthyma gangrenosum (Janda and Abbott, 2010).

2.2.1.3 *Escherichia coli*

The *Escherichia coli* bacterium is a gram-negative rod of about 1.1–1.5 μm x 2.0 – 6.0 μm in size. It grows under aerobic and anaerobic conditions because it possesses two different redox systems (menaquinone and ubiquinone) which enable it to derive energy from catabolic metabolism under both aerobic and anaerobic conditions. Under optimal growing conditions, the rate of cell division of the *Escherichia coli* bacteria is very fast and the number of bacterial cells can double every 20 minutes. However, the circumstances that are ideal for this population dynamics are not achieved in the bacteria‘s normal environment. Pathogenic *Escherichia coli* variants are characterised by the presence of various virulence factors, such as various toxins, particularly in secretion systems (Kaper et al, 2004).

Escherichia coli could pose a huge burden on the individuals' as well as the state's economy. Hence, there is a need for continued surveillance of resistant strains and proper selection and rational use of antimicrobes for treating surgical wound infections in the hospital before this problem escalates into epidemic proportions (Yadav et al, 2012).

2.2.1.4 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram negative, aerobic, rod shaped bacterium with unipolar motility (Ryan and Ray, 2004). It is a common bacterium which can cause disease in animals and humans, found in soil, water, and mostly on man-made environments throughout the world. It thrives not only in normal atmospheres, but also with little oxygen, and has thus colonized in many natural and artificial environments. Because it thrives on moist surfaces, this bacterium is also found on medical equipments including catheters, causing cross infections in hospitals and clinics. It uses a wide range of organic material for food; in animals the versatility enables the organism to infect damaged tissues or people with reduced immunity (Balcht and Smith,1994). Occasionally, *Pseudomonas aeruginosa* can colonize human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *Pseudomonas aeruginosa* in healthy subjects is usually low, but following hospitalization, higher colonization rates can be encountered, especially amongst subjects treated with broad-spectrum antimicrobial agents. Colonization is common gastrointestinal tract of patients receiving anticancer chemotherapy, in the respiratory tract of mechanically ventilated patients, and on the burnt skin of patients (Morrison and Wenzel, 1984; Pollack, 2000).

Studies have shown that such wound infections are universal and that the bacteria types present vary with geographical locations. *Pseudomonas aeruginosa* are opportunistic pathogens and are responsible for a wide range of infections. They are common precipitants of sepsis by virtue of the inflammatory response activated by endotoxins present in the Gram-negative cell wall (Yadav et al, 2012). *Pseudomonas aeruginosa* are hard to treat because this bacterium shows intrinsic and acquired resistance to different antimicrobial compounds (Serrano et al,2017).

2.2.1.5 *Candida albicans*

The relative proportions of organisms causing nosocomial bloodstream infections have changed over the last decade, with *Candida* species now firmly established as one of the most frequent agents. *Candidemia* not only is associated with a high mortality but also extends the length of the hospital stay and increases the costs of medical care (Rauha et al, 2000).

Candida albicans is the most frequently encountered pathogenic human fungal species and commonly colonizes host mucosal and moist skin surfaces . However, under conditions of immune dysfunction, this opportunistic microbe can rapidly transit from commensal to pathogen, causing an array of infections ranging from localized mucosal to severe systemic infections with high morbidity and mortality rates. Oral candidiasis or thrush is the most common opportunistic infection in HIV-infected population with 80–90% of these individuals developing oropharyngeal candidiasis during the course of their illness. In addition, recent longitudinal studies have shown that in the ageing population, *Candida albicans* is even more frequently encountered in the oral cavity, especially in edentulous elderly populations .The success of this species as an opportunistic pathogen which is the result of its repertoire of virulence factors, including the ability to switch between a yeast and hyphal morphology, a property crucial to its pathogenicity (Schlecht et al,2015).An important group of the skin pathogens are the fungi, among which dermatophytes and *Candida spp*. are prominent (Martinez et al, 2012).

2.2.2. Human Skin and Skin Wound

The skin is the human body’s largest organ, colonized by a diverse million of microorganisms, most of which are harmless or even beneficial to their host. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The cutaneous innate and adaptive immune responses can modulate the skin microbiota, but the microbiota also functions in educating the immune system. The development of molecular methods to identify

microorganisms has led to an emerging view of the resident skin bacteria as highly diverse and variable. An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment (Elizabeth et al,2011).

Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries. Irrespective of the nature of the cutaneous injury, acute wounds are expected to heal within a predictable time frame.The treatment required to facilitate, healing will vary according to the type, site, and depth of a wound. The primary closure of a clean, surgical wound would be expected to require minimal intervention to enable healing to progress naturally and quickly (Bowler et al, 2001).

Wound healing happens in three stages. The first is improvement in general resistance and support mechanisms that could be obtained from rejuvenative, adaptogenic, palliative, antioxidant, cleansing, detoxifying, buffering, and lubricous activities. Second, stimulating the repair and regenerative mechanisms to prolong cell life, cell migration and cell binding, remove skin blemishes, and improve tensile strength or elasticity of the skin, improve moisture-holding capacity of skin. Third, therapeutic and nutritional activities including anti-inflammatory, antiseptic, and antimicrobial, protein and collagen synthesis and increased stability of biomembranes. The polyphenols present in the polyherbal extract are capable of promoting rapid epithelialisation of wounds and also the antioxidant and antimicrobial property of the polyherbal extract promotes the healing faster when compared to the individual herb (Narendhirakannan et al, 2012).

2.2.3 Factors Influencing the Growth of Microbes in Fabrics

Textiles made from natural fibres are generally more susceptible to biodeterioration than the synthetic fibres. This is because their porous hydrophilic

structure retains water, oxygen and nutrients, providing perfect environment for microbial growth. Products such as starch, protein derivatives, fats and oils used in finishing of textiles can also promote microbial growth. Microorganisms may attack the entire substrate, that is the textiles fibres or may attack only one components of the substrate, such as plasticizer contained there in, or grow on dirt that has accumulated on the surface of a product (Balouiri et al,2016).

The vast majority of antimicrobials work by leaching or moving from the surface on which they are applied. This is the mechanism used by leaching antimicrobials to poison a microorganism. Besides affecting durability and useful life, leaching technologies have potential to cause a variety of other problems when used in garments (Uddhav et al,2016).

An anti-microbial with a completely different mode of action than the leaching technologies is a molecularly bonded unconventional technology. In this method the antimicrobial agent remain affixed to the substrate-killing microorganisms as they contact the surface to which it is applied. It physically stabs and electrocutes the microorganisms on contact to kill it. Effective levels of this technology do not leach or diminish over time. A variety of antimicrobial finishes have been developed for application to textiles (Matuskova et al,2014).

Cotton textiles in close proximity to the human body provide an ideal living environment for yeast, bacteria and fungi (Payne and Kudner, 1996). Dust, soil and textiles are the sources of nutrients for microorganisms. Perspiration contains amino acids, salts, carboxylic acids and other essential nutrients. Oils or dead skin cells secreted from the skin are also a possible source of carbon. Cotton consists of hydrophilic cellulose and has a high affinity for water. Perspiring human beings have been estimated to give off an average of 0.1liter/hour of water, which accumulated in clothing and bedding. A humid environment will provide enough water to support fungal growth, whereas bacteria need more water and require dampness. Most bacteria and fungi will grow at an ambient temperature of 10-20°C. Certain bacteria prefer slightly warmer conditions of bedding or clothing in close proximity to the skin (McNeil, 1964).

The synthetic antimicrobial agents and metal oxides are very effective against a range of microbes, but it was also associated with side effects and could not be used for medical application. Hence, there is a great demand for eco-friendly antimicrobial finishes on textiles. The herbal extract finished fabrics were considered as significant for the medical textile application (Dyke,2003).

Even mild surface growth can make a fabric look unattractive by the appearance of unwanted pigmentation. Heavy infestation which results in rotting and breakdown of the fibres and subsequent physical changes such as loss of strength or flexibility may cause the fabric to fail in service. The material is attacked chemically by the action of extracellular enzymes produced by the microorganisms for the purpose of obtaining food. Plant fibres such as cotton, flax, jute and hemp are very susceptible to attack by cellulolytic fungi. The complete degradation of cellulose can be effected by enzymes, produced by the fungi known as cellulases. Even though microbes are useful in many ways such as brewing, baking and biotechnology, they can also be harmful to both textile industries and human (Adnan et al, 2010).

2.3. Use of Herbs in Textile Finishing

Plants are the integral part of nature. Nature reflects the creative power of living god. Plants have an almost endless variety of uses to human beings. India is the birth place of indigenous medicine such as siddha, ayurveda and unani, (Suresh et al, 2012). In early days, plant use was restricted to food, medicine and shelter but with change in course of time, man started exploring the potentiality of plants for a number of useful purposes. Hence the dependency on plants increased both directly and indirectly (Ali et al., 2003 and Ali and Qaiser, 2009).

Abutilon indicum is a hairy shrub with golden flowers. Various part of the *Abutilon indicum* have been used in treating various human ailments. The roots are used in treating uterine heamorrhagic discharges. Similarly, seeds are used in the treatment of bronchitis, gonorrhea and piles. Leaves are useful in treating toothache,lumbago, piles and all kind of inflammation. Bark is used as anthelmentic, diueric and alexeteric (Anyensu et al, 1978).

Cassia auriculata commonly known as tanner's cassia, also known as "avaram" in Tamil language is a shrub belongs to the *Caesalpinaceae* family. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits are used as anthelmintic, seeds are used to treat eye troubles and root are used in skin diseases. It is also used for the treatment of ulcers, leprosy and liver disease. The anti-diabetic, hypolipidemic, antioxidant and hepato protective effect of *Cassia auriculata* have been reported. It was also observed that flower and leaf extract of *Cassia auriculata* shown to have antipyretic activity (Maneemegalai and Naveen, 2010).

Cassia fistula is a wild tree and mainly grows on road side throughout India. It is a deciduous medium sized tree growing upto 20 - 40 meters in height. The bark of this plant is rough, grayish and the leaves are compound. It has showy racemes, up to 40cm long with bright yellow fragrant flowers. Fruits of these plants are long and have cylindrical pod. The seeds of this plant are broadly ovate and horizontally arranged in the sweetish pulps, which is having medicinally important value. *Cassia fistula* is also known as Golden shower which was widely used by tribal people to treat various ailment including ringworms and other fungal skin infection. It is used by Malayali tribe in India to treat nasal infection. It is useful against skin diseases, liver troubles, tuberculous glands and in the treatment of rheumatism, hematemesis, pruritus, leucoderma and diabetes. The effects of plant extract on bacteria have been studied by a very large number of researchers in different parts of the world. Plant parts are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavanoids, glycosides etc. which have been found under *in vitro* studies to have antimicrobial properties (Satpute et al, 2015).

Tridax procumbenz is common herb found in India. It is denoted by different names; in English as Mexican Daisy, in ayurvedic as Jayanti, in siddha

and Tamil as Vettukkaaya-thalai and in folk as Akala kohadi. The whole plant was reported to have healing power, treat various ailments and such as bronchial catarrh, wound, dysentery, diarrhea and prevent hair loss. Pharmacological studies have shown that *Tridax procumbenz* possess properties like anti inflammatory, hepato-protective, wound healing, immune modulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects. These components show the presence of the phytochemical and antimicrobial effect of *Tridax procumbenz* (Christudas et al, 2012). There are many natural plant products, which show antimicrobial properties. Extracts from roots, stems, leaves, flowers, fruits and seeds of diverse species of plants exhibit antimicrobial properties. These antimicrobial agents can be used as textile finishing agents (Thilagavati and Krishnabala, 2007).

2.3.1 Plant Authentication

Medicinal plants cover a wide range of plant taxanomy and closely related species. There is an increasing international market for medicinal plants, which are used both for herbal medicine and for pharmaceutical products. Accurate and rapid authentication of plants and their respective adulterants is difficult to achieve at the scale of international trade in medicinal plants. The natural medicines are much safer than synthetic drugs, have gained popularity in recent years, leading to a tremendous growth of phyto-pharmaceutical usage. However, herbal medicines can be potentially toxic to human health and sometimes may cause unknown effects. The recent investigations have revealed that many plants used in traditional and folk medicine are potentially toxic and mutagenic (Matthews et al., 2003)

Due to the complex nature and inherent variability of the chemical constituents of plant-based drugs, it is difficult to establish quality control parameters.Due to the popularity of herbal drugs globally, their adulteration or substantiation aspects are gaining importance at the commercial level. Pharmaceutical companies are procuring materials from traders, who are getting these materials from untrained persons from rural or forest areas. This has given

rise to wide-spread adulteration or substitution, leading topoor quality of herbal formulations.Misidentification of herbs can be non-intentional orintentional. Adulteration can occur due to ignorance or intentional substitution with cheaper plant material and may cause damage to human body. Therefore,authentication at various stages, from the harvesting of the plant material to the final product, is a need of the hour.The general approaches to herb identification are dependent on morphological (Khan et al, 2011).

2.3.2. Textile Material Used for Wound Dressing

The inherent properties of the textile fibres provide room for the growth of microorganisms. Beside the structures of the substrates, the chemical processes may induce the growth of microbes. Humid and warm environments still aggravate the problem. Infection by microbes cause cross infection by pathogen and developments of odour where the fabric is worn next to skin. In addition, the staining and loss of the performance properties of textile substrates are the results of microbial attack. Garments of health care workers are an important aspect that can easily become contaminated (Vaneechoutte et al, 2013).

The textile materials play an important and crucial role in designing appropriate structures for the healthcare and medical industries. There has been a sharp increase in the use of medical textile products not only in the hospital, hygiene and healthcare sectors but also in hotels, homes and other environments where hygiene is required. With the increasing threat from new strains of bacteria and viruses and the growing problems such as Deep Vein Thrombosis (DVT) and leg ulcer, it is vital that newer or enhanced medical devices should be developed to cope up the situation. The demand for medical textile products is enormous both in developed and developing countries (Rajendran and Anand, 2006).

Wound management has recently become more complex because of more insights into wound healing and increasing need to manage complex wound healing in order to obtain both functional and cosmetics results. A wound can be defined as a cut or breakin the continuity of any tissues, caused by injury or operation. Modern dressing are designed to facilitate the function of wound

healing rather than just to cover. Wound healing is the body's natural process of regenerating dermal and epidermal tissues which involves a higher orchestered sequence of complex events, resulting in the restoration of the wounded tissue to the normal or quasi-normal state found prior to wound repair (Gupta et al, 2010)

In general, wound dressing pocesses a moist wound environment. This is the key factor to debridement and is obtained by using occlusive or semi-occlusive absorbent dressings.There are a variety of methods that can be used to dress an exuding wound and keep a moist environment. So the healing of a wound depends not only upon medication but also upon the use of proper dressing techniques and suitable dressing materials. The ideal characteristics of a wound dressing include the following aspects;

- Impermeability to water and bacteria
- Freedom from particulate matter
- Thermal insulation
- Absorption and retention of exudates
- Prevention of trauma on removal
- Removal of toxic substances
- Prevention of dehydration
- Allowing for gaseous exchange
- Pain relief and comfort

Modern dressings are required to create the optimal environment for wound healing. They should be easy to apply and can reduce the nursing time with fewer dressing changes and pains of removal with less adherence between wound surface and dressing layer (Yadie and Hu,2015).

Antimicrobial finish in textiles prevent the growth of bacteria, protect health and prevent diseases. Clothing and textile materials are not only the carriers of microorganisms such as pathogenic bacteria, odour generating bacteria and mould fungi, but also good media for the growth of the microorganisms. Among

various functional ability, the antimicrobial property of fabric is being considered to be important with garments, which are in direct contact with human body (Jayapriya et al,2014).

2.3.3. Phytochemical Screening

Phytochemicals are natural bioactive compounds found in plants and are divided into two groups; primary and secondary compounds. These compounds are classified according to their functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consist of alkaloids, terpenoids, phenolic compounds and many more (Krishnaiah et al, 2009). There are several known phytochemicals and are non-nutritive that have protective or disease preventive properties. Plant produces these chemicals to protect itself, and they can also protect humans against diseases (Okigbo et al, 2008).

Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits (Okwu, 2005). They are not essential nutrients and are not required by the human body for sustaining life. The different phytochemicals such as Volatile oils, Alkaloids, Glycosides, Flavanoids, Tannins and Polyphenolic compounds, Carbohydrates, Proteins, Fixed oils and Fats, Terpenoids (Cowan, 1999), found in medicinal plant parts are precursors for the synthesis of useful medicines (Sofowora,1993).

Phenols are a member of a group of aromatic chemical compounds with weakly acidic properties and are characterized by a hydroxyl (OH) group attached directly to an aromatic ring. The simplest of phenols 32 derived from benzene is also known as phenol and has the chemical formula C_6H_6OH. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens (Okwu and Okwu, 2004).

Phenolic compounds may reduce risks of many infectious diseases. The use of traditional medicine mainly derived from plant sources has become an attractive segment in the management of many lifestyle diseases. Plants produce

phenolic compounds as secondary metabolites involved in diverse processessuch as growth, lignification, pigmentation, pollination, and resistance against pathogens, predators, and environmental stresses (Kyselova, 2011).Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Pandey et al, 2009).

Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. They are known to be synthesized by plants in response to microbial infection and have been found *in vitro* to be effective against a wide array of microorganisms (Harborne, 1973). This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the attractive colours of flowers, fruits and leaves. Based on the variation in the type of heterocycle involved, flavanoids may be divided into six subclasses. Flavanoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage (Kyselova, 2011). They have strong anti-cancer activity and protect against all stages of carcinogens. Flavanoids are well known to reduce the risk of heart diseases in patients (Urquiaga and Leighton, 2000).

Alkaloids rank among the most efficient and therapeutically significant plant substances. Some 5,500 alkaloids are known and they comprise the largest single class of secondary plant substances which contain one or more Nitrogen atoms, usually in combination as part of a cyclic structure. For thousands of years, indigenous groups around the world discovered, through self-experimentation with locally available plant extracts, that they could provide materials for hunting prey, culinary enhancement, amelioration from disease, relief of pain, and healing for 200-year period, many alkaloids became critical components of the global pharmaceutical armamentarium, and tremendous healing has resulted from their clinical application (Amirkia and Heinrich, 2014).

Quinones have aromatic rings with two or more ketone substitutions. The natural quinone pigments range in colour from pale yellow to almost black and

there are over 450 known structures of quinones. These compounds are responsible for the browning reaction in cut or damaged fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin.Quinones are of interest from a medical and toxicological perspective due to their unique reactivity and high prevalence in the environment (Madeo et al, 2013).

Tannin is a general descriptive name for a group of polymeric or phenolic substances capable of tanning leather or precipitating gelatin from a solution, and astringency (Harborne, 1973). They are divided into two groups, namely hydrolyzed and condensed tannins. Many physiological activities such as stimulation of phagocytic cells and wide range of anti-infective action have been assigned to tannins (Okwu and Okwu, 2004).

Terpenoid essential oils are the main compounds found in the volatile steam distillation fraction responsible for the characteristic scent, odour or smell found in many plants. Some essential oils possess medicating properties and are used in the pharmaceutical industry (Krishnaiah et al,2009).

Herbs and spices produce these bioactive compounds which react with other organisms in the environment to exhibit antioxidant activity and inhibit bacterial and fungal growth. The majority of the active compounds are phenols, vitamin C, vitamin E, tannins and carotenes (Aqil et al, 2006; Thitilertdecha et al,2008). Sources of natural antioxidants are primarily plant phenol such as flavanoid that exhibit antioxidant, antimicrobial, anti- carcinogenicity and other biological active compounds (Demiray et al, 2009; Mohan et al, 2008; Sengul et al,2009). The substances that inhibit the growth of pathogens and are least toxic to host cells are considered good medium for development of new antimicrobials. The extraction process of phytochemicals in enormous amount by rapid and accurate methods of screening plants for antimicrobial product development (Banso and Olutimayin, 2001) are recently emerging procedures. Many phytochemicals originally rare in occurrence are of almost universal distribution in the plant kingdom contain physiologically active principles that over the years have been exploited in the traditional system of medicine for the treatment of

various diseases (Adebajo et al, 1983). There is a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes (Eshrat and Hussain, 2002).

Phytochemical investigation of crude plant extracts is very important with regard to their potential pharmacological effects. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude extracts, which could be both qualitative and quantitative in nature (Grover et al, 2014).

2.4. Antimicrobial Finishing on Textiles

Functional finishes of textile materials can be defined as a process of providing functional properties to textile and clothing materials. Functional properties can be obtained either by:

- The fibre itself (characteristics of the polymer or additives before fibre spinning)
- Yarn, fabric or material construction (for instance, with different fibres or different layers)
- Textile finishing.

In many cases, the functional properties involve a surface modification, which can be obtained by means of chemical modification, by applying of a surface layer or by more ecological friendly treatments such as the use of enzymes or physical modification. Some of these properties were developed mainly for “protective” clothing but nowadays they are often present in functional textiles used for “normal” clothing. Many fabric producers are devoting more and more attention to try to put into the market products with new effects that can represent an important added value (De Almeida et al, 2005).

The finishing technologies employed in textile treatments are based on direct incorporation or reaction or binding of functional agents, UV blockers, water

or oil repellents. Recently, these functional finishes are being developed and applied after appropriate modification to enhance activity on textiles (Gulrajani 2013).The functional finishes are classified into Stabilization, Durable press, and Soil release finish. Appearance retention, Abrasion resistance, Anti fibre-shedding, Carpet back coating, Crease-resistance, Comfort-related, Water-proof, Hydrophilic, Softening, Rot-proof, Biological-control, Antimicrobial, Aroma, Flame-retardant, Safety-related, Water-repellent and UV protection finish (Pan et al, 1997; Prayag 1994 and Samantha 1994).

2.4.1 The Need for Antimicrobial Textiles

An important and growing part of the textile industry consists of the medical and related healthcare and hygiene sectors. A hospital contains an enormous amount of textiles with the added threat of high volumes of traffic. Because of the continuous flow of people, particularly those with infectious diseases, both patients and employees are at risk of cross transmission of diseases and other health issues. The increasing rate of drug-resistant bacteria also heightens the importance of finding safe and durable antimicrobial finishes (Chinta, 2013)

The increasing demand for comfortable, aesthetic, durable, functional, and safe textile products dictates the development of new and contemporary techniques of processing and designing textiles (Tomsic et al, 2008). Bacteria and fungi are microbes that can grow on textiles.Garments of healthcare workers are a significant contributor to the spread of infections since they are easily contaminated. Moreover, it has been shown that bacteria can grow and survive on fabrics commonly used in healthcare environments for more than ninety days, contributing to the transmission of diseases (Appidi et al, 2008).

The word Antimicrobial is a general term for any product that kills or controls microbes (Srikanth, 2010).Antimicrobial finishing causes a fabric to inhibit the growth of microbes in textile materials (Kadolph and Sara, 2007).Antimicrobial finished fabrics are important not only in medical applications but also in terms of daily life usage (Erdem and Yurudu, 2008).Common problems in hospital and

healthcare institution is microbial contamination of surface, including textile fabrics, which can lead to infection and cross infections. Hence, it is extremely important that protective clothing and hospital linens meet the demand of antimicrobial protection (Ristic'et al, 2011). Antimicrobial finishing of textiles has become extremely important in the production of protective, technical and decorative textile products. This has provided opportunities to expand the use of textile applications in medical, pharmaceutical, engineering, food and agricultural industries (Simoncic and Tomsic, 2010). The antimicrobial fabric gained significant momentum in the recent past due to its wide acceptance as surgical apparel, baby clothing and undergarment etc. There has been a growing need to impart antimicrobial and infection resistant properties into textiles such as inner clothing which comes into direct contact with human skin because the growth of microbes on it may negatively affect the wearers as well as the textile itself (Yi and Yoo, 2010).

Substances added to the fibre, such as natural-based auxiliaries, lubricants, antistats, and dirt provide a food source for microorganisms. Cotton is more likely to be attacked by fungi. Hence, healthcare is a serious business which is not only influenced medical professionals but also by the manufacturers of diversed medical products (Erkan and Merih, 2004).

Textile materials found different end uses in medical and healthcare application depending on the specific endurance performance (White et al, 2010). Irrespective of the end application of medical textiles,the material should possess basic bio active properties especially antimicrobial. Antimicrobial finishes are currently being used on disposable, nonwoven textiles for the medical industry. Currently, testing is being done to find safe and efficient antimicrobial finishes for woven fabrics (Harrison, 2002). Although people have used natural materials agents to combat diseases for millennia, only in the twentieth century, people started to produce antimicrobial compositions and add them to textile materials (Ramchandran et al, 2004).

2.4.2. Application of Antimicrobial Agents

To impart an antimicrobial ability to textiles, different approaches have been studied, being mainly divided into the impregnation of antimicrobial agents in the textile polymeric fibres or on the polymer surface. Regarding the antimicrobial agents, different types have been used, such as quaternary ammonium compounds, triclosan, metal salts, polybiguanides or even natural polymers. Any antimicrobial treatment performed on a textile, besides being efficient against microorganisms, must be non-toxic to the consumer and to the environment (Morais et al, 2016).

Antimicrobial properties are given to textile materials by various application methods such as by using spun in additives padding, spraying, polymer modification and microencapsulation (Landage, 2012).

Poly herbal extractions are known to express high effectiveness in a vast number of diseases. The therapeutic effect was easily obtained due to the presence of different phytochemicals and the effects are further potentiated when herbals are formulated together in poly herbal extractions. Till date, many researchers had conducted studies on poly herbal extracts to evaluate their effectiveness against various microorganisms (Srivastava et al*,* 2013).A survey study performed in UK noted that the main reason underlying in the use of medical herbs in polyherbal combination has produced effective and favourable outcomes of the treatment (Parasuraman, 2014). Poly herbal extractions are usually found to have wide therapeutic range of application. Most of them are effective even at a very low dose and safer at high dose. Thus, they have minimal risk to and maximum capacity to cure the disease ratio (Joshi, 2000).

2.4.3. Antimicrobial Finishing

The medical textile industries have always played an important role in the protective aspects of fabrics. The fabrics have long been recognized as a good support medium for the growth of microbes. A microbe on textile causes the unwanted effects to both the wearers and textile itself. The negative factor of the microbes has resulted in the development of innovative and hygienic finishes on textiles. The consumers are also demanding for the hygienic clothing which

resulted in antimicrobial textile products. Antimicrobial finish prevents the growth of bacteria. Anti-microbial textiles with improved functionality find a variety of applications such as infection control and barrier control (Rajendran et al,2016).

2.4.4. Antimicrobial Finishing Methods

A major factor that has stimulated interest in antimicrobial finishes using textiles with improved functionality are a variety of health care applications. Antimicrobial finishes using natural source have been the current vogue that promotes natural and eco-friendly life style. Natural products can be selected for biological screening based on medicinal use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today they continue to play a major role in primary early care as therapeutic remedies in many developing countries (Sumathi et al, 2015).

The antimicrobial agents can be applied to the textile substrates by pad and dry curing, exhaust, spray and foam techniques and coating. The substances can also be applied by directly adding into the fibre spinning dope. It is claimed that the commercial agents can be applied online during the dyeing and finishing operations. A variety of methods for enhancing the durability of the finish include:

- In-solubilisation of the active substances in or on the fibre.
- Treating the fibre with resin, cross-linking agents or condensates.
- Micro encapsulation of the antimicrobial agents with the fibre matrix.
- Fibre surface coating.
- Chemical alteration of the fibre by covalent bond formation.

Use of graft polymers, homo polymers and co-polymerization on the fibre (Gopalakrishnan, 2006).

2.4.4.1. Dip and Dry Method

The application of the finish is now extended to textiles used for outdoor, healthcare sector, sports and leisure. Herbal products seem to possess moderate efficacy with no or less toxicity and are less expensive as compared with synthetic drugs. In dip and drying method, the fabric is dipped into the bath containing herbal extract for half an hour at room temperature and then the garment is dried

in the room temperature. This method is called as dip dry method (Sumithra and Raaja, 2014).

2.4.4.2. Exhaust Method

The exhaust method can be followed for coating fabric. The fabric can be mordanted prior to dyeing. The treated fabric was introduced to the plant extracted solution. Exhaustion application is also done in the jigger drum etc. Pad and exhaustion application are noted to be permanent application (Malik et al, 2011). Among various methods tested, exhaust coating was found to be more effective for antimicrobial finishing (Mahesh et al, 2011).

2.4.4.3. Microencapsulation

Microencapsulation may be defined as a micro packaging where in active core material is encapsulated in a polymer shell of limited permeability. It is a process in which tiny particles or droplets are surrounded by a coating to form small capsules, containing of many useful properties. In general, it is incorporated in food ingredients, enzymes, cells or other industrial based products too on a micro metric scale (Singh et al,2010). Moreover, it is the formation of a barrier to avoid chemical reactions and to enable the controlled release of the ingredients (Vilstrup, 2001).

The micro encapsulation technique was brought to use for the first time in 1940 by B.K. Green, for the production of No Carbon Paper - NCR (Alat and Sarat ,2005).The development of micro encapsulation products started in the 1950s with research into pressure–sensitive coatings for the manufacture of carbonless copying paper. The textile industry has, however, been slow to envisage applications for innovative micro encapsulation techniques. Micro encapsulation technologies offer many opportunities to improve the properties of textiles or to give them new functions (Dixit et al, 2006).

The objective of this technology is either to protect the active core material from the external environment until the right stimulus is encountered. In this technique, tiny droplets of benefit laden products such as moisturizers, fragrances, deodorizers,vitamins or repellents are packed in microscopically small

capsules. It is vital that microencapsules are stable and durable (Ramalingam and Subramanian,2006).

The term microencapsulation is appropriate since, the particles are very small the particle sizes between 3-800 mm are known as microcapsules or microspheres (Achwal, 2003). The microcapsules are produced by depositing a thin layer of polymers on small solid liquid particles, or dispersion of solid in liquid. The core materials in the shell may be released by friction, pressure, diffusion through the polymer walls or by the diffusion of the polymer coating. (Jin et al, 2008)

The following are the characteristics of microcapsules (Saravanamuthu,2010).

- Microcapsules can be made in size ranging from few microns to thousand microns in diameter or even larger.
- Rate of release from microcapsules depends largely on the polymer wall structure, which in-turn is influenced by the conditions employed in the preparation
- Microcapsules show good thermal stability
- Higher is the ratio of loading fraction, the better is the efficiency.

The effect of microencapsulation is based on the selection of methods (Nelson,1991); such as core and wall of the polymer, wall thickness, wall permeability, particle size range and release mode of the contents etc. Manufacturing methods for microencapsulation can be categorised namely chemical encapsulation and mechanical encapsulation. In chemical encapsulation, the production and isolation of capsules is done in a liquid medium and involves chemical or phase change separation (Gomez and Genovez, 1997). The mechanical encapsulation technique is characterised by continuous operation, restricted diversity in size of the nucleus particle and there is a need for specialized mechanical equipment like spray dryer for the formation and isolation of capsules (Gomez and Baptista, 2001).

There are several other types of microencapsulation techniques such as the centrifugal extrusion process. Pan coating method and Air suspension coating (Aggarwal et al,1998), Spray-drying (Mauriello et al, 1999), Hot melt encapsulation (Erkan et al, 2004), Interfacial polymerization (Ziegler, 1951) and Coacervation technique (Lazko et al, 2004).

Microencapsulation was developed as a technique over sixty years ago, but it is only over the last decade that it has become common in textile dyeing, printing and finishing (Ian, 2003). It has attracted the interest of the dyeing, printing and finishing in textile wet processing method, for the last decade (Bairagadar and Katkar,2009). The microcapsules can introduce important new qualities to garment and fabrics, such as stability and controlled release of active compounds. Microencapsulation is a unique technique which facilitates the controlled release of the finishing as and when required and also enhance durability (Chinta et al, 2013).

2.4.4.4. Nanoencapsulation

Nano technology is defined as the art and science of manipulating matters at the Nano-scale to produce novel and unique materials and products (Butola and Mishra, 2007). It is also defined as the use of structures with at least one dimension of Nano-meter size for the manufacture of devices, materials, or systems with new or significantly enhanced properties due to their Nano-size. It offers great opportunities in all fields of science and technology, textiles, material science, electronics, mechanical, optics, energy, medicine, and aerospace (Cho et al, 2006).

The particle size below 1mm are known as Nano particles (Jin et al, 2010).In 1974, Nario Taniguchi coined the term nanotechnology for management of submicron particles.Nature has created the building blocks of life in nanoscale such as DNA, RNA, amino acids, sugars, and hormones (Weiss et al, 2006). Inspired by nature's creation, man has engineered nanomaterial for the progress and well being of mankind. In 1959, Richard Feynman proposed the concept of nanostructures, The term "nano" refers to a magnitude of 10^{-9} m (Quintanilla et

al,2010). Nanotechnology has emerged as one of the most promising scientific fields of research for decades. It deals with the production, processing, and application of materials with sizes less than 1,000 nm (Sanguansri and Augustin, 2006). Additionally, nanotechnology has also improved the solubility nature of water, heat stability, and oral bio-availability of bio-active compounds (Huang et al,2010, McClements et al, 2009 and Silva et al, 2012).

Nanotechnology has become an umbrella term for a wide range of processes and technologies that can manipulate or exploit materials with an organized structure at the nanometer scale (Maskayet al, 2006). The influence of Nano technology in the textile finishing area has brought up novel and innovative finishing application technique. It has the potential to generate novel bulk materials with new properties in textile coating and finishing (Schmitt and Benjamin 2008). Discrete nanoparticles or molecules of finishes can ideally be brought individually on selected spots of textile materials in a specific alignment and trajectory through electrostatic, thermodynamic, or other technical methodologies. Gulrajani, studied and concluded that nanotechnology is making significant involvement in the field of textiles. The five main areas are Nano colouration, Nano finishes, Nano fibre, Nano filteration, and Nano composites. The types of Nano finishes are given below:

- Hydrophobic Nano finishes
- Self-cleaning Nano finishes
- Photocatalytic self cleaning
- Antimicrobial finishes (Gulrajani, 2006).

The use of nanotechnology has increased rapidly in the textile industry and also nanotechnology has real commercial potential for the textile industry. The fact that conventional methods used in fabrics finishing never lead to long-lasting effects, and will lose their functions after laundering or wearing is one of the main reasons for increased use of nanotechnology in the textile industry. Nanotechnology can deliver high durability for fabrics, since Nano-particles have high surface energy and a large surface area-to-volume ratio, thus offering

improved affinity for textiles and leading to an increase in durability of the function. Also, Nano-particles coating on fabrics will not affect their hand feel or breathability (Wong et al, 2006).

According to Yadav et al (2006), coating of nano-particles on the surface of fibre or fabrics is one of the methods to create high active surfaces with distinctive properties and also to attain high durability function for the fabrics. Nanotechnology offers new and enhanced means of imparting a range of functional performance in the fabrics. In fact, textile industry is one of the first manufacturing industry to come up with finished products that are improved through nanotechnology based functional finishing (Radhakrishnaiah, 2005).

Various properties imparted to textiles using nanotechnology include wrinkle resistance, water repellence, anti-bacterial effect, soil resistance, anti-static, UV-protection, flame retardation, improvement of dye ability, electrical conductivity, photo catalytic ability, photo oxidizing capability against biological and chemical species, UV absorption, self-decontaminating and blocking functions for both military and civilian health products and so on. Nano metal oxides such as Al_2O3_3,TiO_2, ZnO, SiO_2, MgO and ceramics are used in textile finishing for modifying the surface properties and imparting functional properties (Mahltig et al, 2006).

In recent years, more attention has been given to the potential application of innovative technologies, especially nanotechnology, the wave of future, as well as the application of smart nano-materials (Dastjerdi and Montazer,2010). Following are the applications of nano materials;

- Enhancing the performance and functional properties of the current textile products
- Developing smart and intelligent textiles with novel functions
- Satisfying the growing needs of textile users for hygienic clothing and active wear
- Allowing for great opportunities and options to develop innovative textile processes and products with high-value added

The roles of green chemistry in nanotechnology and nano science fields are very significant in the synthesis of diverse nano-materials and synthesis of silver nano-particles using herbal extracts.There is a growing need to develop environmental friendly processes for nano-particle synthesis that do not use toxic chemicals. Therefore, demand for an environmentally sustainable synthesis process has led to a few biomimetic approaches. Biomimetics refers to applying biological principles in materials formation. One of the fundamental processes in biomimetic synthesis involves bioreduction. Biological methods of nano-particle synthesis using microorganisms , enzymes , fungus , and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods. Sometimes the synthesis of nano-particles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures (Patel et al, 2014).

2.5. Antimicrobial Assessment

Antimicrobial nature of plant origin have enormous therapeutic potential and have been used since time immemorial (Sharma and Kumar,2008). The use of natural products and search for drugs derived from plant phytochemicals with good therapeutic properties is as ancient as human civilization and for a long period of time, mineral, plant and animal products were the main sources of medicines (De Pasquale,1984). The antimicrobial activity of plants has been attributed to the presence of some active constituents in the extracts.The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases (Chaman et al, 2013). Antimicrobial activity for herbal extracts has been deliberated in a variety of research works (Mohanasundari et al, 2007). Various test procedures have been used to evaluate the antimicrobial activity (Ramachandran et al, 2004).

2.5.1. Agar Well Diffusion Assay

The determination of antimicrobial susceptibility testing has been done using the agar well diffusion method to detect the presence of anti-bacterial or anti-fungal activities of the plant samples, A sterile swab was used to distribute

bacterial and fungal culture evenly over the appropriate medium prepared. A standard caliper was used to measure the zone of inhibition. The antimicrobial activity was thus determined qualitatively by antimicrobial well diffusion method (Delahaye et al, 2009).

In Agar Well Diffusion Method, a cork borer is Sterilized by auto claving or by rinsing in alcohol followed by sterile water. Nutrient agar plate and Potato Dextrose Agar (PDA) plate is prepared. Aseptically punch (4mm) holes in the agar using a cork borer. Using a wax pencil, mark the underside of the Petri plate to label the wells. Cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. *Pseudomonas aeruginosa, Staphylococcus saprophyticus, Escherichia coli and Aeromonas hydrophila* were swabbed on Agar plates and *Candida albicans* was swabbed on PDA plates.

Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. Then the extracts of respective source (60 µl each) were added in 2 wells of petri plates. The ethanolic solvent was used as control whereas streptomycin and nystatin was used as reference for bacterial and fungal species respectively. The plates were incubated at 37ºC for 24 hrs. The zones of inhibition were measured in millimeters, using a ruler on the underside of the plate. The zone size was recorded (Chaman et al, 2013).

2.5.2. AATTC 147-2004

The Parallel Streak Method (AATCC 147- 2004) has filled a need for a relatively quick and easily executed qualitative method to determine antimicrobial activity of diffusible and non-diffusible antimicrobial agents on treated textile materials. In the “classical” Parallel Streak Method (for diffusable agents), the agar surface is inoculated making it easier to distinguish between the test organism and contaminant organisms which may be present on the unsterile specimen. The Parallel Streak Method has been proven effective over a number of years of use in providing evidence of antimicrobial activity against both Gram positive and Gram negative bacteria. A modified Parallel Streak Method can be used to evaluate the antimicrobial activity of non-diffusable agents. Thereby, a piece of

textile is pressed onto an agar plate and the test bacteria are inoculated over the specimen by three or four parallel streaks (Binovation, 2010).

The utilization of antimicrobials dates back to ancient Egypt and these were used in the preservation of mummies. The initial antimicrobial textile material, in recent history, was developed by Lister in 1867.The recipe follows the procedure: from the inoculums of *Staphylococcus sapropyticus, Areomonas hydrophila, Pseudomonas aeroginosa* and *Candida albicans* using a 4 mm inoculating loop, one loopful of the inoculums was transferred to the surface of agar plates, making five parallel streaks on the central area of a plate without a refilling 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 hr 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 (Mishra et al, 2016).

2.5.3. Wound Scratch Assay

Plants produce a diverse range of bioactive molecules and secondary metabolites that make them rich sources for different types of medicine. Many medicinal plant species present worldwide are used in the traditional medicine as the treatment for skin diseases caused by fungi and bacteria (Chanda et al, 2010). Presently scientists are keenly working on to evaluate the cure from plant origin. It is due to their high specific healing properties, healing nature, non-toxicity property and cost effectiveness, several plants and their products are used in folk medicine in treatment of wounds and have been reported to promote healing (Bagali et al, 2006).

Wound is a substantial trauma where the skin is torn, cut or punctured. By exposing to air, opportunistic and accidental microorganisms entering the site of the wound, which leads to wound contamination and finally development of infection. Dermal wound is a common pathologic condition and shall be defined as any break in the integrity of the skin. It is associated with high degree of morbidity

due to blood loss, pain, edema, inflammation and loss of functionality. Cut wounds are majorly characterized by migration and proliferation of cells like fibroblastic cells, endothelial cells, deposition of connective tissue, angiogenesis, re-epithelization and finally reducing the size of the wound (Patil, 2010).

The wound healing assay is a powerful tool, which allows the researcher to study cell migration and cell interactions in an *in-vitro* laboratory setting. In some cases also single cell migration can be analyzed. Cell migration plays an important role in many complex physiological and pathological processes. This is also called a scratch assay because it is done by making a scratch on a cell monolayer and capturing images at regular intervals by time lapse microscope (Rodriguez et al, 2005).

Extent of wound healing was determined by the cells migrating into the denuded area. Healing of wounds involves the activity of a complex network of blood cells, cytokines and growth factors, resulting in re-establishment of normal skin tissue condition. The interest in evaluating the utility of plant extracts for wound healing process has been increased during the last decades. The significance of plant secondary metabolites as potential agents that interfered through various wound repair stages has been confirmed, both *in vitro* and *in vivo* (Ariano et al, 2005).

In addition fibroblasts also plays a critical role in generating immune response to a tissue injury. They are the important players in initiating inflammation response in the presence of invaded microorganisms. They induce chemokine synthesis through the presentation of receptors on their surface. Immune cells then respond and initiate a cascade of events to clear the invasive microorganisms (Smith et al, 1997). Receptors on the surface of fibroblasts also allow regulation of hematopoietic cells and provide a pathway for immune cells to regulate fibroblasts.The wound healing effect of the polyherbal extract was analyzed by *In vitro* Wound scratch assay in fibroblast cell lines. A fibroblast is a type of cell that synthesizes the extracellular matrix and collagen, the structural frame work (stroma) for animal tissues, and plays a critical role in wound healing.

Fibroblasts act as the most common cells of connective tissue in animals. Cell proliferation was monitored at different time points such as 1, 4, 12, 24 and 72 hours and images of the migrated cells were observed under the inverted phase contrast microscope (Liang et al, 2007).

The analyzed and exploration of coating herbal medicine in the wound dressing materials, various extracts of the medicinal substances from different plants sources were applied on the cotton fabric and their evaluation for antimicrobial property carried out. The results confirmed that extracts impart excellent antimicrobial property when applied alone as well as in polyherbal combination (Khurana et al,2016). The medicinal properties of the herbal extracts by wound scratch assay in the fibroblast cell lines concluded that this can be used as a promising scientific approach and platform to differentiate between plant extracts known for their wound healing and their anti-inflammatory properties (Fronza et al,2009). Further concluded, the polyherbal formula demonstrated high potential as therapeutic agent in wound healing (Gaspar et al, 2015).

2.5.4. Fourier Transform Infrared Spectroscopy (FTIR)

Spectroscopy has emerged as one of the major tools recently for biomedical applications and has made significant progress in the field of clinical evaluation. FTIR spectroscopic studies can lead to significant improvements both in the quantity and quality of research and their outcomes. FTIR mainly deals with non-aqueous samples (Movasaghi et al,2015).

Infrared Spectroscopy or FTIR is a standard method of analytical pharmacy and chemistry which provides the images vibration of the atoms in the compound. Therefore, it is also referred to as vibrational spectroscopy. IR (Infra Red) spectrum is obtained by passing infra red radiation through the sample and determining the fraction of the incident radiation that is absorbed at a particular frequency. Fourier transformation is a mathematical operation demonstrated by 'Jean Fourier' which converts the frequency domain into time domain. The instrument consists of a interfero meter, fixed mirror, a movable mirror, beam

splitter. A beam emitted by a source is split into two by the beam splitter, 50% of the incident radiation will be reflected to one of the mirrors while 50% will be transmitted to the other mirror. The two beams are reflected from these mirrors, returning to the beam splitter where they recombine and interfere to give constructive interference or destructive interference, depending on the difference in the optical paths between two arms of interfero meter. The signal is then recorded by the detector (Dole et al, 2011).

FTIR-ATR (Attenuated Total Reflection-Infrared Spectroscopy) has been used extensively in textiles for the analysis of the coated surfaces of functional textiles (Meilert et al, 2005). Studying the surface chemistry of the photo catalytic self-cleaning cotton by coating TiO_2 was done using ATR-IR. The surface of polyester grafted with acrylic acid has been characterized using ATR-IR (Kawase et al, 1991). There were also reports of the use of Attenuated-Total-Reflectance (ATR) FTIR spectroscopy for the identification of cellulosic fibre and characterisation of their state of degradation (Garside and Wyeth, 2007). The speed and sensitivity of the FTIR spectroscopy allows rapid analysis of micro-samples down to the nanogram level, making the FTIR unmatched as a problem-solving tool in organic analysis. The FTIR microscope accessory allows spectra from a few nanogram of material to be obtained quickly, with little sample preparation, resulting in more data at lower cost. In some cases, thin films of residue are identified with a sensitivity that rivals or even exceeds electron or ion beam-based surface analysis techniques. This has enabled a wider use of this in textile field (Shaikh et al, 2014).

2.5.5. SEM Analysis

Scanning Electron Microscope (SEM) is used to identify morphological structure of fibre, yarn or fabric. SEM assessment is also used to identify the uniformity of finishing above the specimen. The photographic images taken from SEM analysis of microencapsulated, nanoencapsulated and washed samples were observed under different magnifications. Due to their small size in nature, a

nanoparticle demonstrates original material properties, which are extensively different from those of their bulk counterparts (Bindhu et al, 2016).Advances in microscope technology have improved the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes in herbal drug standardization (Singaravelu et al, 2007)

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

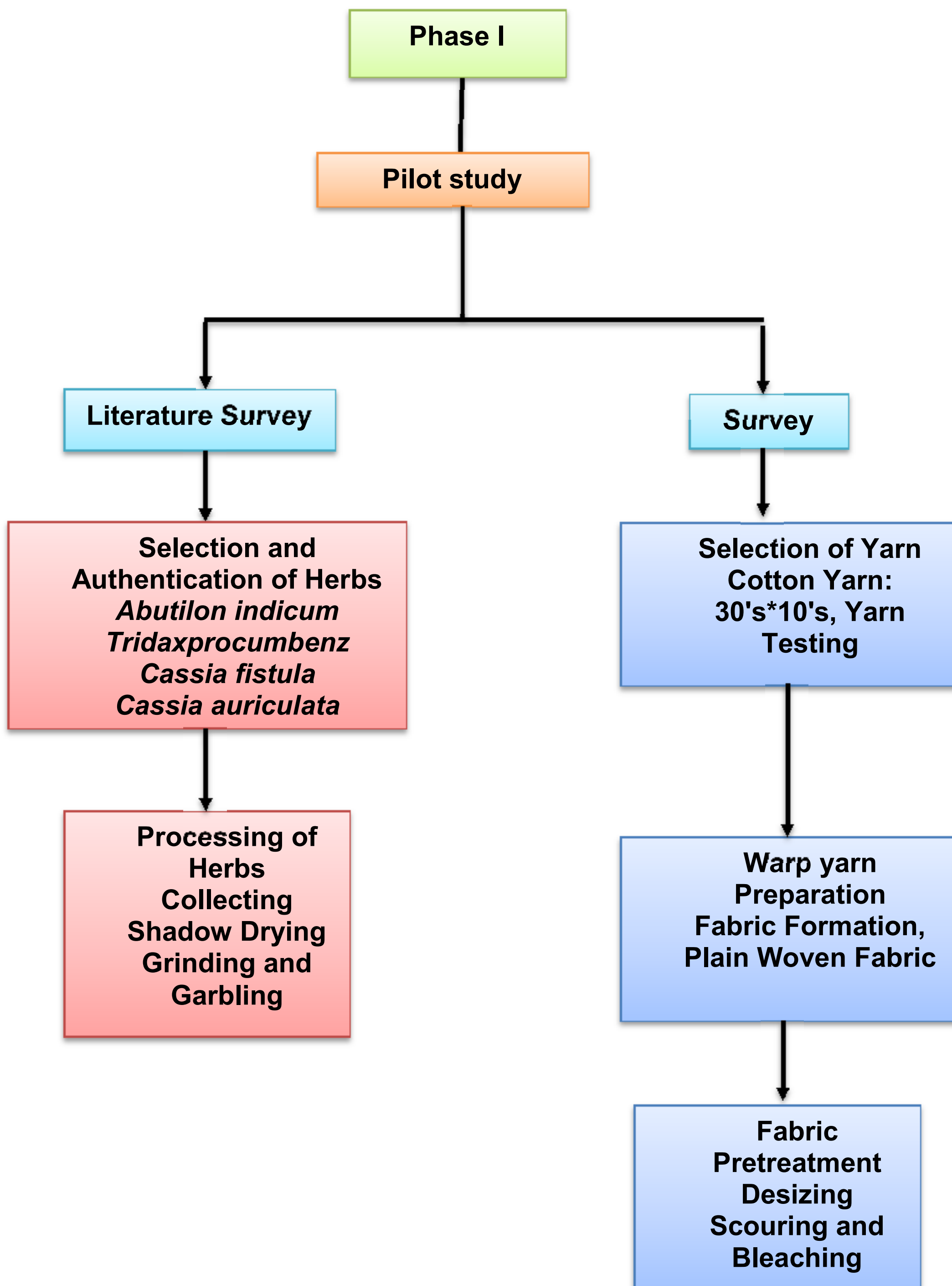
3.14 Product Development and Evaluation

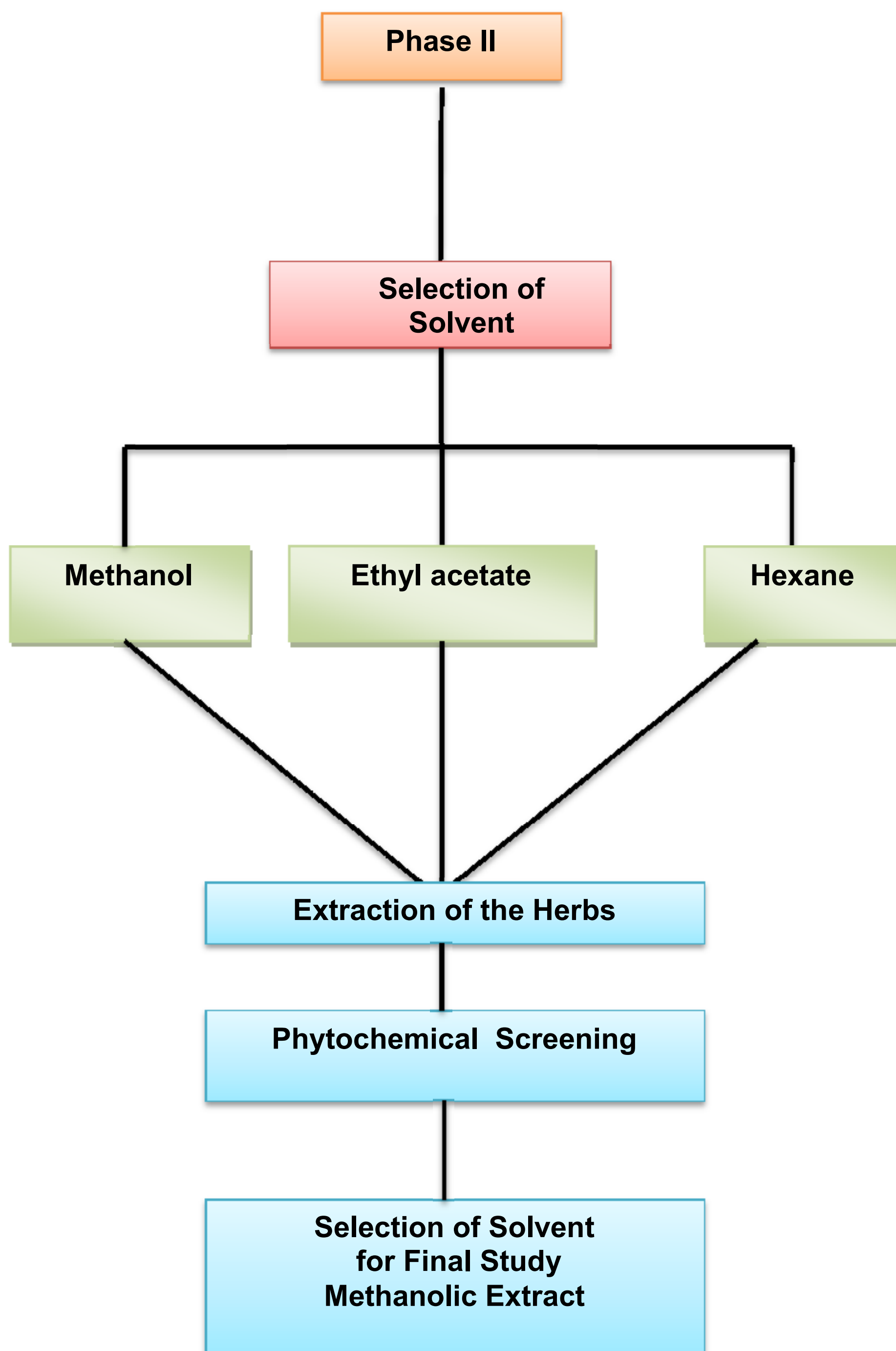
3.14.1 Microbial Filteration Test

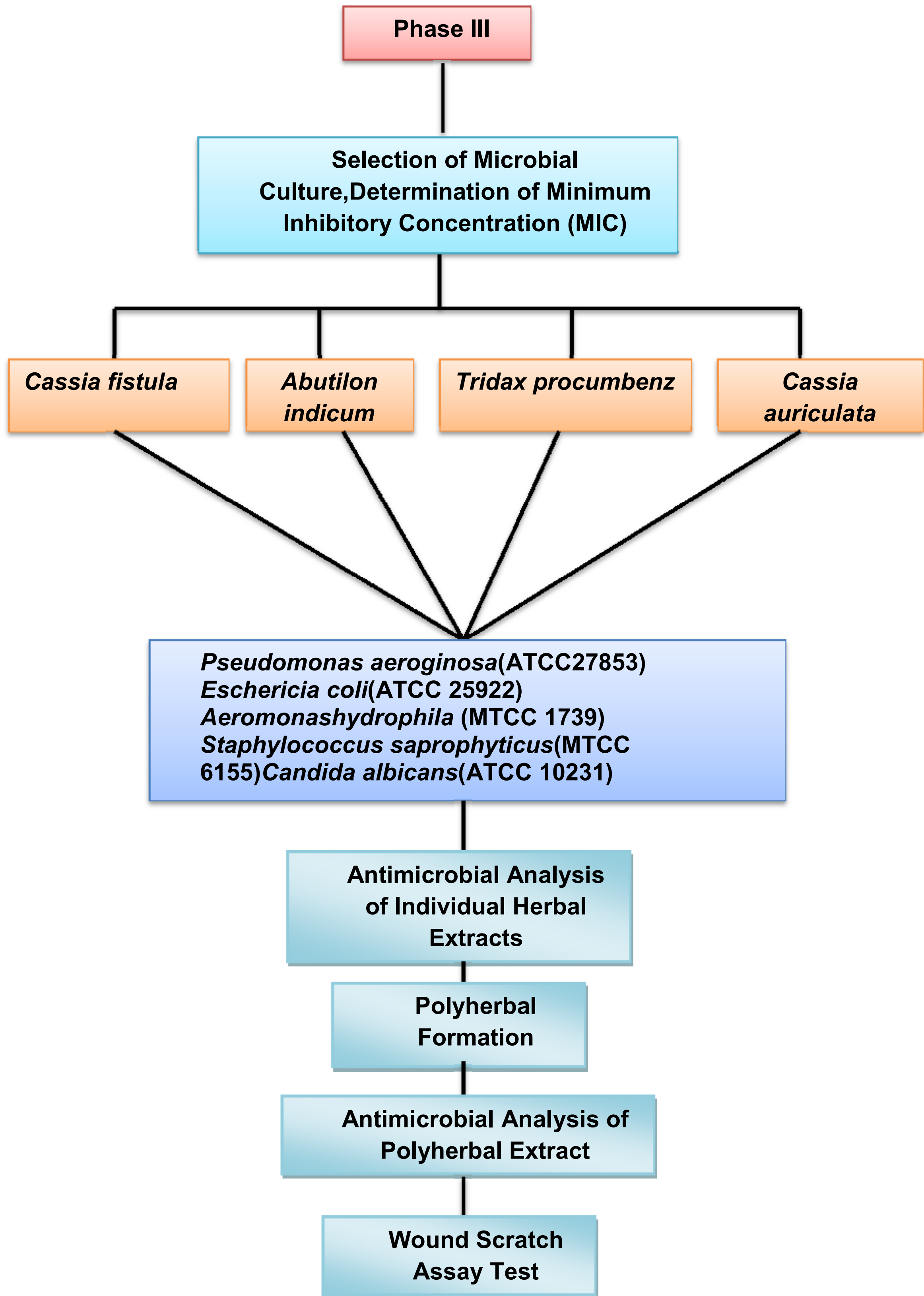
3.14.2 Bandaids Toxicity Test

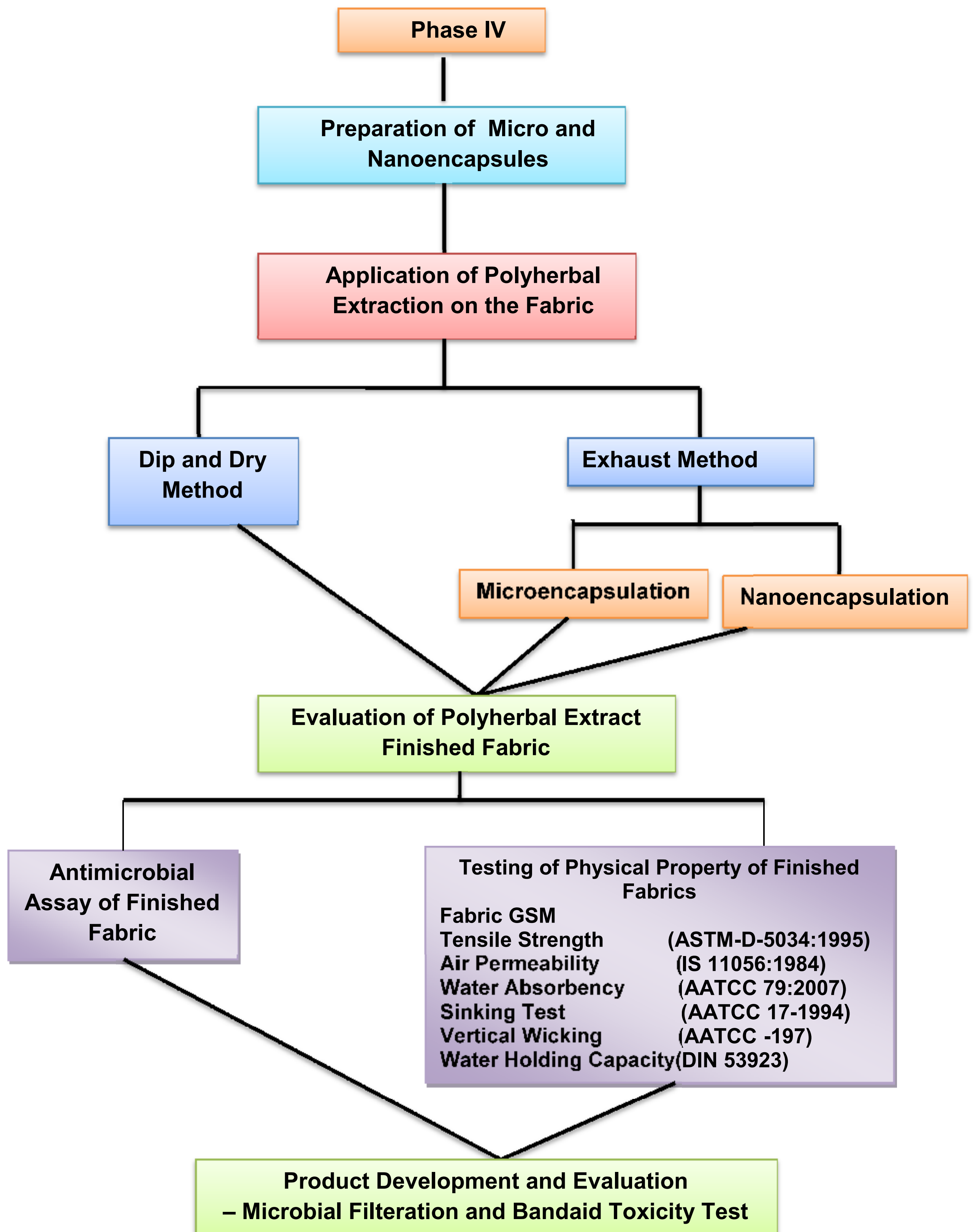
3.15 Statistical Analysis

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

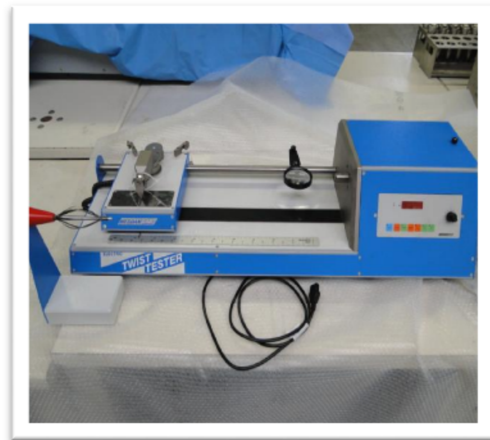


Plate VIII Yarn twist tester

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±2°c. 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±2°cwas 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/D1422M)	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 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.

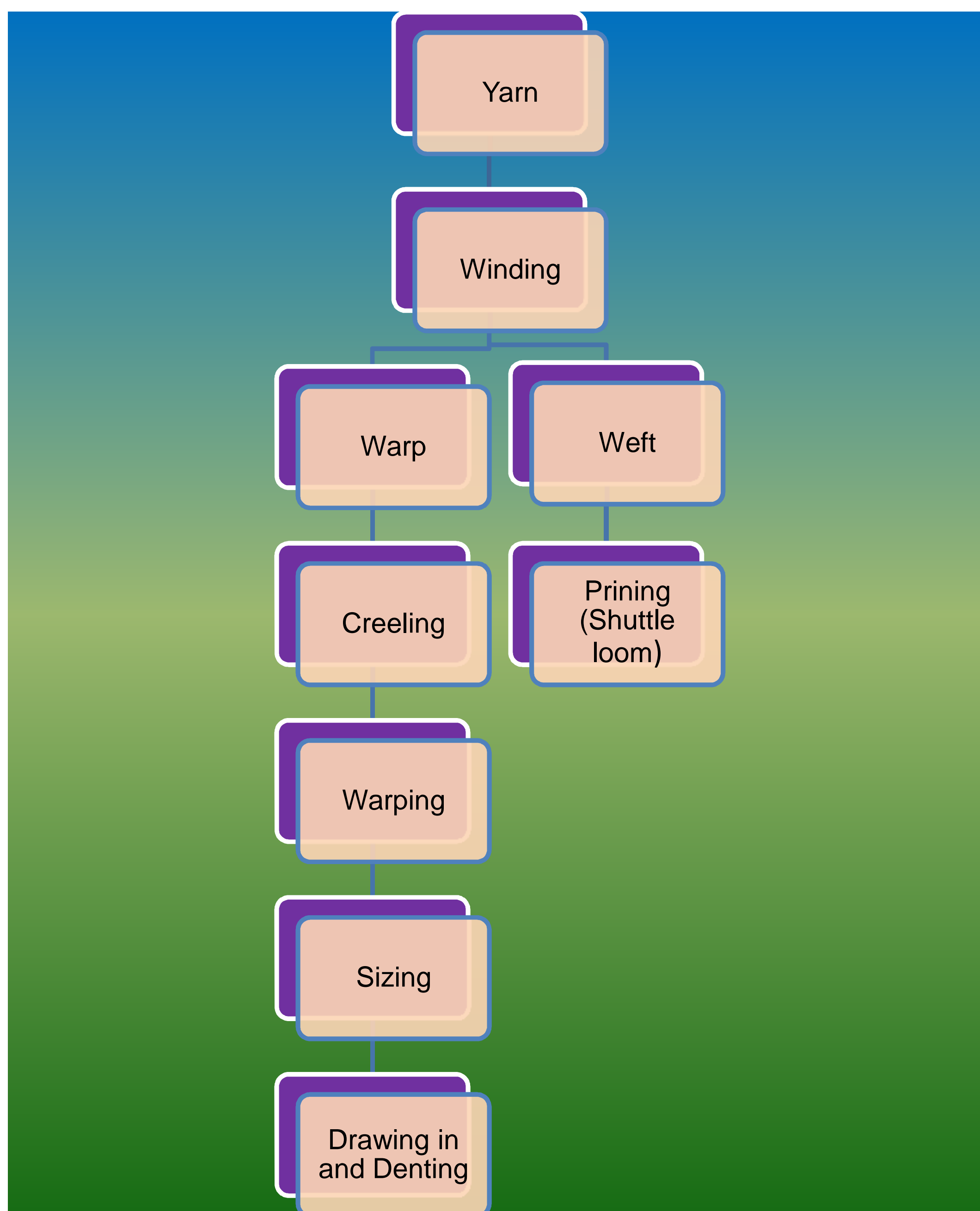


Figure I

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	30ºc
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	80ºc
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*

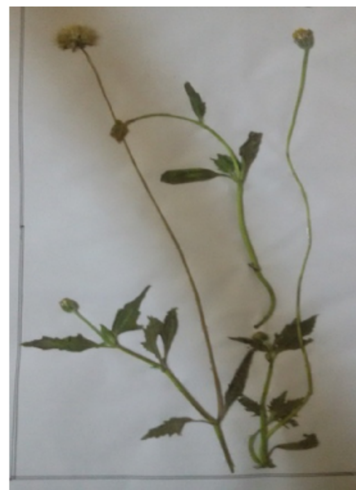


Plate XIII *Tridax procumbenz*

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

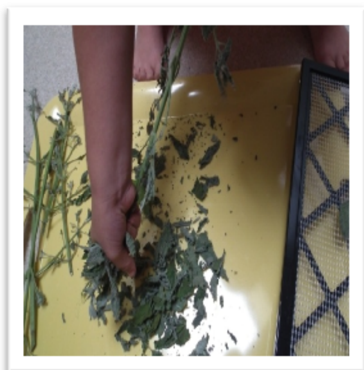


Plate XVII Garbling

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*

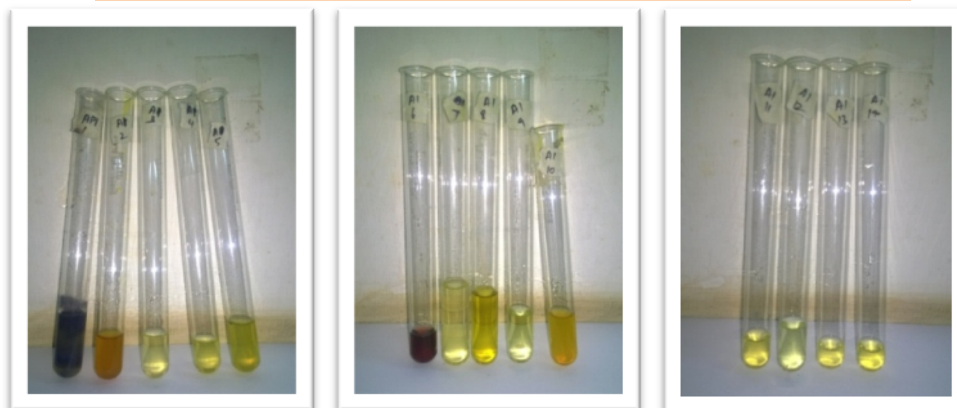


Plate XX (a) Hexane Extract of *Abutilon indicum*

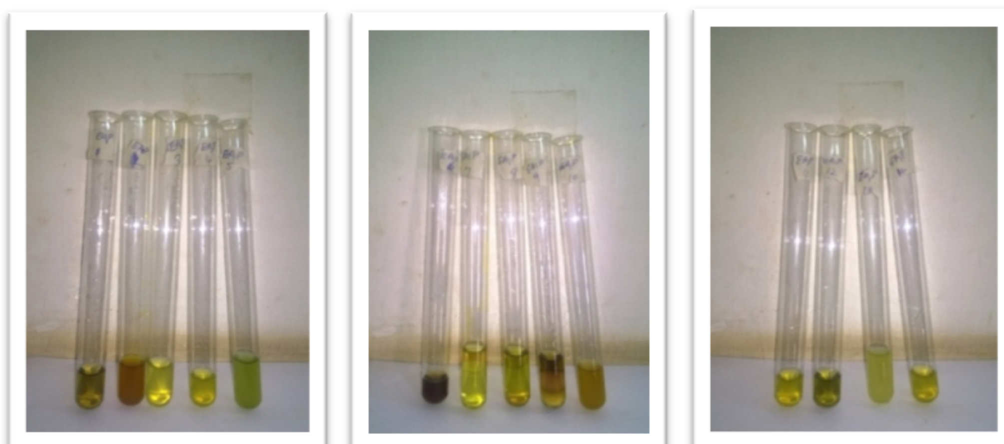


Plate XX (b) Ethyl acetate Extract of *Abutilon indicum*

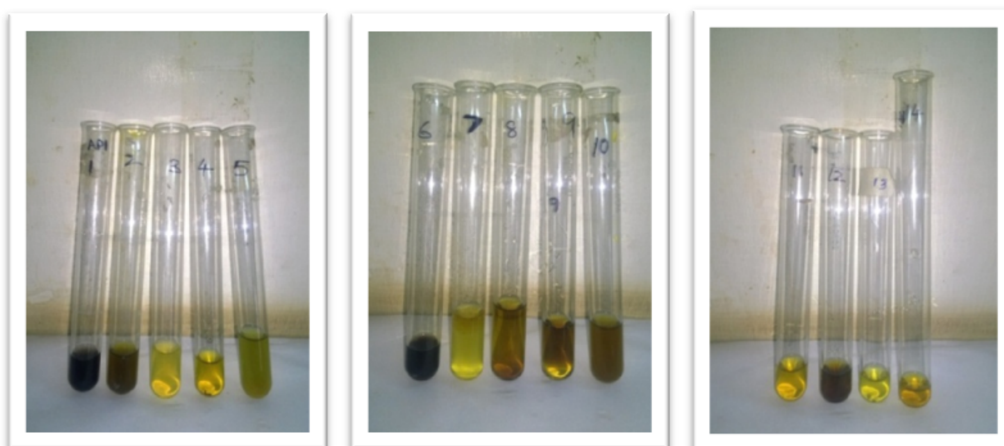


Plate XX (c) Methanol Extract of *Abutilon indicum*

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*

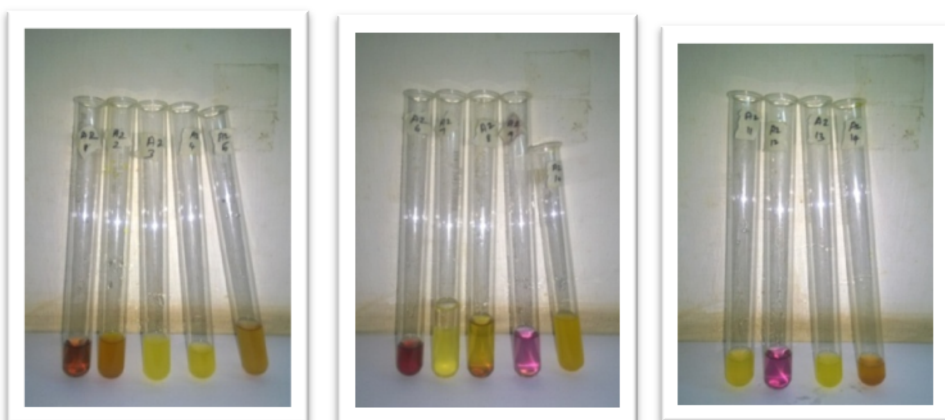


Plate XXI (a) Hexane Extract of *Cassia auriculata*

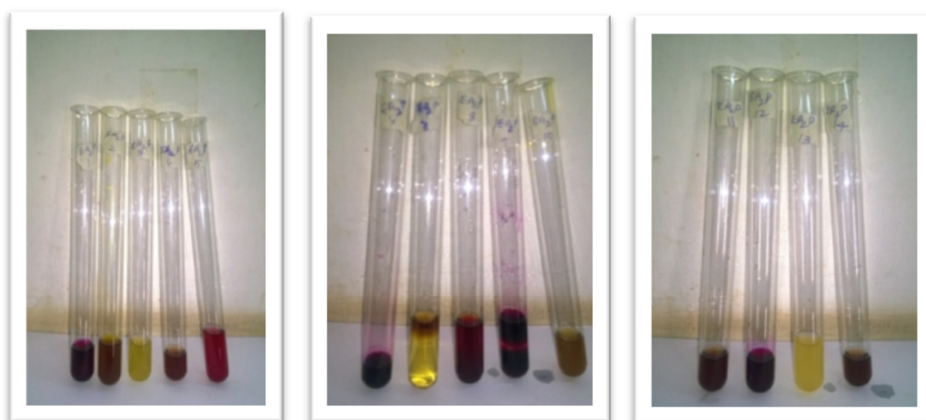


Plate XXI (b) Ethyl acetate Extract of *Cassia auriculata*

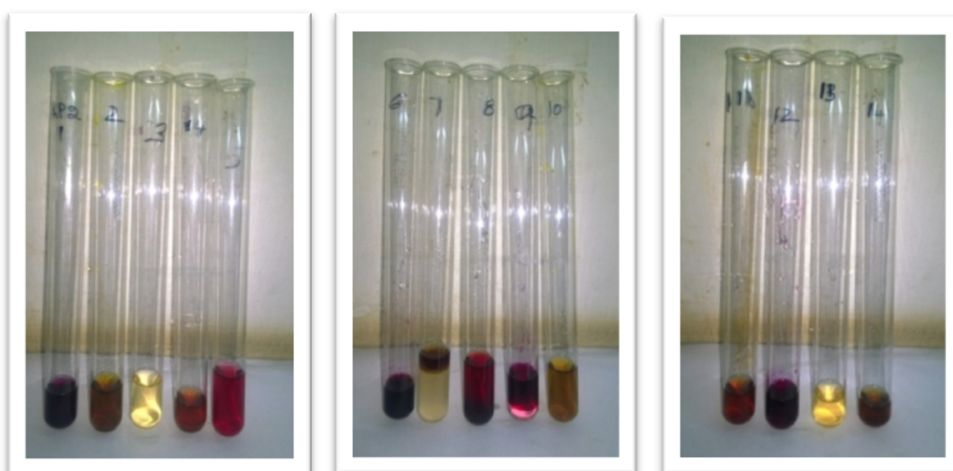


Plate XXI (c) Methanol Extract of *Cassia auriculata*

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*

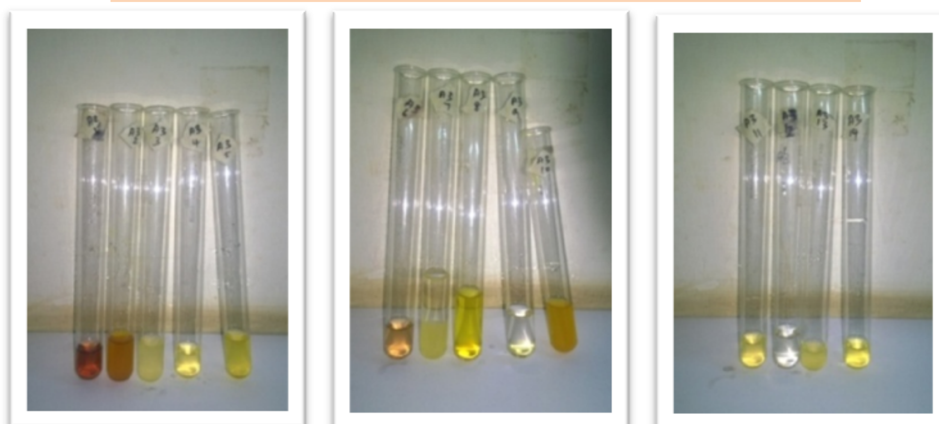


Plate XXII (a) Hexan Extract of *Cassia fistula*

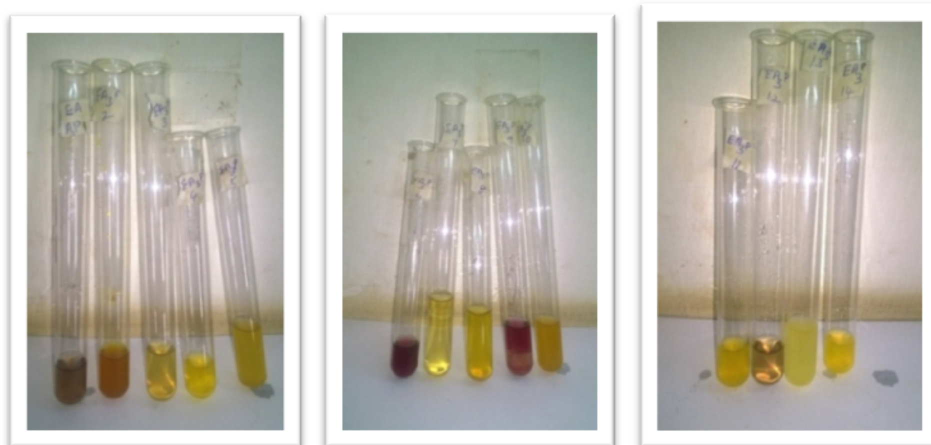


Plate XXII (b) Ethyl Acetate extract of *Cassia fistula*

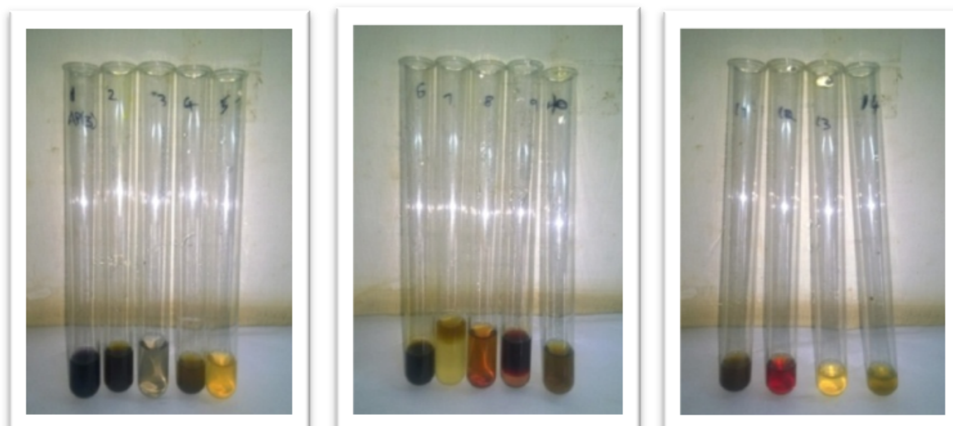


Plate XXII (c) Methanol Extract of *Cassia fistula*

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*

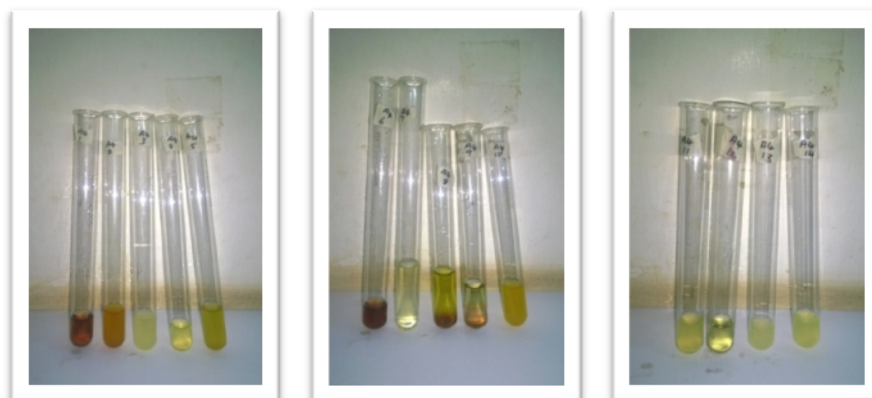


Plate XXIII (a) Hexane Extract of *Tridax procumbenz*

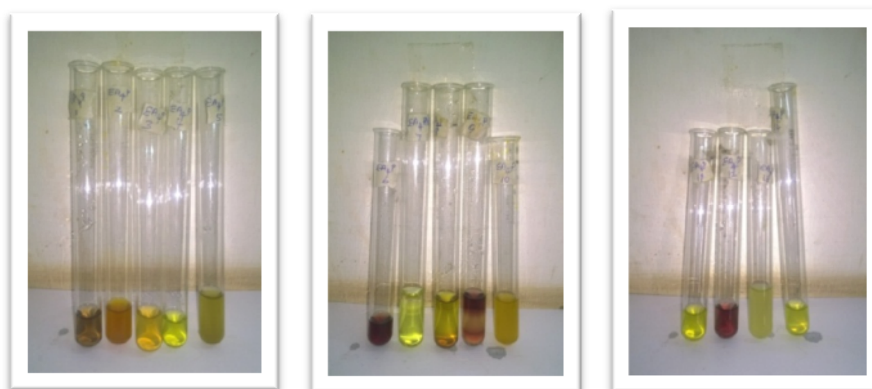


Plate XXIII (b) Ethyl acetate Extract of *Tridax procumbenz*

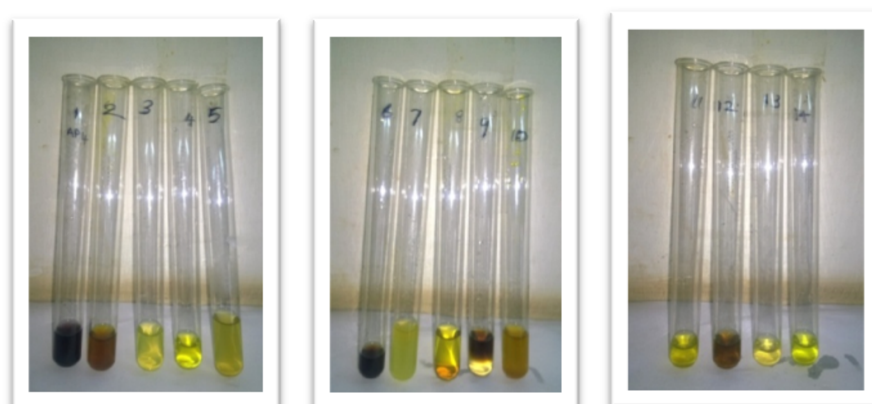


Plate XXIII (c) Methanol Extract of *Tridax procumbenz*

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*

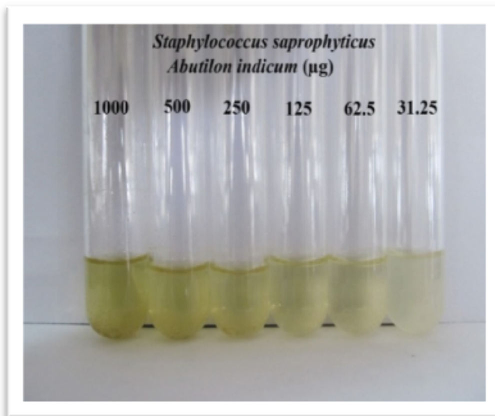


Plate XXIV(a) *S. sapropyticus*

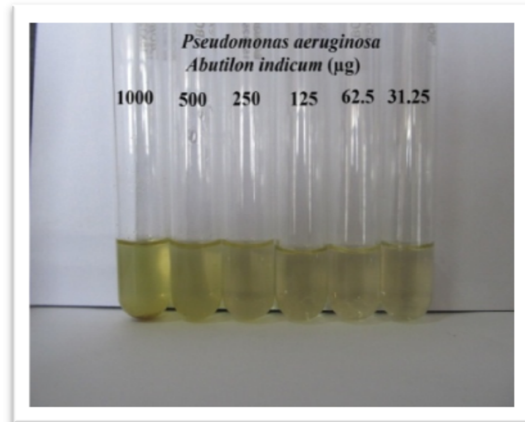


Plate XXIV(b) *P. aeroginosa*

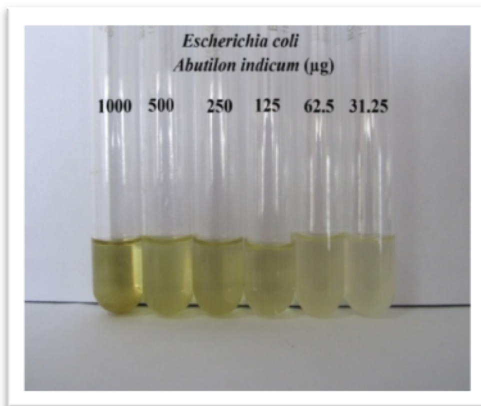


Plate XXIV(c) *E. coli*

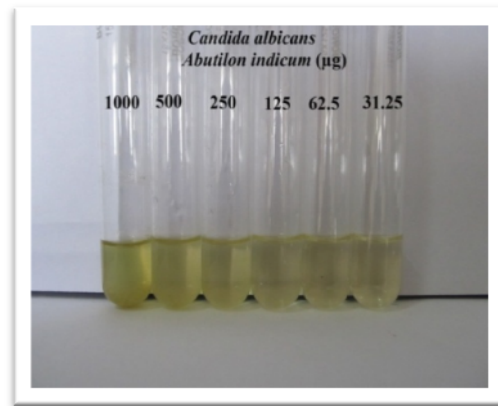


Plate XXIV (d) *C. albicans*

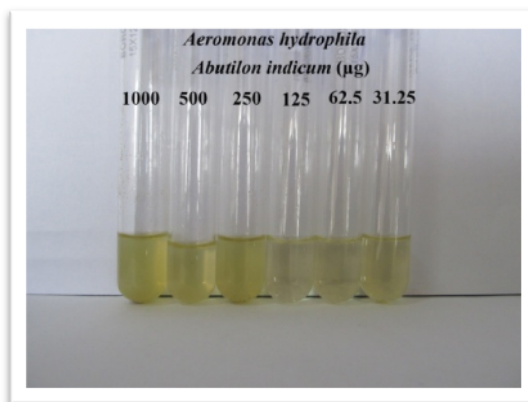
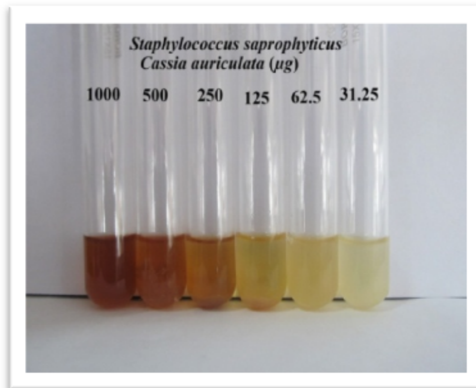


Plate XXIV (e) *A. hydrophila*

MIC of *Cassia auriculata*



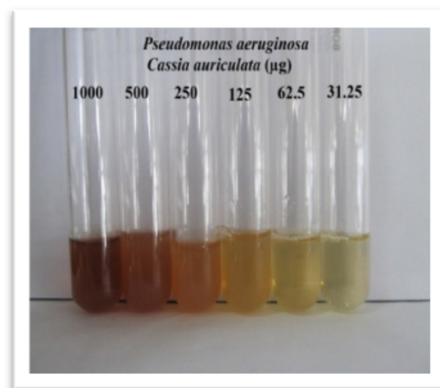
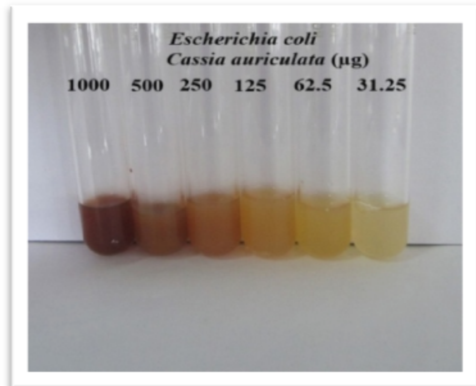


Plate XXV(a) *S.Saprophyticus* **Plate XXV(b) *P. aeroginosa***



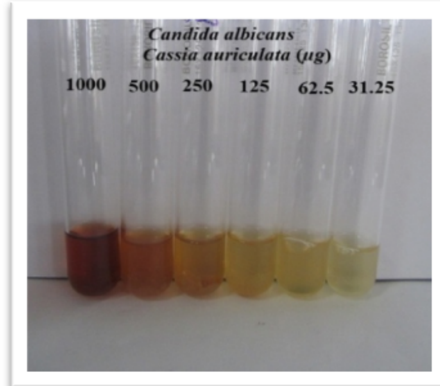


Plate XXV(c)*E. Coli* **Plate XXV(d)*C. albicans***

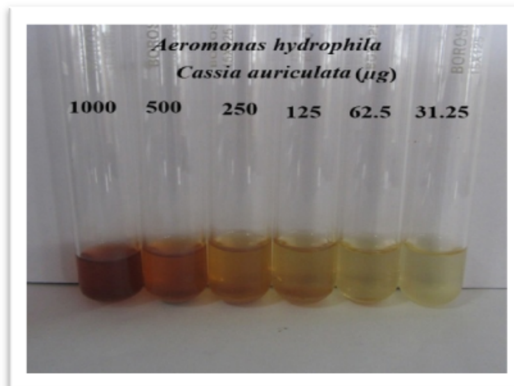


Plate XXV(e)*A. hydrophila*

MIC of *Cassia fistula*

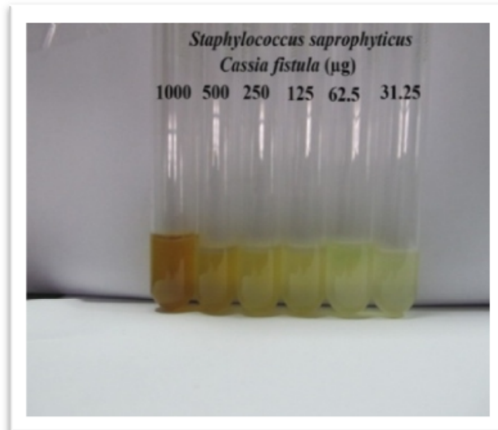


Plate XXVI(a)*S.saprophyticus*

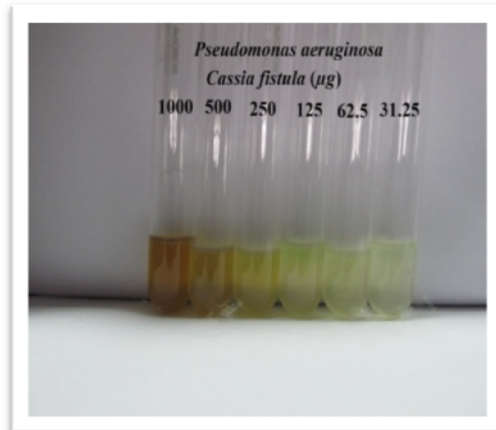


Plate XXVI(b)*P. aeroginosa*

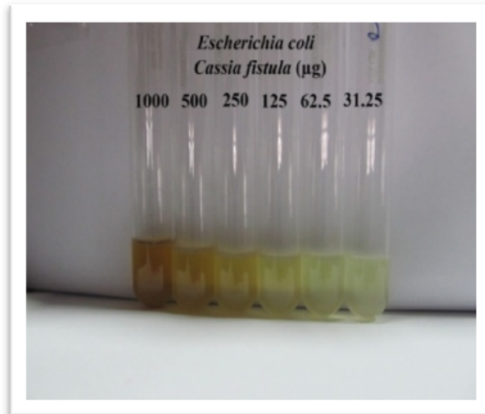
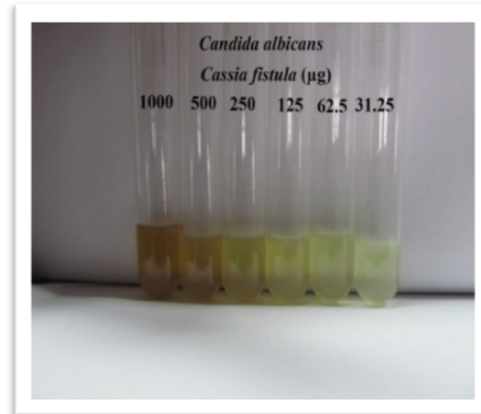


Plate XXVI(c)*E. coli*



PlateXXVI(d)*C. albicans*

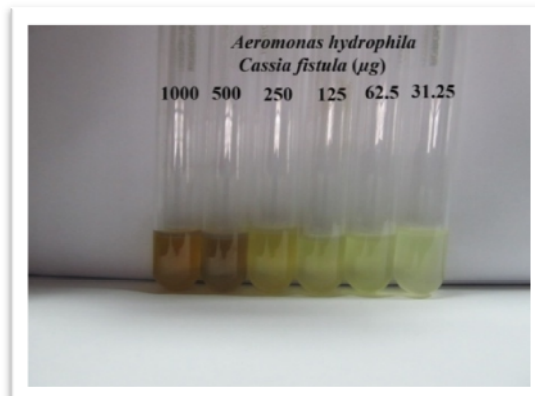
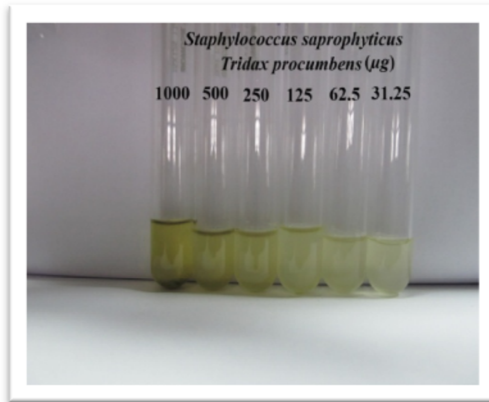


Plate XXVI(e)*A. hydrophila*

MIC of *Tridax procumbenz*



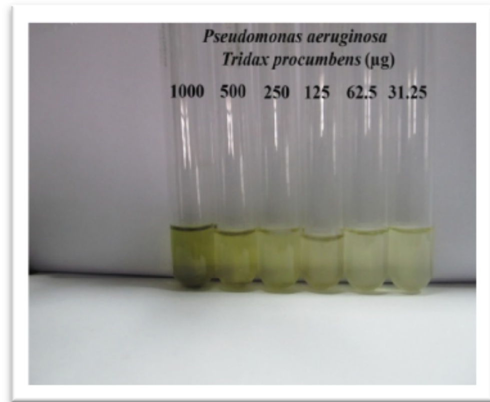
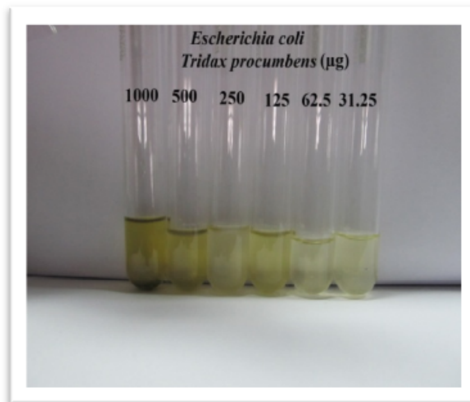


Plate XXVII(a)*S. Saprophyticus*

Plate XXVII(b)*P. Aeroginosa*



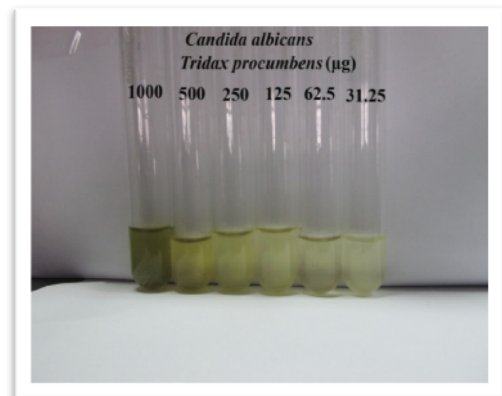


Plate XXVII (c)*E. coli*

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.

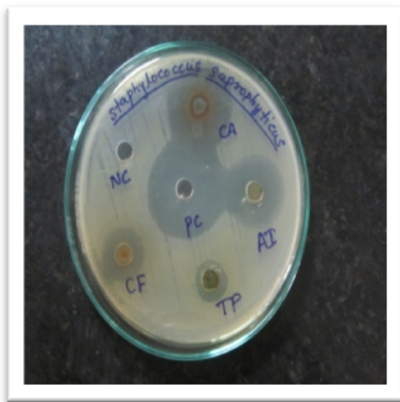
The extract of *Cassia auriculata* showed better antimicrobial activity than *Tridax procumbenz, Cassia fistula* and *Abutilon indicum.*Levofloxacin was used as positive control and distilled water was used as a negative control. Considering the MIC, value the herbal extract was taken for test. The *Tridax 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

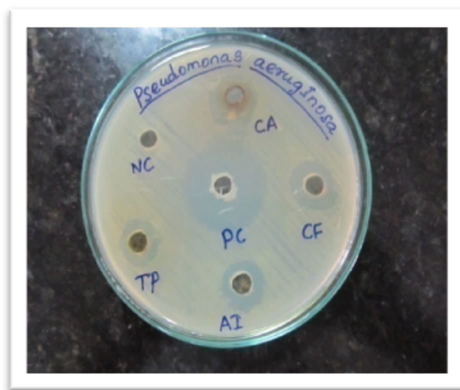
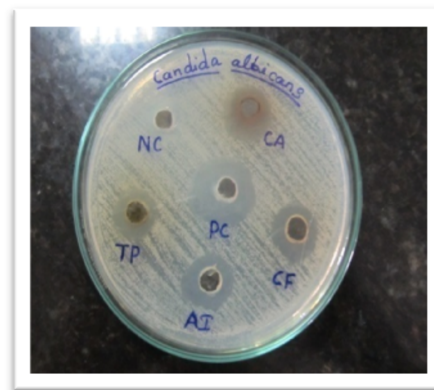


Plate XXVIII(d)
P. aeroginosa



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 37⁰C 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

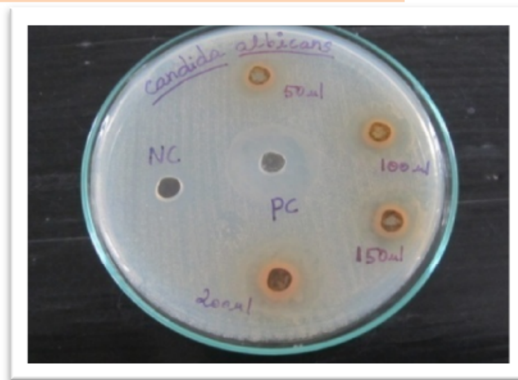


Plate XXIX (b)
C. albicans

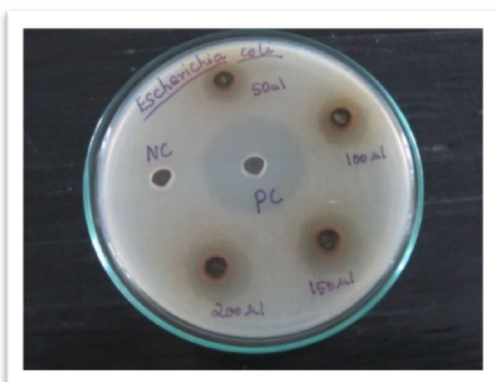


Plate XXIX (c) *E.coli*

Plate XXIX (d)*A.hydrophila*

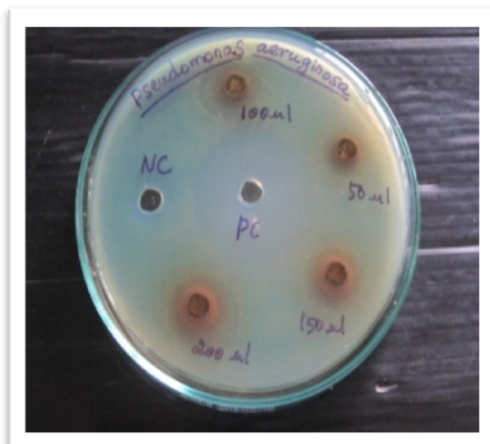


Plate XXIX (e)
P.aeroginosa

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

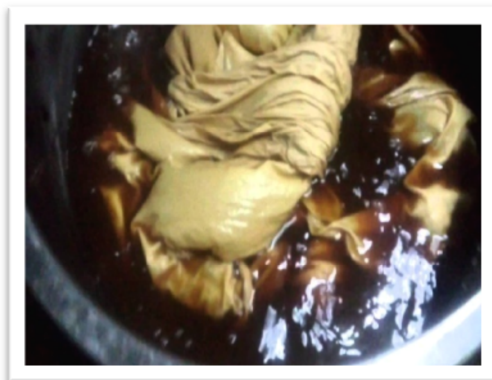


Plate XXXI

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 55°c in the hot air oven and let to cure for 30 minutes.

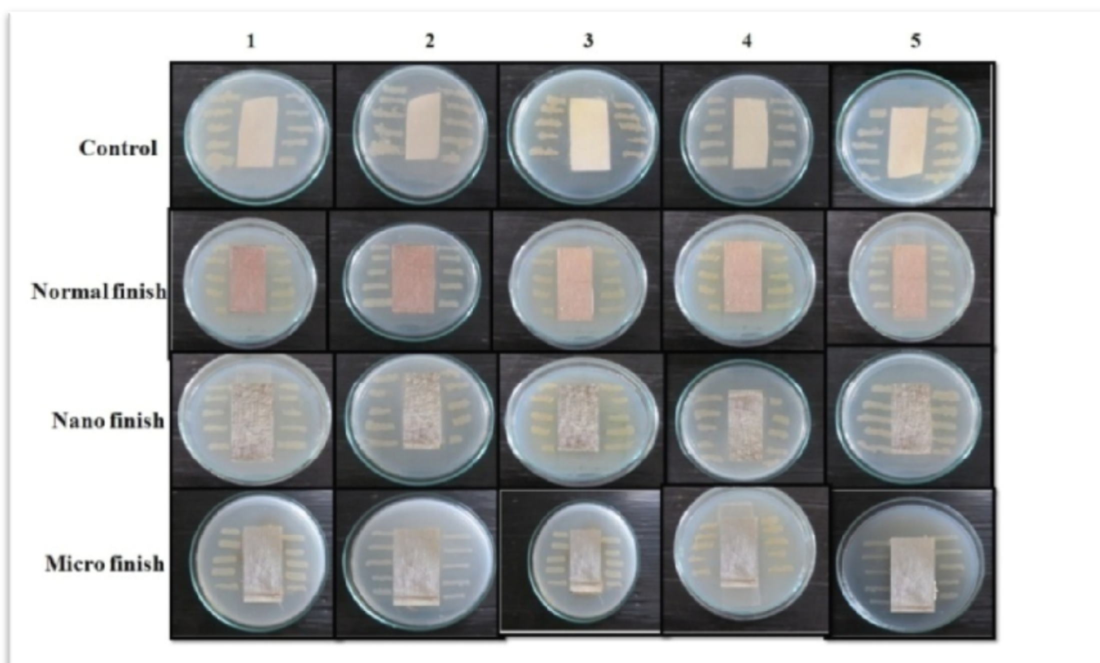
3.10 Antimicrobial Activity by AATCC 147 (AATCC 147 Test Method - 1993)

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 37°C. 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/m^2. 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±2°c 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±2°c.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±2°c. 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 90° 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°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

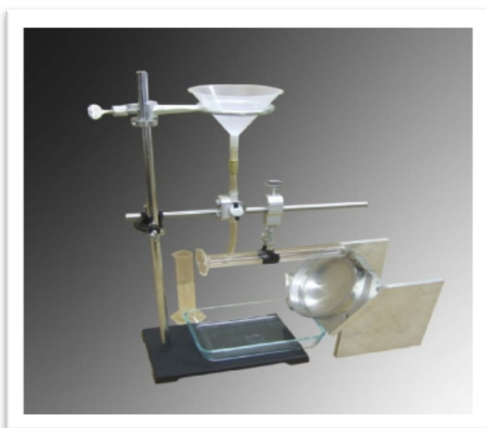


Plate XXXVI Absorbency tester

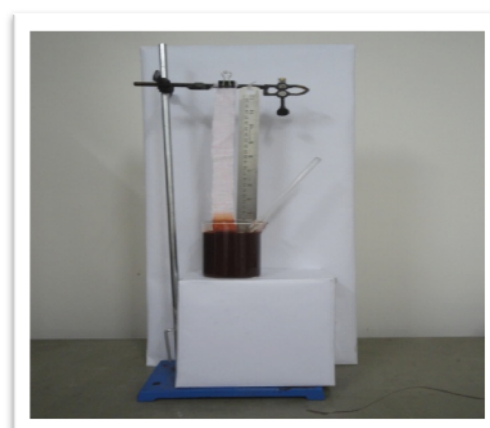


Plate XXXVII Vertival wicking tester

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.

Bacterial reduction R (%) = A – B/A X 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 0th hour and 18th 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.

4

RESULTS AND DISCUSSION

The results and discussion of the work entitled **"Developing Herbal Antimicrobial Finish Cotton Fabric for Wound Dressing"**are presented as follows;

4.1 Properties of Commercialy Available Wound Dressing Bandaids

4.1.1 Inteview Schedule

4.1.2 Properties Best Suitable for Wound Dressing Bandages Used for AcuteWound

4.2 The Yield % Obtained from Each Plant Extract

4.3 Determination of Minimum Inhibitory Concentration (MIC)

4.4 Antimicrobial Activity of the Herbal Extract

4.5 Antimicrobial Analysis for Poly Herbal Extract

4.6 Wound Scratch Cell Line Assay

4.7 Antimicrobial Assessment of Polyherbal Finished Fabric

4.8 SEM Analysis of Polyherbal Finished Fabric

4.9 Assessment of Finished Fabric Using Fourier Transform Infrared Spectroscopy

4.10 Physical Properties of Polyherbal Finished Fabric

4.10.1 Physical Properties of the Commercial Bandaids and Control Fabric

4.10.2 Fabric Weight of Polyherbal Finished Fabric

4.10.3 Tensile Strength of Polyherbal Finished Fabric

4.10.4 Sinking Test of Polyherbal finished fabrics

4.10.5 Water Holding Capacity of Polyherbal Finished Fabric

4.10.6 Air Permeability of Polyherbal Finished Fabric

4.10.7 Absorbency of Polyherbal Finished Fabric

4.10.8 Vertical Wicking of Polyherbal Finished Fabric

4.11 Microbial Filteration Test

4.11.1 Bacterial Filteration Test

4.11.2 Fungal Filteration Test

4.12 Bandaid Toxicity Test

4.1 Properties of Commercialy Available Wound Dressing Bandaids

Self adhesive band aids require certain parameters in common which was collected through the market survey. In market survey it was found that the respondents expressed that the herbal antimicrobial bandaid for minor cut, burns and scratches should be made with plain woven cotton fabric of yarn count 30's in the warp yarn and 10's in the weft yarn. As per the study certain quality parameters such as absorbency, water holding capacity, air permeability, tensile strength needs to be taken care. The size varies from standard size has to 3/4 inch width and three inch length. Considering the length three inch length and two inch width had been adopted for the study. Other specification such as quality, good adhesion, perfect finishing, water proof, herbal finished, easy unwind of the bandaid and wound dressing materials for minor injuries and major injuries were also included in the question and this is presented in Appendix I

4.1.1 Inteview Schedule

The details of the parameter required for the wound dressing bandaids as mentioned by the physisans is mentioned in Table VII.

Table VII

Details of the Parameters Required Wound Dressing Bandaids

List of Bandages	Types of Wounds	No of Respondents%
Hydro colloid	Burns	28
	Wounds that emitting liquids	04
	Necroticwounds	28
	Pressure ulcers	44
	Varicose ulcers	28
Hydro gel	Leaking wounds	24
	Painful or necrotic wounds	44
Alginate dressing	High amounts of drainage	36
	Burns	24
	Venous ulcers	36
	Packing wounds	16
	Higher state pressure ulcers.	24
Collagen	Chronic wounds	32
	Pressure sores	20
	Transplant sites	36
	Surgical wounds	16
	Ulcers	24
	Burns or injuries with a large surface area	52
Foam	Injuriesexhibitodour	16
	Absorbs exudates for wound surface.	48
	Used on surgical incision sites	28
	Burns	16
	Ulcers	40
	IV sites	20
Cloth	Cover open wounds	52
	Grazes	12
	Cuts	12
	Areas of delicate skins	24

From the Table VII it is clear that Hydro colloide, Hydrogel, Alginate dressing,Collagen, Foam and Cloth dressings are best for pressure ulcers, painful or nercotic wounds, high amount of drainage and venous ulcers, transplant sites, burns or injuries with a large surface areas, absorbes exuades for wound surface and cover open wound respectively.

Hence it could be concluded that each wound dressing material was different and can be used for different kind of wounds.

4.1.2 Properties Best Suitable for Wound Dressing Bandages Used for AcuteWound

The properties best suitable for wound dressing use for acute wounds are given in Table VIII

Table VIII

Properties Best Suitable for Wound DressingBandages Used for Acute Wound

Best Suitable Properties for Wound Dressing	No of Respondents%
Non-breathable	nil
Breathable	92
Easy wearable	88
Comfortable	92
Self-adhesive	40
Suitable for sensitive skin type	80
Reduce pain	56
Remove dead tissue	68
Cooling effect on burning wounds	64
Absorb excess liquid	68
Containing sodium and seaweed fibres	16
Helping to bring the wound edges together	68
Aiding the growth of new blood vessels	72
Effectively speeding up healing	84
Allow water vapour to enter	20
Keeping the area moist	28
Promoting faster healing	72
Antimicrobial property	92
prevent infection	84
Used to dress all shapes and sizes	72
Biodegradable	60

The Table VIII contains clearly shows that breathable, comfortable and antimicrobial properties are most desirable factors for wound dressing as expressed by 92%of respondents respectively. This is followed by easy wearable, effectively speading up healing prevent infection and suitable for sensitive skin type as mentioned by 88,84 and 80% of the respondents respectively.

The hydrocolloid wound dressing are in relation to the results of (Pott et al,2014) in his paper “The Effectiveness of Hydrocolloid Dressing Versus Other Dressing in the Healing of Pressure Ulcers in Adult and Older Adults a Systematic Review and Meta Analysis”.

4.2 The Yield % Obtained from Each Plant Extract

The results pertaining to the percentage yield of the plant parts of *Abutilon indicum, Cassia auriculata, Cassia fistula* and *Tridax procumbenz* upon various solvents obtained from the dry weight sample of the herbs are presented in the Table IX and Figure II.

Table IX

Yield % Obtain from Each Plant Extract

S. No	Plant Sample	Extract	Weight of sample (gram)	Dry weight of the concentrate (gram)	Yield %
1	*Abutilon indicum*	Hexane	100	1	1
		Ethyl acetate	100	1	1
		Methanol	100	2	2
2	*Cassia auriculata*	Hexane	100	1	1
		Ethyl acetate	100	1	1
		Methanol	100	2	2
3	*Cassia fistula*	Hexane	100	1	1
		Ethyl acetate	100	1	1
		Methanol	100	2	2
4	*Tridax procumbenz*	Hexane	100	1	1
		Ethyl acetate	100	1	1
		Methanol	100	2	2

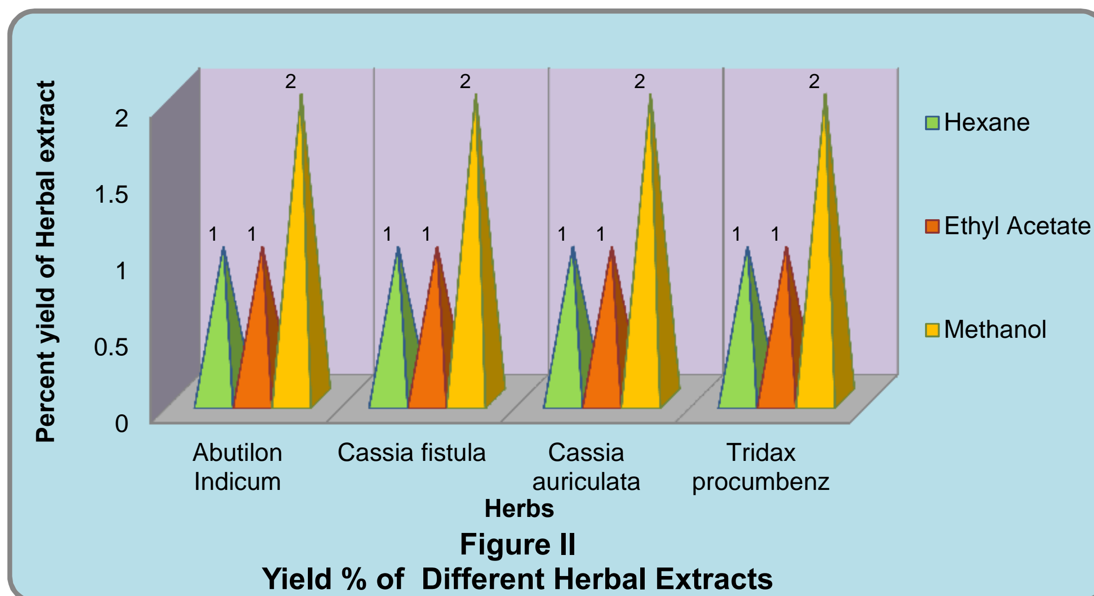


Figure II
Yield % of Different Herbal Extracts

From the Table IX and Figure II, it is evident that the percentage yield of herbal concentrate upon different solvents exhibit similar results except the herbal concentrate yield of Methanolic extract. Irrespective of the types of herbs, all the extracts done with methanol proved to have the maximum yield, as 2 gram. Hence, it was selected as the solvent for the study.

4.3 Determination of Minimum Inhibitory Concentration (MIC)

The outcome of the MIC value of *Abutilon indicum, Tridax procumbenz*, *Cassia fistula* and *Cassia auriculata* extracts are given in Table X and Figure III.

Table X
MIC Values of Herbal Extracts

S. No	Methanolic Extraction	MIC Values (µg /ml) of Herbal Extract				
		1	2	3	4	5
1	***Abutilon indicum***	250	125	250	250	1000
2	***Tridax procumbenz***	500	125	500	250	500
3	***Cassia fistula***	250	250	250	500	500
4	***Cassia auriculata***	62.5	125	250	250	62.5

1. *Candida albicans,* 2. *Pseudomonas aeruginosa* ,3. *Escherichia coli* ,
4. *Staphylococcus saprophyticus*,5. *Aeromonas hydrophila*

It is clear from the above Table X and Figure III that Methanolic extract was subjected to serial dilution method in which samples were diluted to the concentrations of 1000, 500, 250, 125, 62.5, 31.25 µg/ml and 100µl. The test culture of *Candida albicans, Pseudomonas aeroginosa, Staphylococcus saprophyticus, Escherichia coli* and *Aeromonas hydrophila* were inoculated with the equal amount of nutrient bath and herbal extract.It was then incubated at 37°C for 24hours and tested for Minimum Inhibitory Concentration. From the results obtained it was clear that *Tridax procumbenz* showed a breaking point at 500µg/ml where as remaining herbs *Cassia fistula, Cassia auriculata* and *Abutilon indicum* showed a breaking point at 250µg/ml.

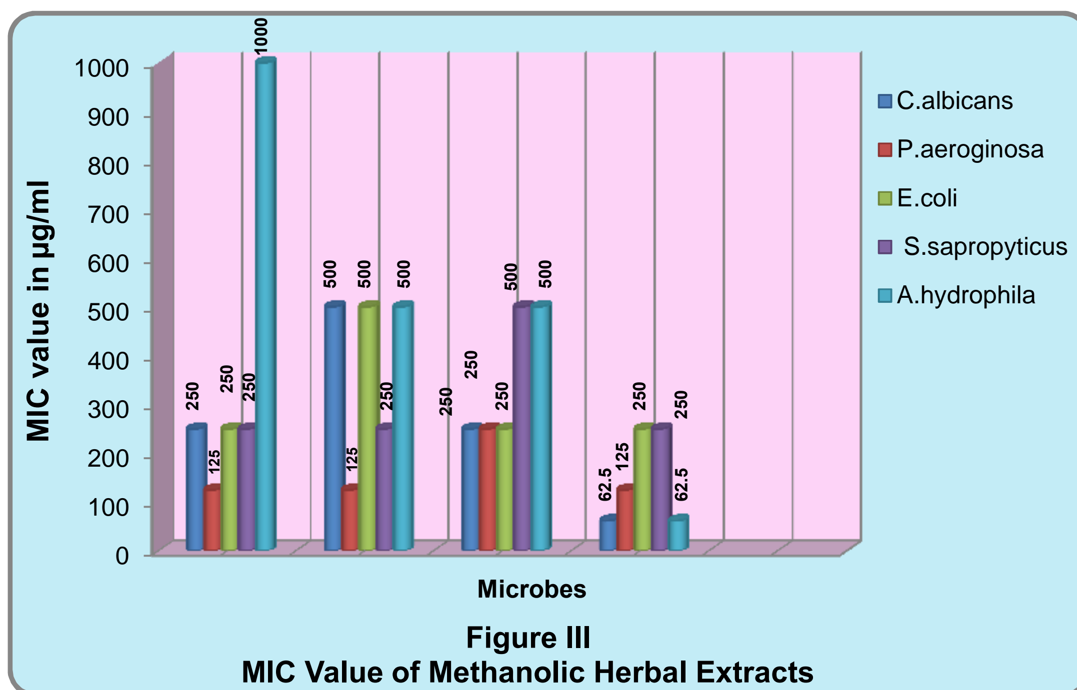


Figure III
MIC Value of Methanolic Herbal Extracts

4.4 Antimicrobial Activity of the Herbal Extract

The results for the zone of inhibition of Methanolic herbal extracts of *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* obtained are shown in the Table XI and Figure IV.

Table XI

Antimicrobial Activity of Herbal Extract by Well Diffusion Method

S. No	Sample	Zone of Inhibition (mm)									
		1		2		3		4		5	
1	Distilled water	-		-		-		-		-	
2	Levofloxacin	22	100	25	100	30	100	28	100	31	100
3	*Tridax procumbens* (500 µg/ml)	13	59%	14	56%	13	43.3%	12	42.8%	13	41.9%
4	*Abutilon indicum* (250µg/ml)	16	72.7%	15	60%	20	66.6%	23	82.1%	15	48.3%
5	*Cassia fistula* (250µg/ml)	15	68.1%	18	72%	16	53.3%	17	60.7%	19	61.2%
6	*Cassia auriculata* (250µg/ml)	19	86.3%	20	80%	22	73.3%	25	89.2%	26	83.8%

1.*Candida albicans*2.*Pseudomonas aeruginosa*3. *Escherichia coli*

4. *Staphylococcus Saprophyticus*5.*Aeromonas hydrophila*

From the Table XI and Figure IV it is proven that, the minimum Zone of Inhibition value obtained by the *Abutilon indicum, Cassia fistula,Cassia auriculata* and *Tridax procumbenz* extracts against the microbial pathogens such as, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus Saprophyticus*, and *Aeromonas hydrophila* were evaluated.

Cassia auriculata(250µg/ml) proved to have better zone value such as 19, 20,22,25 and 26 mm respectively. *Abutilon indicum* (250µg/ml) also showed higher zone of inhibition as 20 and 23 for microbes.*Escherichia coli* and *Staphylococcus saprophyticus* respectively.On comparision with the negative control, it was clear that the fabrics were able to control the growth of microbes as far as the positive is concered.

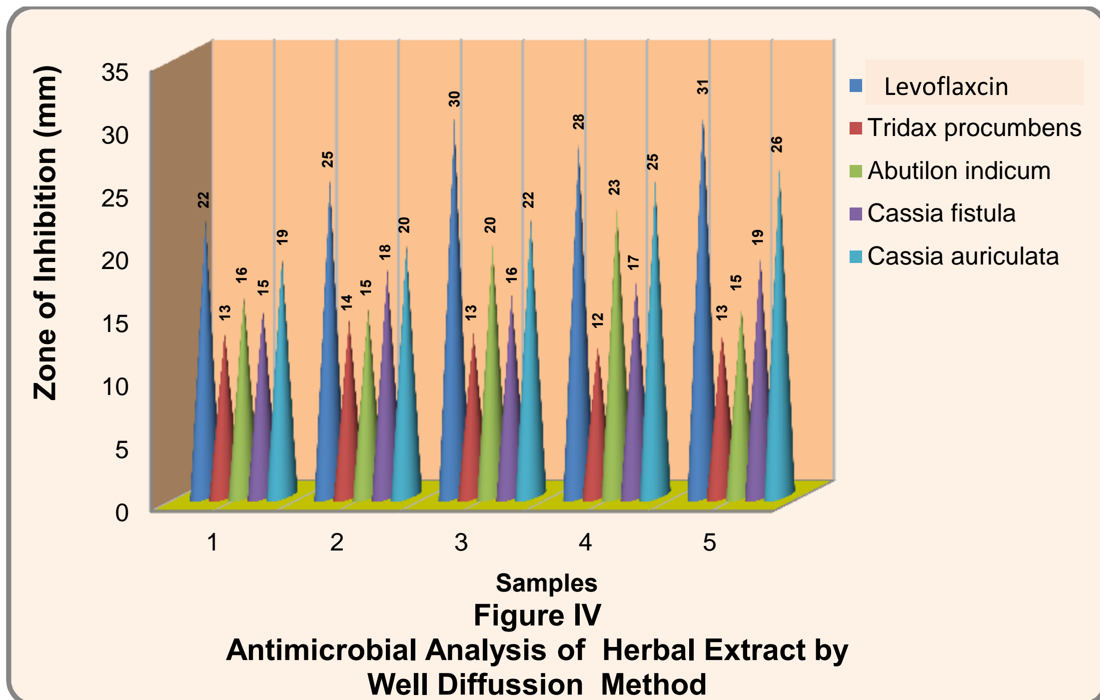


Figure IV
Antimicrobial Analysis of Herbal Extract by Well Diffussion Method

4.5 Antimicrobial Analysis for Poly Herbal Extract

The antimicrobial analysis of polyherbal extract obtained against *Candida albicans, Staphylococcus saprophsyticus, Pseudomonas aeroginosa, Escherichia coli* and *Aeromonas hydrophila* are discussed in Table XII and Figure V.

Table XII

Antimicrobial Activity of Polyherbal Extract by Well Diffusion Method

S. No	Sample		Zone of Inhibition (mm)									
			1		2		3		4		5	
1	Distilled water		-		-		-		-		-	
2	Levofloxacin		22	100	25	100	30	100	28	100	31	100
3	Polyherbal extract	50 µl	14	56%	12	38.7%	11	39.2%	15	75%	10	45.4%
		100 µl	18	72%	15	48.3%	17	60.7%	19	95%	15	68.1%
		150 µl	20	80%	17	54.8%	20	71.4%	21	105%	18	81.8%
		200 µl	22	88%	20	64.5%	22	78.5%	23	115%	20	90.9%

1. *Candida albicans* 2. *Pseudomonas aeruginosa* 3.*Escherichia coli*
4.*Staphylococcus saprophyticus*5.*Aeromonas hydrophila*

From the result it was identified that the polyherbal extract (50 µl,100µl,150µl,200µl) prepared at the ratio of 1:2:1:1 of 80 % concentration treated against the selected microbial pathogens such as *Candida albicans, Staphylococcus Saprophyticus, Pseudomonas aeroginosa, Escherichia coli* and *Aeromonas hydrophila* 200µl of poly herbal extract showed better zone of inhibition followed by 150µl, 100µl and 50µl and can be seen in Table XII and Figure V.

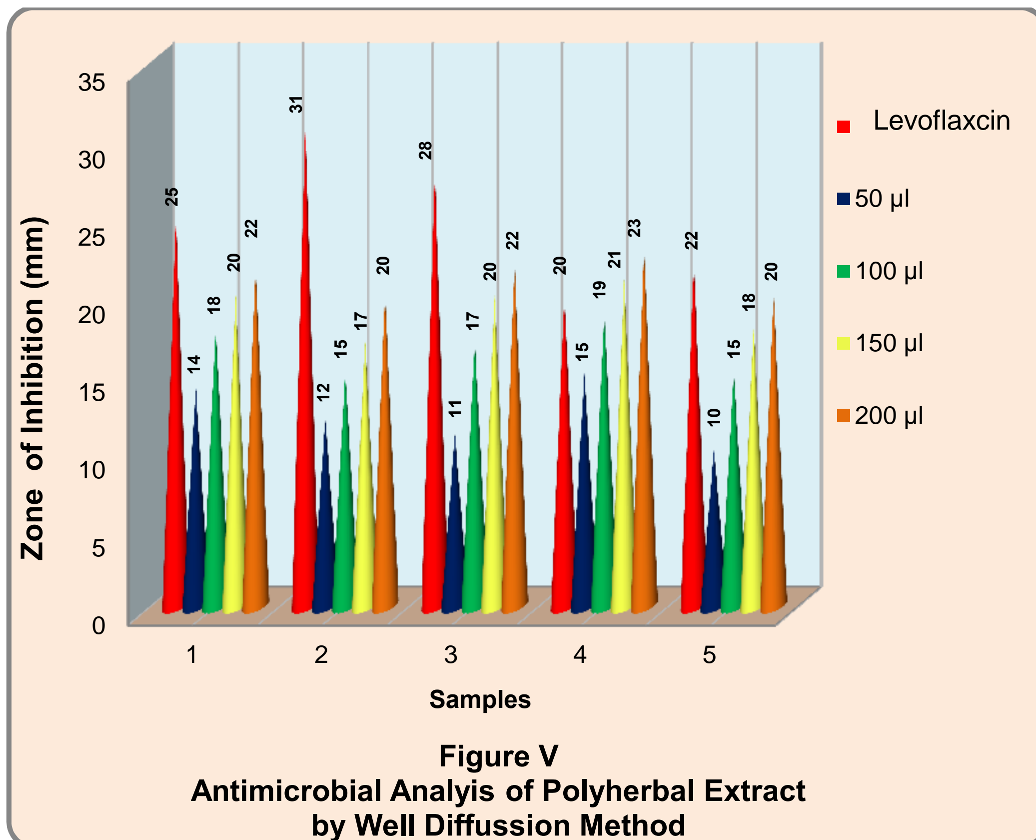


Figure V
Antimicrobial Analyis of Polyherbal Extract by Well Diffussion Method

4.6 Wound Scratch Cell Line Assay

The results pertaining to the wound scratch cell line assay conducted with polyherbal extract are shown in Plate XXXIX

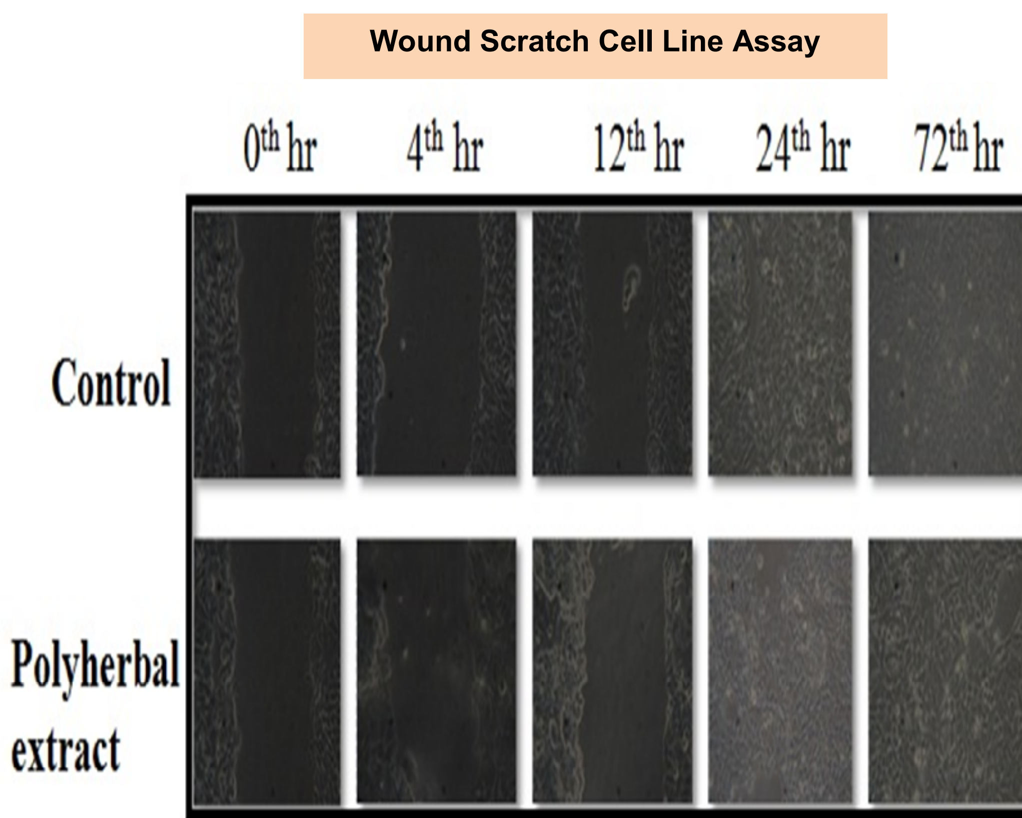


Plate XXXIX

For the study, cells were thoroughly rinsed with 1 X phosphate buffer salin (PBS) to remove cellular debris and treated with 80% concentration (25 µl) of Polyherbal extract. Cell proliferation was monitored at different time points such as 1, 4, 12, 24 and 72 hours and images of the migrated cells were taken at above mentioned time points using digital camera (Nikon, Tokyo, Japan) connected to the inverted phase contrast microscope (Radical instruments, India). Extent of wound healing was determined by the distance traversed by cells migrating into the denuded area, and the process result have been shown in the Plate XXXIX.

4.7Antimicrobial Assessment of the Polyherbal Finished Fabric

The antimicrobial assessment of the poly herbal finished fabrics are illustrated in the Table XIII and Figure VI.

Table XIII

Antimicrobial Activity of Various Types of Polyherbal Finished Fabrics

S. No	Type of polyherbal finished fabrics used	Diameter of Zone of Inhibition (mm)				
		S. saprophyticus	*P. aeruginosa*	*C. albicans*	*E. coli*	*A. hydrophila*
1	C	**13**	**16**	**11**	**19**	**10**
2	DDF	**21**	**25**	**20**	**23**	**20**
3	MEF	**32**	**29**	**31**	**32**	**25**
4	NEF	**34**	**33**	**32**	**35**	**29**

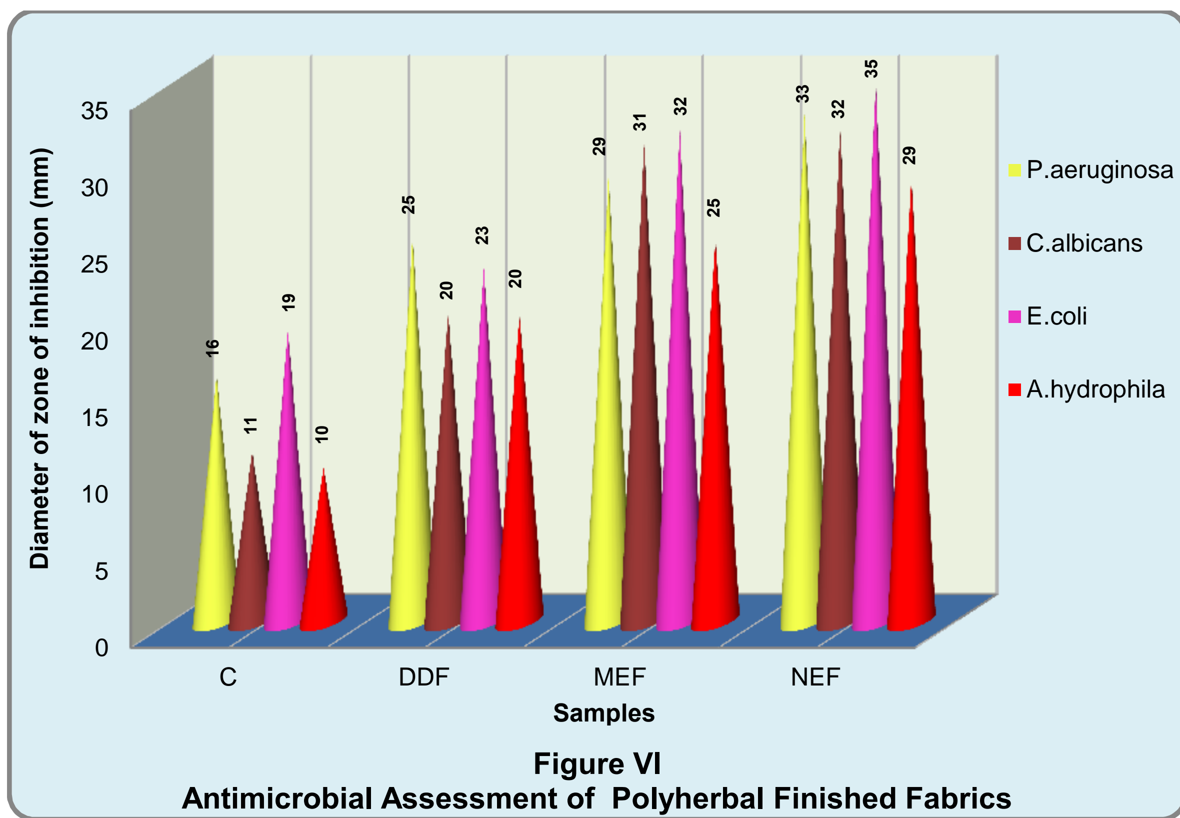


Figure VI
Antimicrobial Assessment of Polyherbal Finished Fabrics

The Antimicrobial assessment for polyherbal finished fabric such as Dip and Dry, Microencapsulation and Nanoencapsulation against microbial pathogens such as *Staphylococcus saprophyticus,Pseudomonas aeroginosa, Candida albicans, Escherichia coli* and *Aeromonas hydrophila we*re evaluated by AATCC 147 Agar well Diffusion method. Sample NEF showed the maximum zone of inhibition as 34,33,32,35 and 29 mm for the microbes *Staphylococcus Saprophyticus,Pseudomonas aeroginosa, Candida albicans, , Escherichia coli* and *Aeromonas hydrophila* respectively, followed by MEF and DDF. Hence it could be concluded that NEF polyherbal Finish had the maximum control over microbes.

4.8 SEM Analysis of Polyherbal Finished Fabrics

The SEM sample of MEF and NEF Figure VII (a,b) and Figure VIII (a,b) indicate the surface morphology of the polyherbal finished fabrics. The range of particle size of herbal extract was observed between 3.328µm of RSD 09 28 and similarly the particle size of nanocapsules had been identified between 171.43nm to 345.25nm. From the figure it is proved that the MEF and NEF particle deposition on the fabric had been embedded firmly on the fabric surfaces of MEF and NEF

SEM Analysis of MEF and NEF Finished Fabric

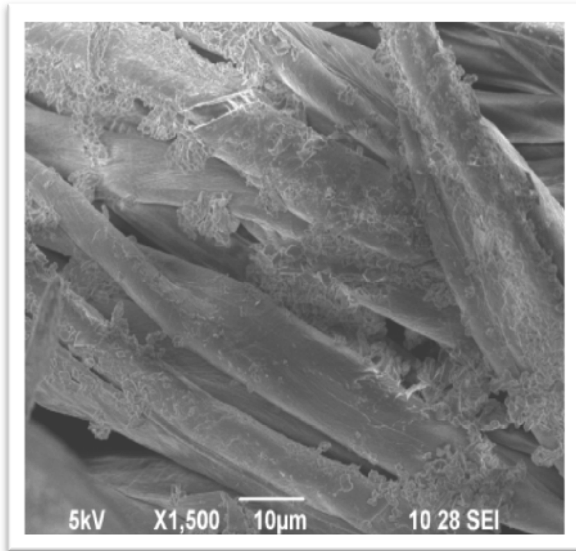


Figure VII (a)

SEM of Microencapsulated Fabric

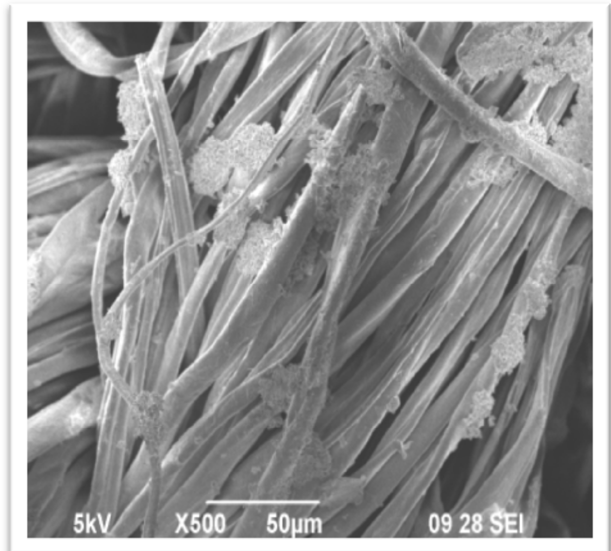


Figure VII (b)

SEM of MicroencapsulatedFabric

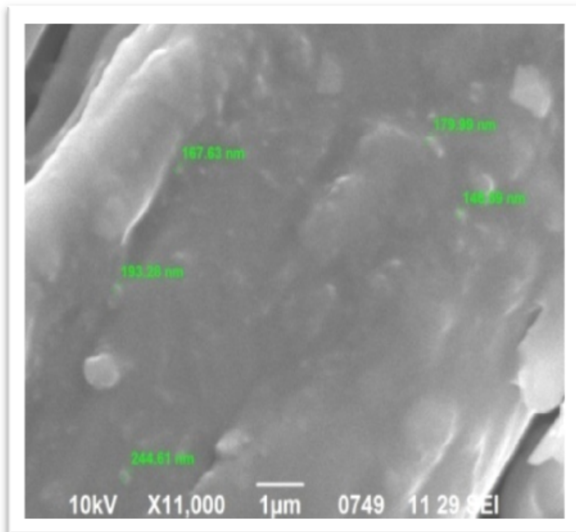


Figure VIII (a)

SEM of Nanoencapsule Fabric

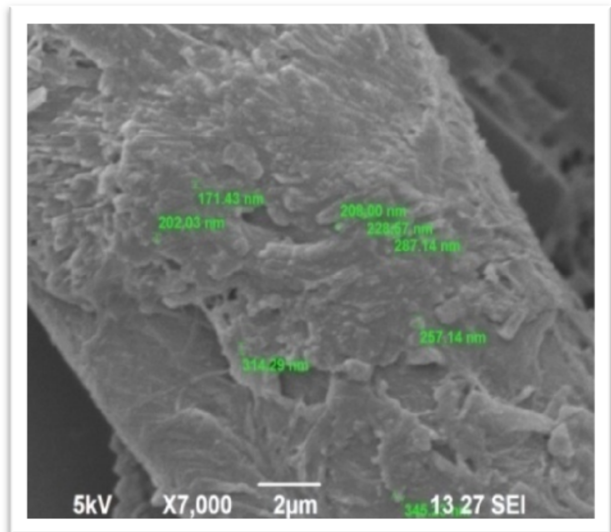


Figure VIII (b)

SEM of Nanoencapsule Fabric

4.9 Assessment of Finished Fabric Using Fourier Transform Infrared Spectroscopy

The results of FTIR images of the polyherbal finished samples with the 80% concentration of herbal extract are shown in the Figure IX, Figure Xand Figure XI.

FTIR Spectroscopy of Polyherbal Finished Fabric

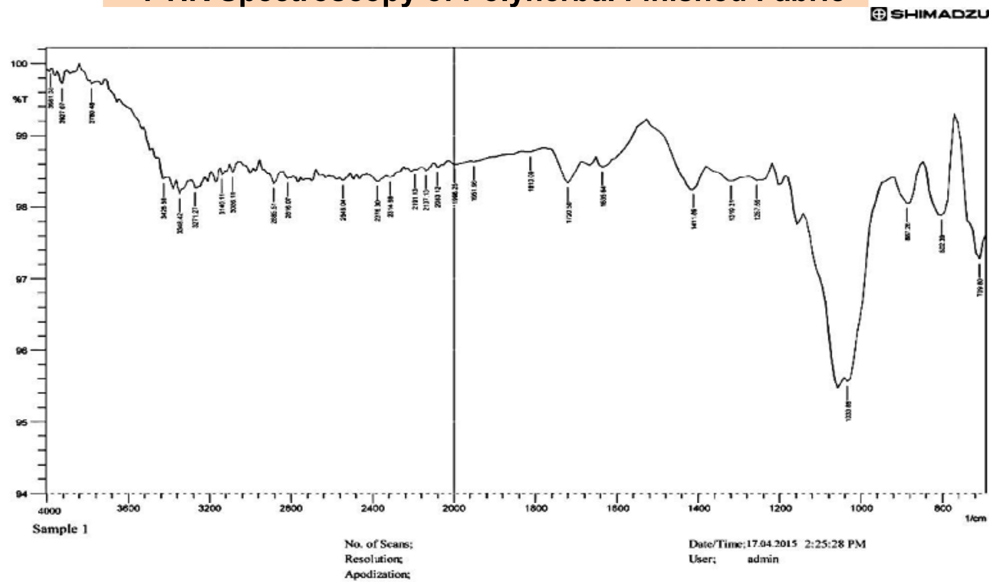


Figure IX
FTIR of Dip and Dry Fabric

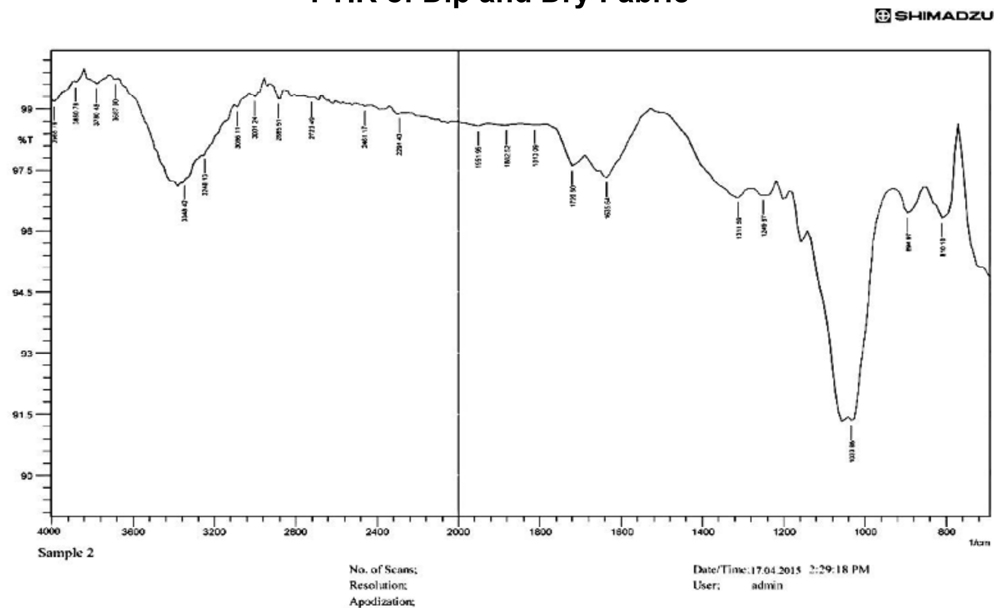


Figure X
FTIR of Microencapsulated Fabric

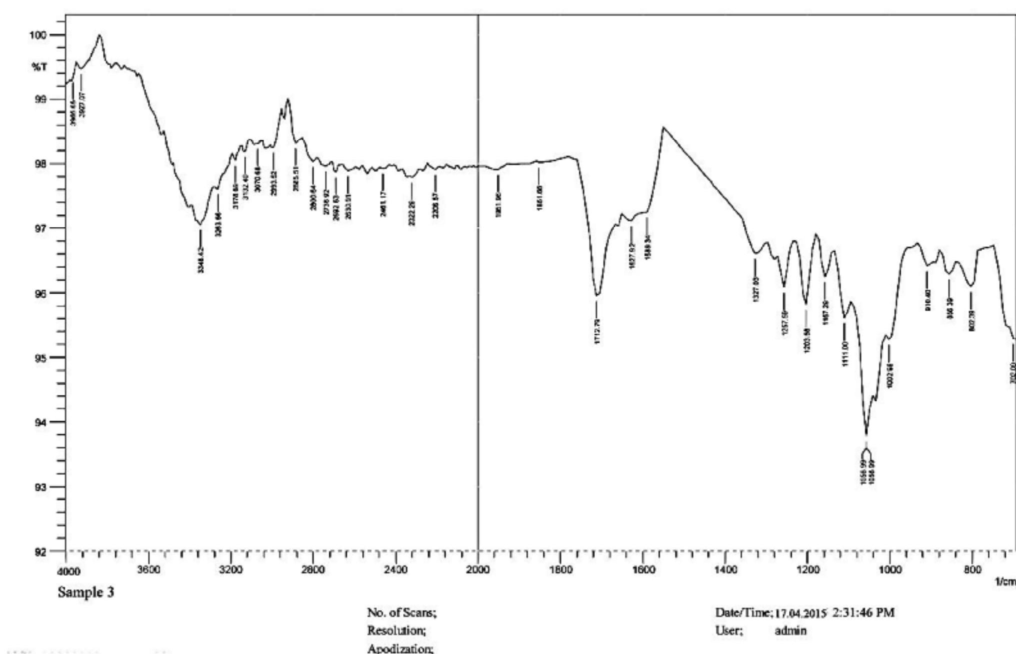


Figure XI

FTIR of Nanoencapsulated Fabric

Regarding the FTIR results from the Figures IX, X and XI the range between 1724cm^{-1} to 2915, 540.935 to 690 and 1242 showed the presence of carbohydrates, alkalides and phenolic compounds. In Figure IX, the presence of spectrum 1846.51 showed the presence of acid halides. In Figure IX the ranges between 1320-1000 proved the presence of carboxcylic, ester acid and ether. The bands of 1452.85 in Figure X showed the presence of alkenes. Similarly the presence of 3360-3549 cm^{-1} in the Figure IX, X and XI show the presence of alcohols. Hence from the FTIR, it was clear that the spectral band proved the presence of phytochemical compounds in herbal finishing fabrics. Hence it could be concluded that the finishing has enhanced the antimicrobial activity.

The Antimicrobial assessment for polyherbal finished fabric such as Dip and Dry, Microencapsulation and Nanoencapsulation against microbial pathogens such as *Staphylococcus saprophyticus,Pseudomonas aeroginosa, Candida albicans, Escherichia coli* and *Aeromonas hydrophila*were evaluated by AATCC

147 Agar well Diffusion method. The sample NEF showed the maximum zone of inhibition as 34,33,32,35 and 29 mm for the microbes *Staphylococcus saprophyticus,Pseudomonas aeroginosa, Candida albicans, , Escherichia coli* and *Aeromonas hydrophila* respectively followed by MEF and DDF. Hence it could be concluded that NEF polyherbal finish had the maximum control over microbes.

4.10 Physical Properties of Polyherbal Finished Fabrics

4.10.1 Physical Properties of the Commercial Bandaids and Control Fabric

The physical property of the commercial bandaid was assessed in need for comparision with that of the polyherbal finished fabric. The properties such as fabric weight, tensile strength, absorbency, air permeability and vertical wicking were tested analysed and presented in Table XIV.

Table XIV

Physical Properties of the Control Fabric

S.No	Fabric property	100 % cotton 30s Ne and 10s Ne
1	Tensile strength warp (kgf)	32.79
2	Tensile strength weft (kgf)	58.27
3	Yarn thickness warp (mm)	0.25
4	Yarn thickness weft(mm)	0.71
5	Air permeability(cm3/cm2/s)	79.3
6	Vertical wicking warp (cm)	0.412
7	Vertical wicking weft (cm)	0.410
8	Fabric GSM	174
9	Water absorbency(sec)	3

In the above Table XIV, the physical property of 30sN and 10sNe plain weave cotton fabric selected for the study is shown. The tensile strength of the

warp and weft is identified as 32.79 (Kgf) and 58.72(Kgf) respectively. The yarn thickness for the warp and the weft is 0.25mm and 0.71mm respectively. Similarly, the air permeability of the woven fabric is 79.3 $cm^3/cm^2/s$. The vertical wicking in the warp and weft direction is 0.412cm and 0.410 cm. The fabric GSM is 174 and the water absorbency is three seconds. These properties are suitable for wound dressing material.

On comparing these properties with the study "Comfort Properties and Dyeing Behavior of Cotton/Milkweed Blended Rotor Yarn Fabrics" carried out by Karthik et al , 2017 were in the tensile strength (21.3 Kgf), yarn thickness of warp and weft (0.42mm,0.59mm) and GSM (154) of the 20sNe and 19sNe fabric proves to be lesser than the present study. Hence, with the variation in counts the fabric prepared by the investigator could be a much better fabric for wound dressing bandaid. However, the air permeability (156.61$cm^3/cm^2/s$) of the woven fabric is lesser than the fabric quoted by Karthik et al, 2017. This may be due to the reason of using lesser yarn count.

In a nutshell it could be concluded that the fabric designed by the investigator for the present study is suitable for wound dressing bandaids.

4.10.2 Fabric Weight of Polyherbal Finished Fabric

The weight of the fabric before and after the fabric finishing had been presented in Table XV and Figure XII.

In the Table XV and Figure XII, the fabric weight of the C, DDF,MEF and NEF fabrics are given which confirms an increase in weight irrespective of the type of finish. The results confirmed that the MEF fabric showed increase in the fabric weight as 175.02 grams when compared to the C, DDF and NEF. Weight of the DDF fabric and NEF fabrics were similar to the weight of the controlled fabric. The increase in the fabric weight proves the adhesion of polyherbal extract to the fabric.

Table XV

Fabric Weight of Polyherbal Finished Fabric

S. No	Samples	Fabric GSM
1	C	174.182 ± 1.45
2	DDF	174.186 ± 1.57^{ns}
3	MEF	175.02 ± 1.73 ^{ns}
4	NEF	174.191 ± 1.96 ^{ns}
	'p' Value	0.523^{ns}

ns- not significance

The result of the analysis of variance of the fabric weight shows that there is no significant variance between the control sample and the herbal finished samples. The minimum increase in the weight of the treated fabric when compare to the control proves the presence of the polyherbal extract in the fabric.

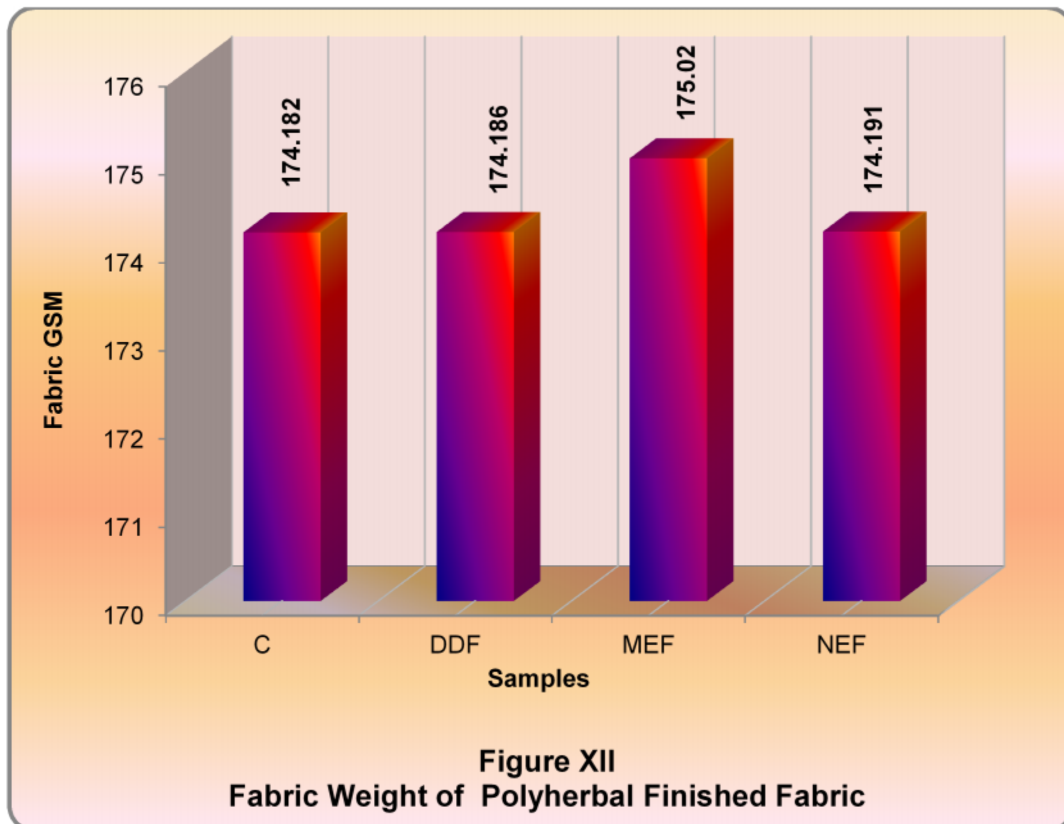


Figure XII
Fabric Weight of Polyherbal Finished Fabric

4.10.3 Tensile Strength of Polyherbal Finished Fabric

The assessment of tensile strength (Kgf) of C, DDF, MEF and NEF finished fabrics in warp and weft direction are presented inTable XVI and FigureXIII (a,b).

Table XVI

Tensile Strength of Polyherbal Finished Fabrics

S. No.	Samples	Warp strength	Warp elongation	Weft strength	Weft elongation
		(Kgf)	(%)	(Kgf)	(%)
1.	C	32.79 ± 1.33	19.22	58.27 ± 1.82**	12.77
2.	DDF	40.86 ± 1.50**	7.57	35.33 ± 1.08**	10.75
3.	MEF	45.52 ± 2.05**	9.73	39.47 ± 1.43**	20.69
4.	NEF	40.20 ± 1.86**	6.53	35.96 ± 1.28**	10.82
'p' value		0.012		0.003	

** - Significant at 1% level

From the analysis of results obtained by the warp direction, the MEF finished fabric with 45.52 Kgf showed maximum tensile strength followed by DDF finished, NEF with 40.86 and 40.20 Kgf respectively. When compared with the finished fabrics, the tensile strength for the original control fabric was lowest with a value of 32.79 Kgf. Hence it could be concluded that the strength of the fabric in warp direction increased on finished fabrics over the original samples.

As far as the analysis of result obtained by test the strength in the weft direction of the fabrics, the maximum strength was noted in original fabric with 58.27 Kgf, followed by MEF, NEF and DDF finished with 39.47 and 35.96 and 35.33 Kgfrespectively. When compared with the finished fabrics, the tensile strength for the original control fabric was highest. Hence it could be concluded that the strength of the fabric in weft direction decreased on finished fabrics over the original samples.

The elongation of the samples in the warp direction increased in NEF sample but decreased in the DDF and MEF samples when compared to the original as 6.53, 9.73 and 7.57% respectively. The increase in elongation in NEF sample may be due to the higher absorption of herbal extracts.

In case of weft elongation sample MEF showed an increase by 20.69% but samples DDF and NEF showed a decrease by 10.75 and 10.82% respectively when compared with control which was 12.77%.

The analysis of variance for the tensile strength of the sample C and polyherbal finished fabrics such as DDF, MEF and NEF are put forth in the Table XVI. The comparison of the tensile strength in both warp and weft directions between treated and the control fabrics showed significant difference at 1% level. The variance noted in the physical property of the treated fabric compare to that of the control fabric proves that finishing processes had significant effect on the tensile strength of the fabric.

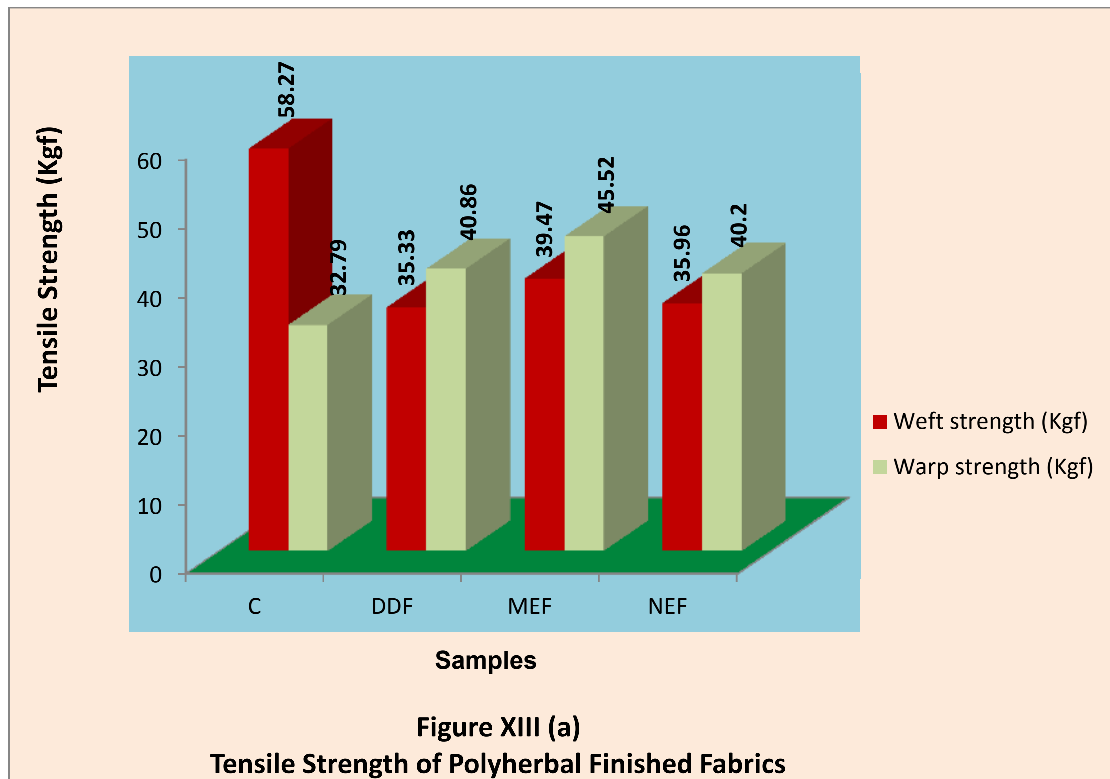


Figure XIII (a)
Tensile Strength of Polyherbal Finished Fabrics

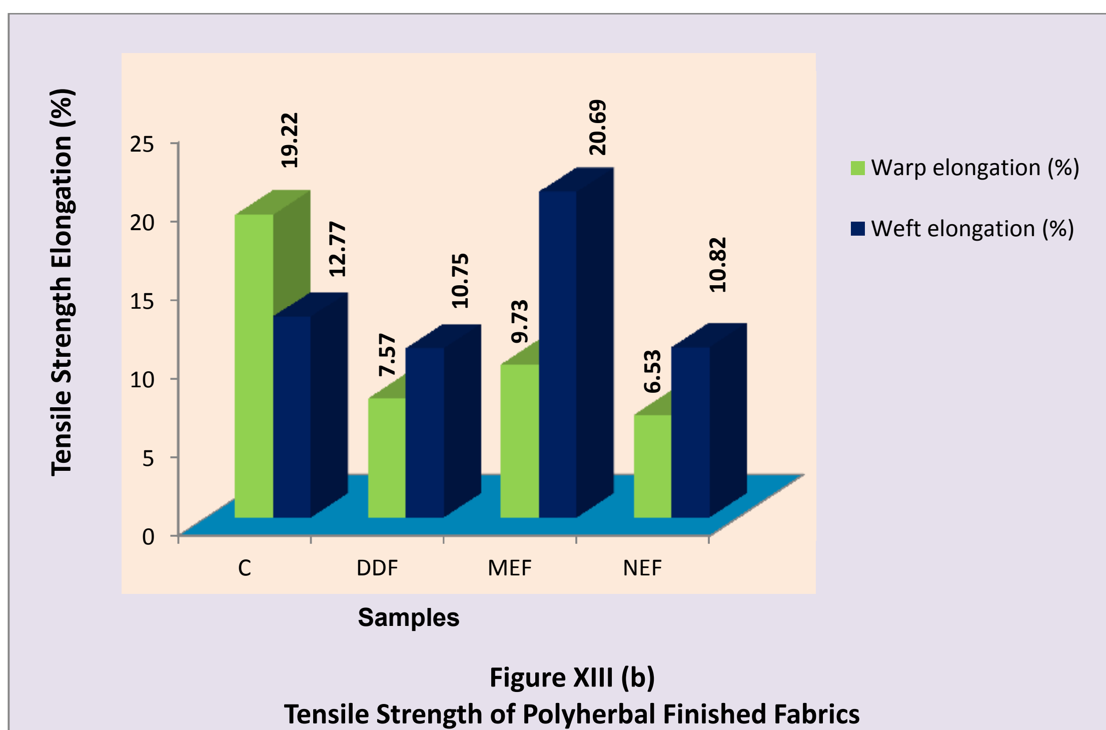


Figure XIII (b)
Tensile Strength of Polyherbal Finished Fabrics

4.10.4 Sinking test of polyherbal finished fabrics

The assessment of the sinking test is depicted in the Table XVII and Figure IX show the analysis of variance of sinking test of polyherbal finished fabrics.

Table XVII
Sinking Test of Polyherbal Finished Fabrics

S. No	Samples	Average sinking time (sec.)
1.	C	3 ± 0.45
2.	DDF	2 ± 0.29**
3.	MEF	1 ± 0.18**
4.	NEF	1 ± 0.18**
'p' value		0.015

** - Significant at 1% level

From the Table XVII and Figure XIV it is clear that the sinking time (seconds) for herbal finished samples reduced except for MFC sample. The

sinking time of the C was recorded to be 3 seconds. Where as the DDF, MEF and NEF samples had taken lesser time to sink as 2,1 and 1 second respectively. It can be inferred from the test the herbal extract finish has enhanced the wettability of the finished fabric DDF and NEF.

The results obtained by the analysis of variance for sinking test in second showed 1% significant variance between the control and polyherbal finished fabrics. The difference in the sinking time of the C,DDF,MEF and NEF finished fabrics emphasize that the processing and treatment given to the fabric affected the sinking property of the fabrics.

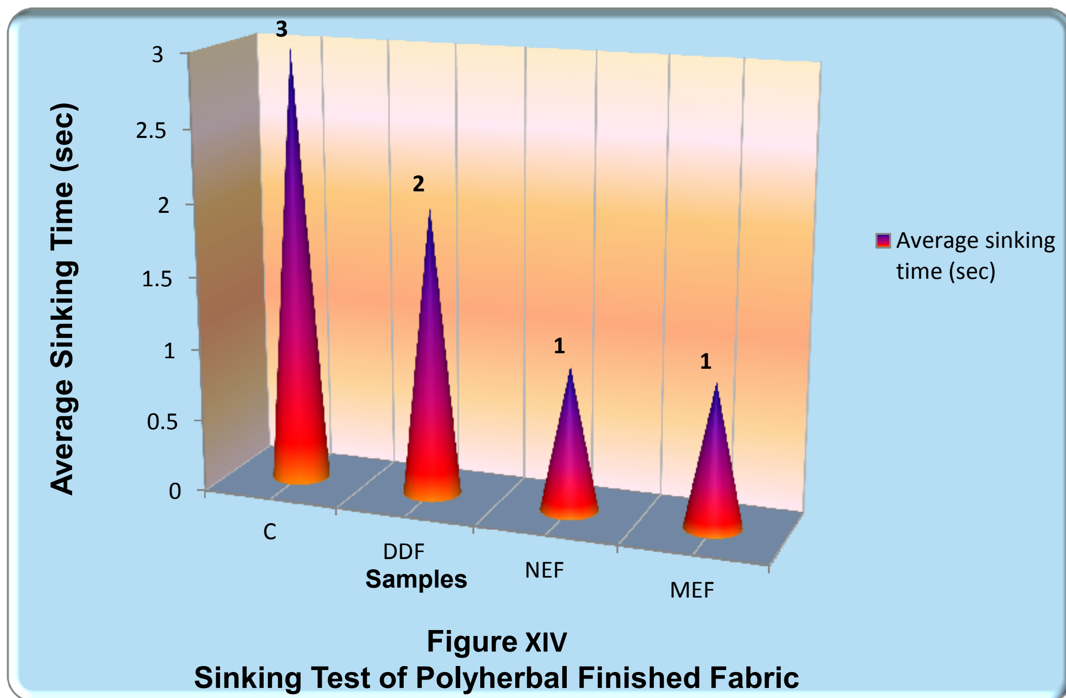


Figure XIV
Sinking Test of Polyherbal Finished Fabric

4.10.5 Water Holding Capacity of Polyherbal Finished Fabric

The following Table XVIII and Figure XVrevealthe analysis of variance of the polyherbal finished fabric.

Table XVIII

Water Holding Capacityof Polyherbal Finished Fabrics

S.No	Samples	Water holding capacity (%)
1.	C	51.25 ± 3.29
2.	DDE	70 ± 4.16**
3.	MEF	66 ± 3.78**
4.	NEF	53 ± 2.37^{ns}
'p' value		0.014

** - Significant at 1% level

From the Table XVIII and Figure XV it is evident that the water holding capacity of the finished fabrics has increased. With respect to water holding capacity, it was least in the sample cand had a capacity of 51.25%. The holding capacity increased for the herbal finished fabrics with the highest record for DDF finished fabric with a capacity of 70%. This was followed by MEF and NEF finished fabrics with a holding capacity of 66% and 53% respectively. Hence it could be concluded that the water holding property of the fabric samples on finishing, improved to a great extent. Increasing in the water holding capacity might be due to the herbal components present in the fabric and also due to the compact arrangement of yarns during the finishing processes, which permits the water to enter into the fabric easily.

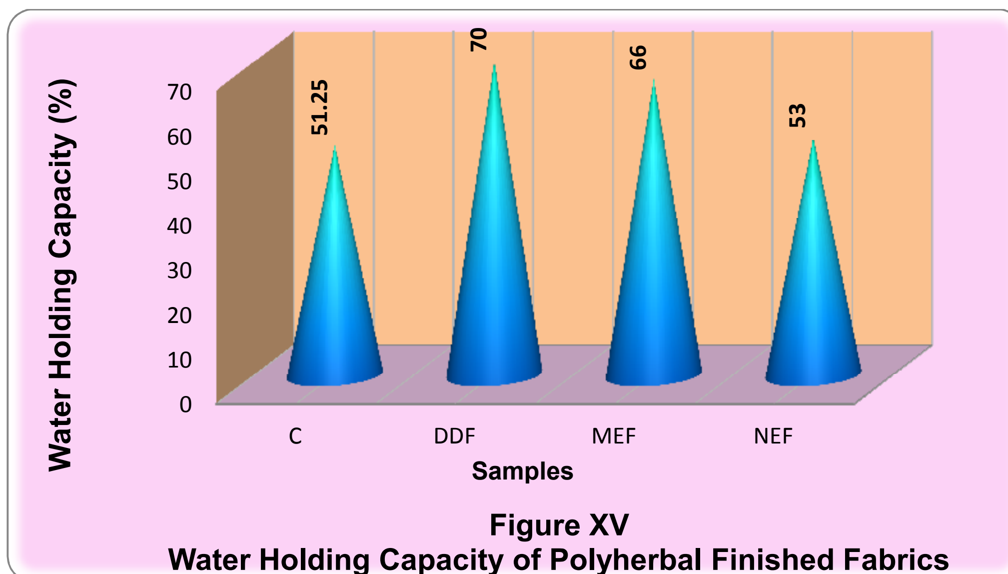


Figure XV
Water Holding Capacity of Polyherbal Finished Fabrics

From the results obtained from the analysis of variance, it was observed that there was no significant variance in the water holding capacity of the C.The finished fabrics such as DDF,MEF shows 1% variancewhereas NEF fabric shows minimum varinance in water holding capacity compare to other samples. The result showedthat the fabric treatment exhibited some significant variance in the water holding capacity of the polyherbal finished fabric.

4.10.6 Air permeability of Polyherbal Finished Fabric

The results of air permeability of the control and finished fabric of DDF, MEF and NEF are presented in the Table XIX and Figure XVI.

Table XIX

Air Permeability of Polyherbal Finished Fabrics

S. No	Samples	Air Permeability($cm^3/cm^2/s$)
1.	C	79.3 ± 3.67
2.	DDF	76.4 ± 2.98*
3.	MEF	79.1 ± 3.46^{ns}
4.	NEF	74.2 ± 3.79**
'p' value		0.004

** - Significant at 1% level; ns - Not significant

From the Table XIX and Figure XVI it is found that the air permeability of the finished fabrics was comparable. Sample C and MEF showed an air permeability of values 79.3 and 79.1cm^3/cm^2/srespectively. However, the air permeability of the DDF and NEF has reduced to 76.4and 74.2cm^3/cm^2/s respectively. The reduction in air permeability proved the finish to enhance movement of air, which in turn will help in the wound/cut/burn healing. Hence it could be concluded that fabris are suitable for bandaid preparation.

The analysis of variance for air permeability shown in Table XIX shows 1%variancelevel had been shown between the control and finished fabrics which identified that the fnishing processes showed notable change in the physical properties of the finished fabric.

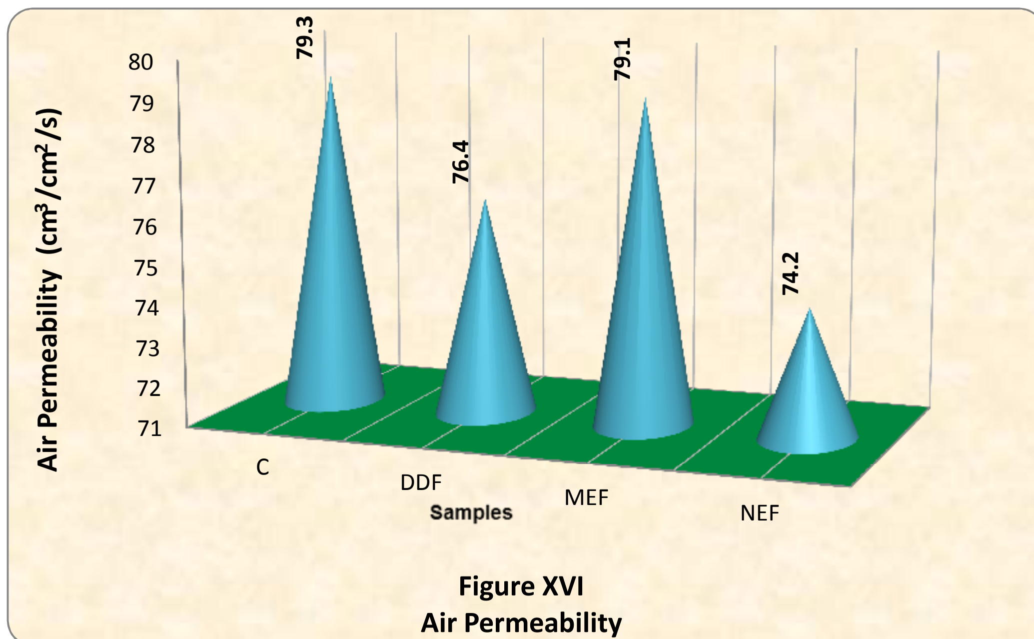


Figure XVI
Air Permeability

4.10.7 Absorbency of Polyherbal Finished Fabric

The results of the water absorbency capacity of the controlled samples DDF, MEF and NEF finished fabrics are given in the Table XX, Figure XVII show the analysis of variance of the absorbency rate of polyherbal finished fabric.

Table XX

Water Absorbency of Polyherbal Finished Fabrics

S. No	Samples	Absorbency(sec.)
1.	C	3 ± 0.29
2.	DDF	2 ± 0.19**
3.	MEF	1 ± 0.15*
4.	NEF	1 ± 0.15*
	'p' value	0.032

** - Significant at 1% level

From the Table XX and Figure XVII, it is found that the sample C took 3 seconds to absorb a droplet of water. Where as, DDF, MEF and NEF samples had taken 2,1 and 1 seconds respectively to absorb a water droplet which have got reduced when compared with controlled fabric. This result revealed that the herbal extract finished fabrics DDF and NEF have better absorbency when compared to controlled sample. Hence it could be conducted that Nano finishing has enhanced the property of water absorbency.

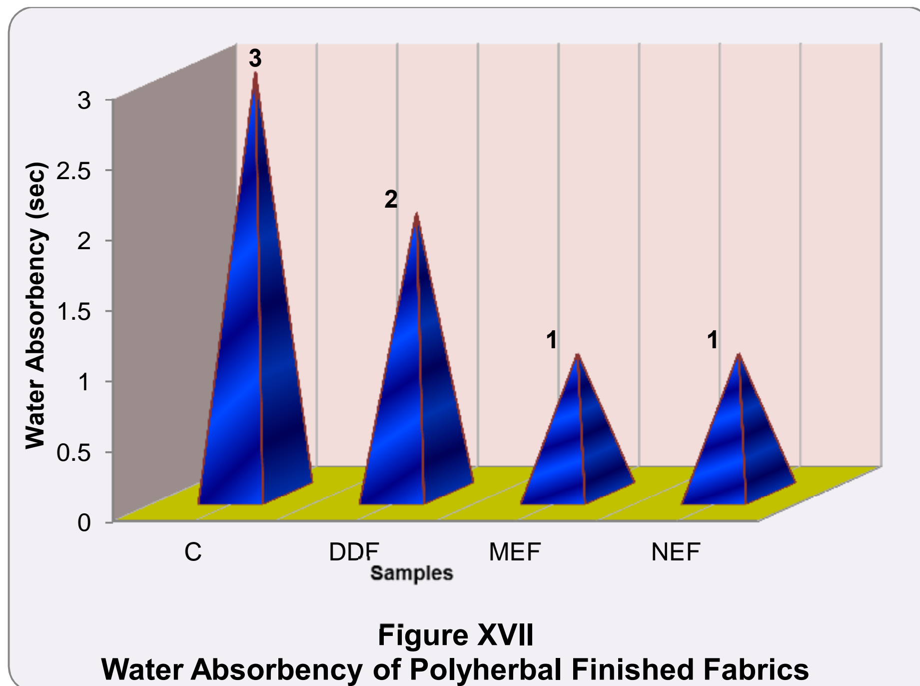


Figure XVII
Water Absorbency of Polyherbal Finished Fabrics

The statistical analysis of variance on the water absorbency of the control and the treated fabric shows 1% level of variance which provides the result that the fabric processing andfinishing processes showed a identifiable effect on the time duration taken in water absorbency .

4.10.8 Vertical Wicking of Polyherbal Finished Fabric

The results of vertical wicking of the sample C and DDF, MEF and NEF for the warp and weft direction are given in the Table XXI and Figure XVIII show the analytical variance of vertical Wicking of herbal finished fabric.

TableXXI

Vertical Wicking of Polyherbal Finished Fabrics

S. No	Samples	Time exponent k (warp)	Time exponent k (Weft)
1	C	0.412 ± 0.07	0.410 ± 0.05
2	DDF	0.396 ± 0.05*	0.395 ± 0.03*
3	MEF	0.415 ± 0.03^{ns}	0.440 ± 0.02^{ns}
4	NEF	0.459 ± 0.06*	0.430 ± 0.04
	’p’ value	0.039	0.042

* - Significant at 5% level; ns - Not significant

The Table XXI and Figure XVIII epict an increase in vertical wicking of DDF, MEF and NEF method of finishing on sample.further it is seen that in the warp direction, the wickabilty of DDF sample was noted for 0.39 minutes. As for the weft direction, the wickabilty of DDF sample was noted as 0.39 minutes, which increased gradually to 0.410, 0.430 and 0.440 minutes in C, NNF and MEF samples respectively. Hence it could be concluded that the rate of water absorbency proved to be increased in the MEF and NEF finished samples compared to the DDF samples.

Analysis of variance for vertical wicking of the control and herbal finished fabrics show significance at 5% level, which prove that the pretreated and finishing processes shows a remarkable effect in the physical property of the finished fabric when compared to that of controlled samples.

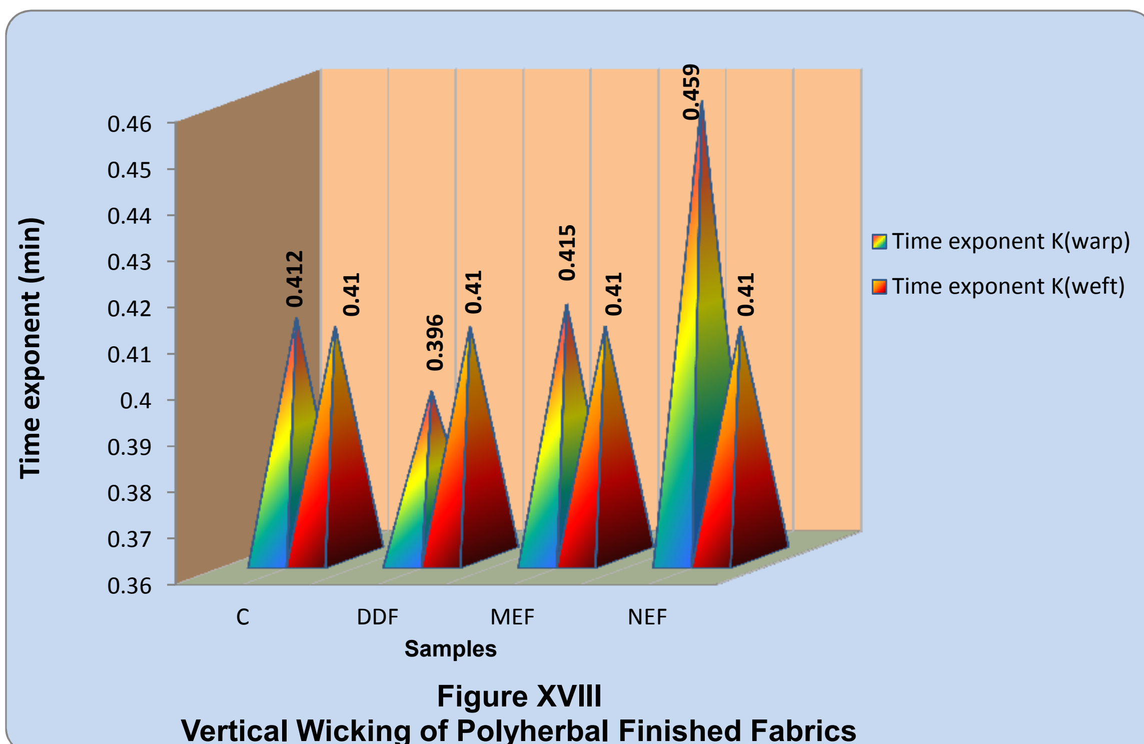


Figure XVIII
Vertical Wicking of Polyherbal Finished Fabrics

4.11 Microbial Filteration Test

4.11.1 Bacterial Filteration Test

From the Table XXII and Plates XL,XLI,XLII and XLIII results it confirms that the samples tested with *Escherichia coli* ATCC 25922, *Staphylococcus sapropyticus* ATCC 6538, *Aeromonas hydrophila* ATCC 100-2004 and *Pseudomonas aeroginosa* ATCC 100-2004 for bacterial filteration shows better filteration when compare to the control untreated sample. The Dip and Dry finished fabric show 80% to 85% of bacterial reduction. Were as, Microfinished and Nanofinished samples shows more or less same bacterial reduction properties as 90% to 95% and 93% to 96%

Table XXII

Bacterial Filteration Test for Control and Treated Bandaids

S. No.	Samples	Bacterial Filter (Reduction - %)			
		Escherichia coli ATCC 25922	*Staphylococcus sapropyticus* ATCC 6538	*Pseudomonas aeroginosa* ATCC 100-2004	*Aeromonas hydrophilla* ATCC 100-2004
1	Control	Nil	Nil	Nil	Nil
2	Dip-dry finished	80	84	85	80
3	Micro finished	94	95	95	90
4	Nano finished	95	93	96	95

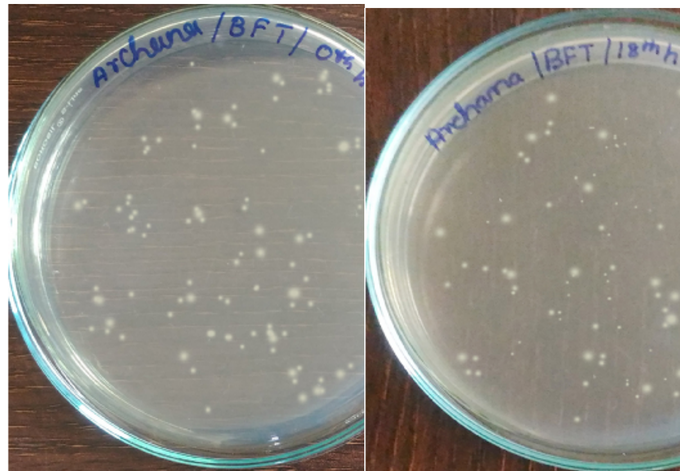
From the Table XXII, it is evident that the three finished test samples, Dip-dry, Microcapsules and Nanocapsules finished medical textile materials exhibited excellent antibacterial properties. As per the test, the bacterial numbers were quantitatively filtered by the test materials during the analysis. The incubation period of 18 hours had greatly influenced in reducing or filtering the number of test bacteria by the samples. This was significantly evident from Table XXII. Microcapsules finished samples exhibited 94% and 95% of bacterial reduction when tested against the respective test bacteria *Escherichia coli* ATCC 25922,*Staphylococcus sapropyticus*ATCC 6538, *Aeromonas hydrophila* ATCC 100-2004 and *Pseudomonas aeroginosa* ATCC 100-2004. The Microencapsulated finished samples showed 94,95,95 and 90 per cent reduction in bacteria colonies respectively. Whereas, Nanofinished samples showed 95, 93, 96 and 95 per cent reduction in bacterial colonies respectively. Dip-Dry finished showed comparatively less reduction percentage than the other finished samples. This may be due to the difference in the durability properties of the antibacterial agents finished onto the sample materials. Hence from the Table XXII it is clear that all the finished fabric showed reduction in bacterial colonies irrespective of the method of treatment for all the different type of bacteria.

Bacterial Filteration Test for ControlSamples

The test result is show in in Plate XL which indicated no variation in the number of colonies (Colony Forming Unit -CFU) of the test organisms. This proves that the control fabric had not been finished with the polyherbal extract.

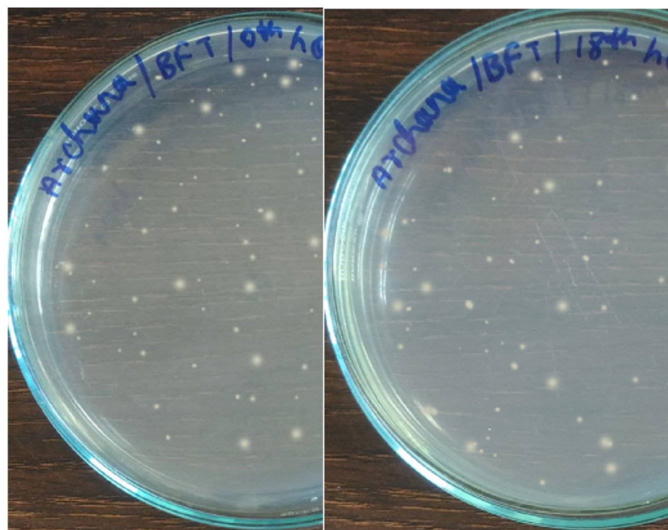
Bacterial Filteration Test for Control Samples

***Escherichia coli* ATCC 25922**



0th hour plate 18th hour plate

***Staphylococcus Saprophyticus* ATCC 6538**

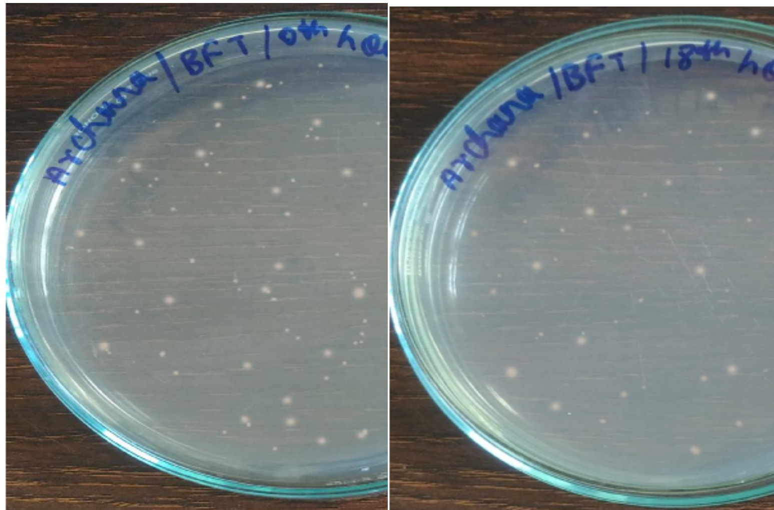


0th hour plate 18th hour plate

Plate XL

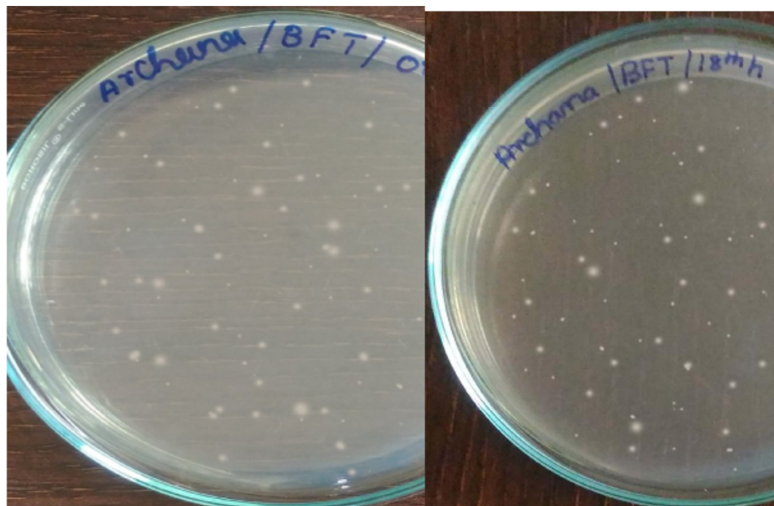
Bacterial Filteration Test for Control Samples

Pseudomonas aeruginosa



0th hour plate 18th hour plate

Aeromonas hydrophila



0th hour plate 18th hour plate

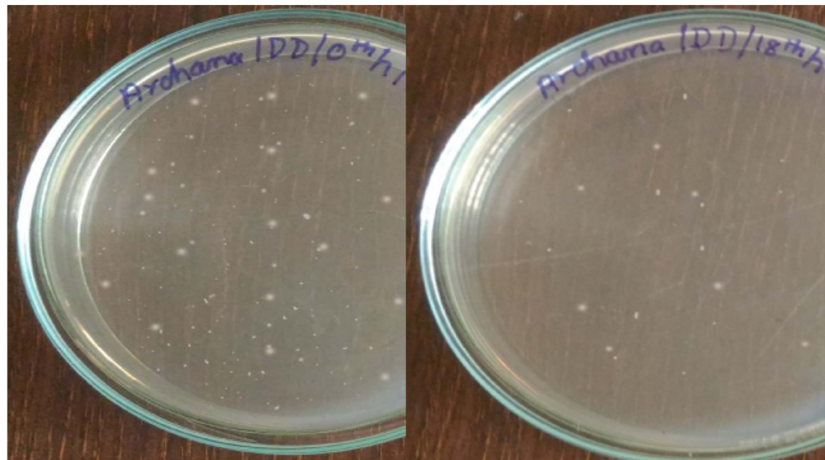
Plate XL

Bacterial Filteration Test for Dip-Dry Finished Samples

The test results in Plate XLI shows the variation in the number of colonies (CFU) of test organisms such as *Escheritia coli, Staphylococcus sapropyticus, Aromonashydrophila* and *Pseudomonas aerognosa* and is evident from the image. 0th hour plate showing more CFU than 18^{th}hour plate. The variation was due to the presence of polyherbal finishing on the bandaid.

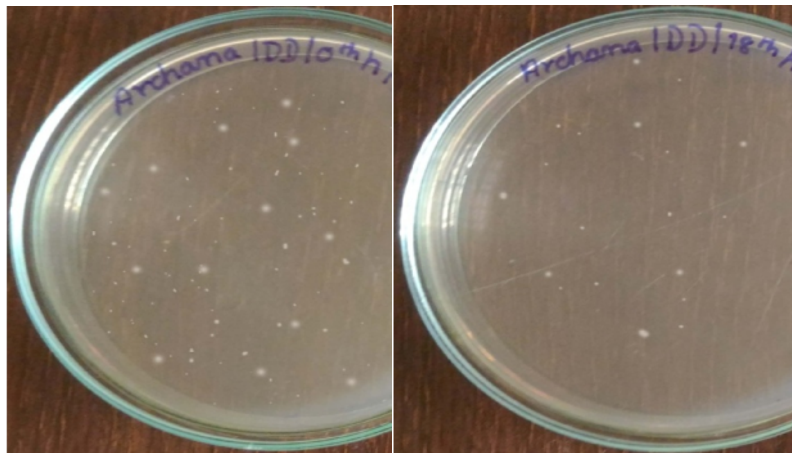
Bacterial Filteration Test for Dip-Dry Finished Samples

Escherichia coli **ATCC 25922**



0th hour plate 18th hour plate

Staphylococcus Saprophyticus **ATCC 6538**

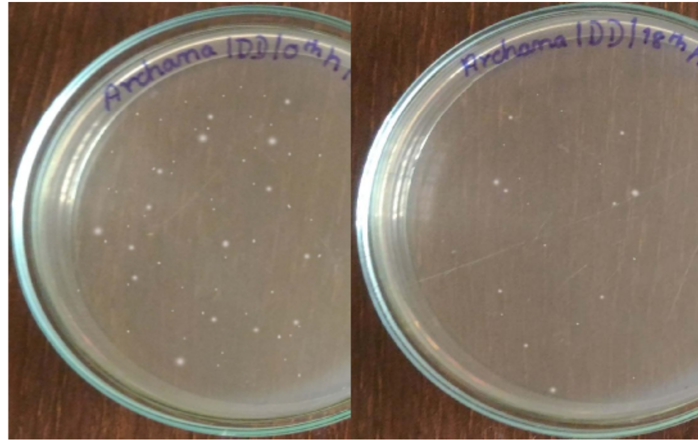


0th hour plate 18th hour plate

Plate XLI

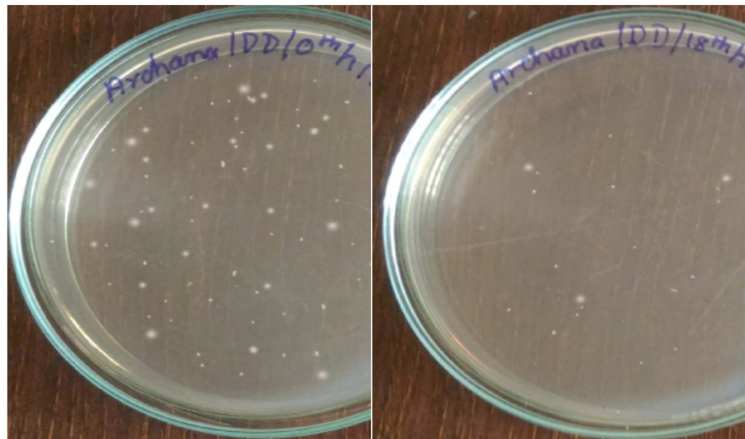
Bacterial Filteration Test for Dip-Dry Finished Samples

Pseudomonas aeruginosa



0th hour plate 18th hour plate

Aeromonas hydrophila



0th hour plate 18th hour plate

Plate XLI

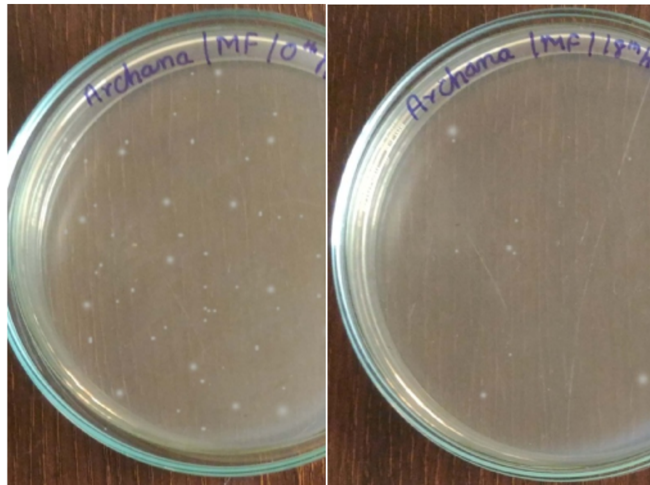
Bacterial Filteration Test for Microcapsule Finished Samples

The result shown in Plate XLIII indicates the number of colonies (CFU) of test organisms such as *Escheritia coli, Staphylococcus sapropyticus, Aromonashydrophila* and *Pseudomonas aerognosa*. 0th hour plate showing more CFU than 18th hour plate. The difference was due to exposure of organisms to the

polyherbal finish on the bandaid for 18hours, whereas fabrics exposed to 0 hours did not have any antibacterial effect on the test organisms.

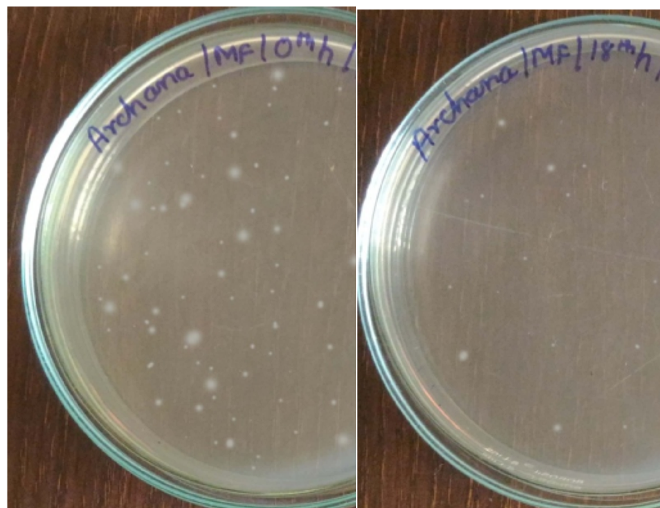
Bacterial Filteration Test for Microcapsule Finished Samples

Escherichia coli **ATCC 25922**



0th hour plate 18th hour plate

Staphylococcus Saprophyticus **ATCC 6538**

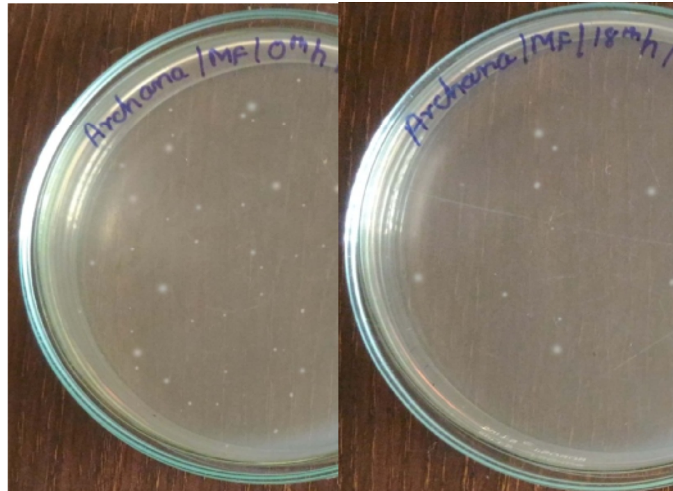


0th hour plate 18th hour plate

Plate XLII

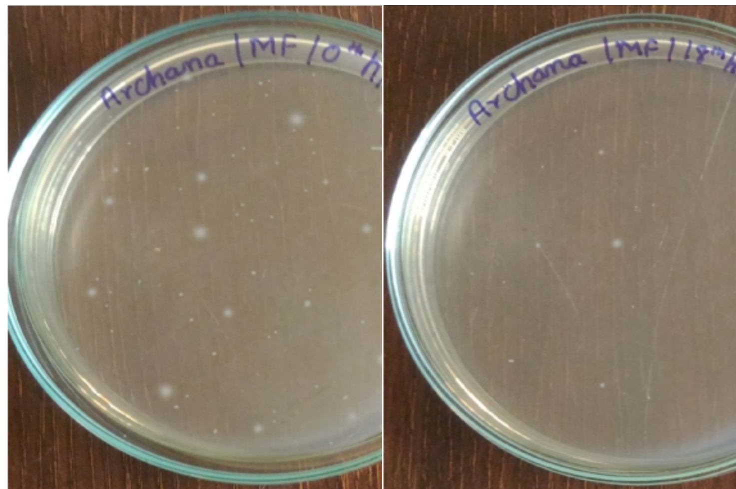
Bacterial Filteration Test for Microcapsule Finished Samples

Pseudomonas aeruginosa



0th hour plate 18th hour plate

Aeromonas hydrophila



0th hour plate 18th hour plate

Plate XLII

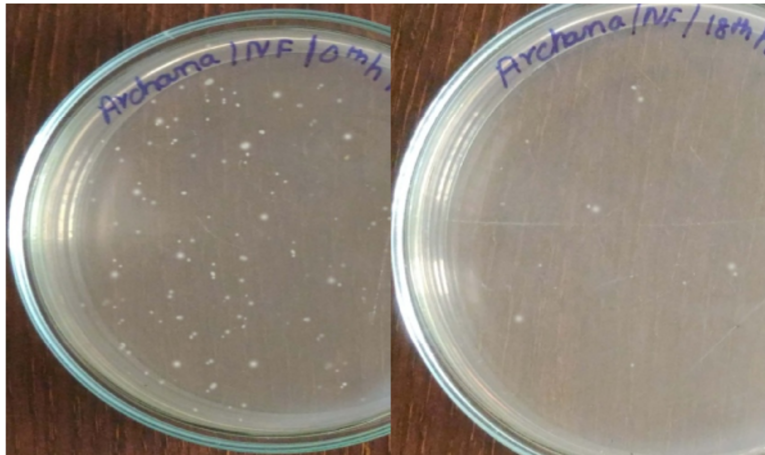
Bacterial Filteration Test for Nanocapsule Finished Test Samples

The result shown in Plate XLIIII indicates the number of colonies (CFU) of test organisms such as *Escheritia coli, Staphylococcus sapropyticus, Aromonashydrophila* and *Pseudomonas aerognosa*. 0th hour plate showing more

CFU than 18th hour plate. The difference was due to exposure of organisms to the polyherbal finish on the bandaid for 18hours, whereas fabrics exposed to 0 hours did not have any antibacterial effect on the test organisms

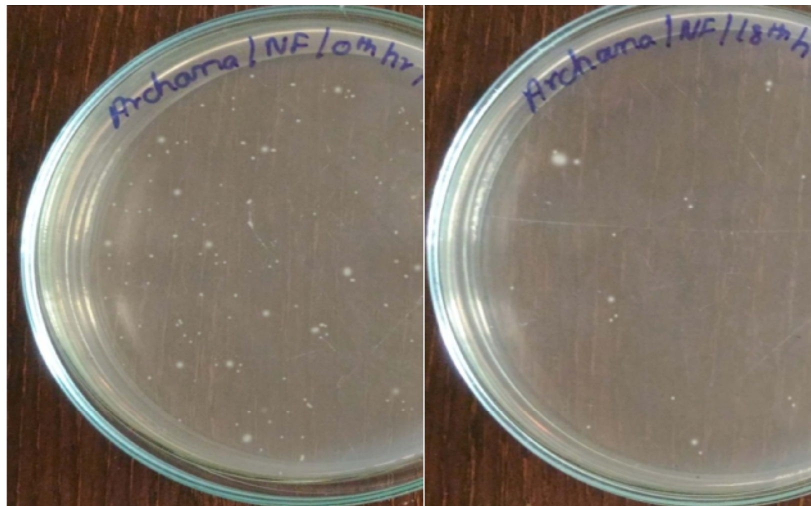
Bacterial Filteration Test for Nanocapsule Finished Test Samples

***Escherichia coli* ATCC 25922**



0th hour plate 18th hour plate

***Staphylococcus Saprophyticus* ATCC 6538**

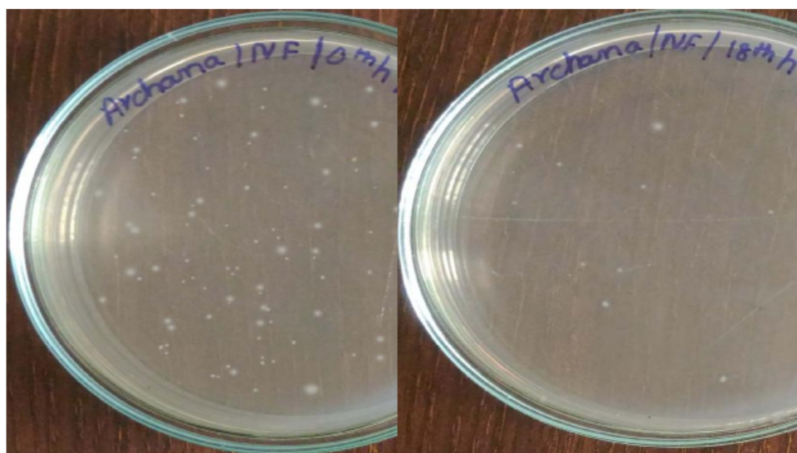


0th hour plate 18th hour plate

Plate XLIII

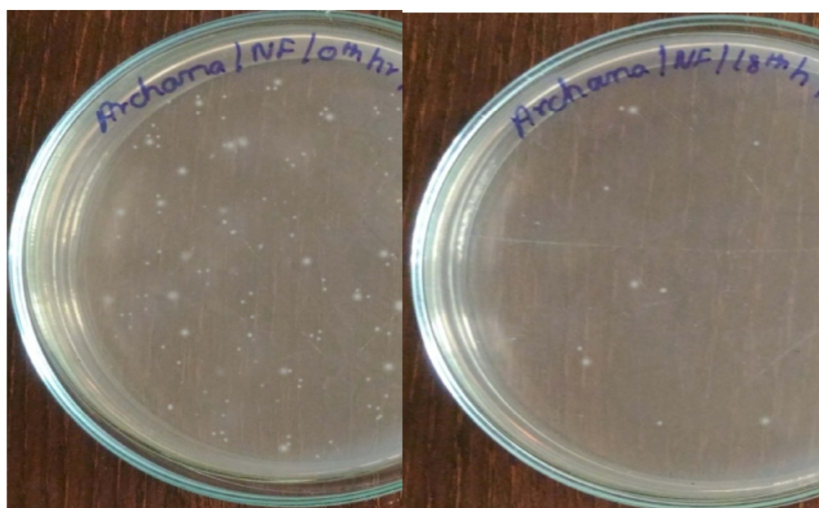
Bacterial Filteration Test for Nanocapsule Finished Test Samples

Pseudomonas aeruginosa



0th hour plate　　　18th hour plate

Aeromonas hydrophila



0th hour plate　　　18th hour plate

Plate XLIII

4.11.2 Fungal Filteration Test for Control and Treated Samples

From the Table XXIII and Plate XLIV, it is confirmed that the samples tested with *Candida albicans* for fungal filteration shows better filteration when compared to the control untreated sample. The Dip and Dry finished fabric

showed 85% of fungal reduction where as, Microfinished and Nanofinished samples showed more or less similar fungal reduction properties as 90% and 96%.

Table XXIII

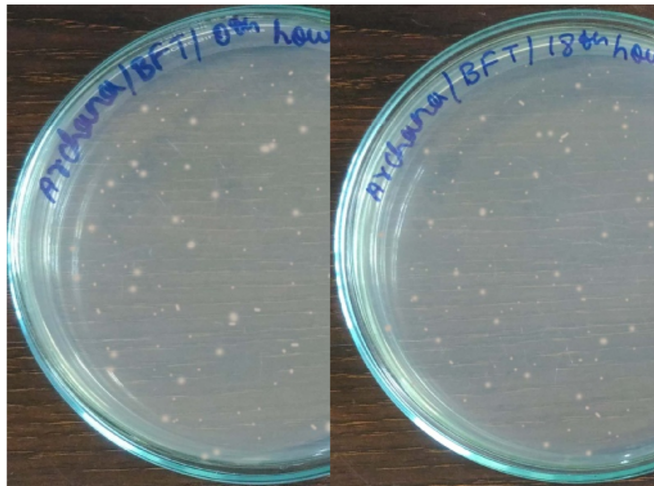
Fungal Filteration Test for Control and Treated Bandaids

S. No.	Test Samples	Fungal Filter (Reduction - %)
		Candida albicans
1	Control	Nil
2	Dip-dry finished	85
3	Microcapsule finished	90
4	Nano finished	96

Among the three finished test samples, microcapsules and nanocapsules finished bandaids materials exhibited excellent anti-fungal properties. As per this test, the fungal numbers were quantitatively filtered by the test materials during the analysis. The incubation period of 18hours had greatly influenced in reducing or filtering the number of test fungi by the bandaids. This was significantly evident from Table XXIII. Dip and dry bandaid exhibited 85 per cent of fungal reduction when tested against the respective test fungi *Candida albicans*. Whereas, microencapsules and nanoencapsules finished bandaid showed a reduction of 90 and 95 per cent of bacterial reduction respectively. From the Table XXIII it is identified that Dip and dry shows less reduction percentage compared to that of Micro and Nano finished bandaids. This may be due to the difference in the durability properties of the anti-fungal agents finished onto the bandaid materials. This result also proved that the finished samples had antifungal property against the selected fungi.

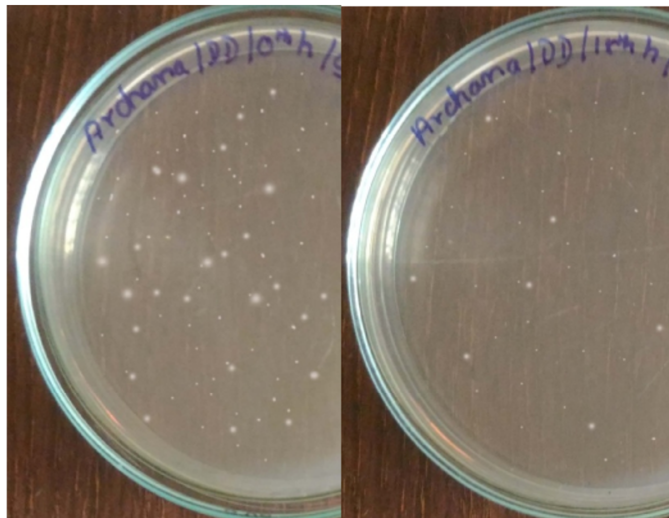
Fungal Filteration Test for Control and Finished Samples

Fungal Filteration Test for Control Samples
Candida albicans



0th hour plate 18th hour plate

Fungal Filteration Test for Dip-Dry Finished Samples
Candida albicans



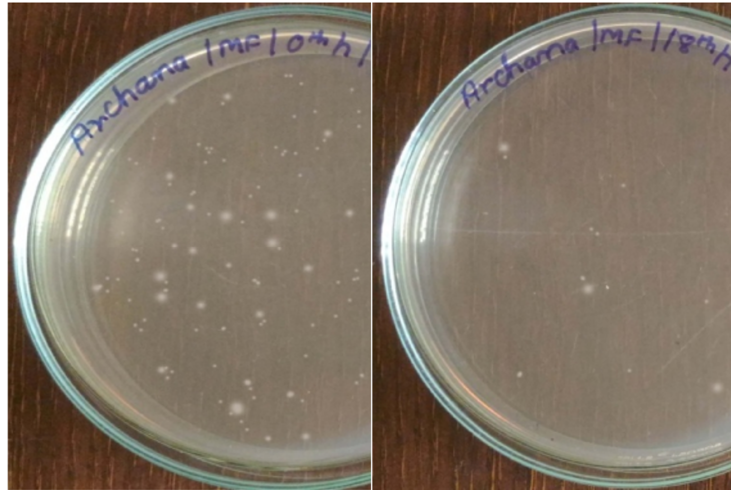
0th hour plate 18th hour plate

Plate XLIV

Fungal Filteration Test for Control and Finished Samples

Fungal Filteration Test for Microcapsule Finished Samples

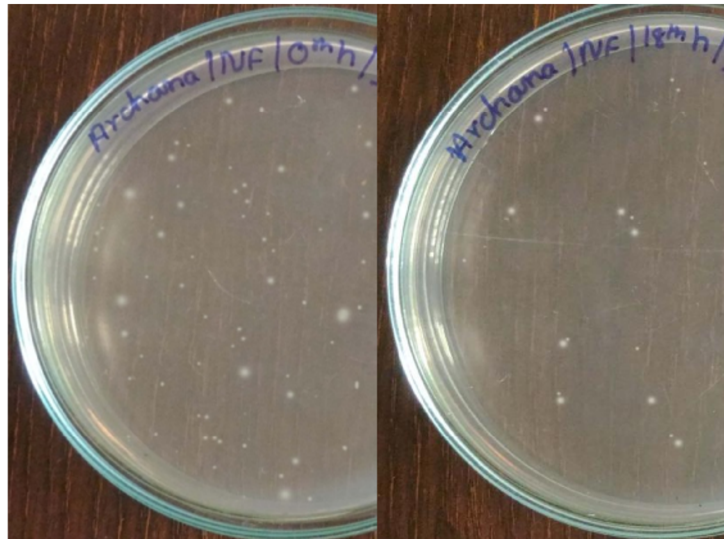
Candida albicans



0th hour plate 18th hour plate

Fungal Filteration Test for Nanocapsule Finished Test Samples

Candida albicans



0th hour plate 18th hour plate

Plate XLIV

From the results, difference in the number of colonies (CFU) of test organisms was evident from the image. 0th hour plate showing more CFU than 18th hour plate. The difference was due to exposure of organisms to the antibacterial nanoparticles for 18hours, whereas fabrics exposed to 0hours does not have any antibacterial effect on the test organisms.

4.12 Bandaid Toxicity Test

The results shown in Table XXIV reveals the concentration, % of cell inhibition and % of viable cell for Dip and dry, Micro and Nano encapsulated finished Bandaids.

Table XXIV

Bandaid Toxicity Test for Control and Treated Banaids

S. No.	Samples	Concentrations (µg/ml)	% cell inhibition (L_{929})	% viable cells (L_{929})
1	Control	10	12.6	87.3
2	Microcapsule samples	55	14.5	85.3
3	Dip-Dry samples	55	12.4	87.1
4	Nano particle samples	60	11.3	88.2

All the four bandaids used in the study were subjected for toxicity test after exposing the required concentrations on the mouse fibroblast cell lines (L_{929}). From the Table XXIV it is clear that during the analysis, the antibacterial agents finished onto the sample swatches (extracts from microcapsule finished swatches, dip-dry swatches and nanoparticle finished swatches) did not exhibit any toxicity for the mouse fibroblast cell lines (L_{929}). This was evident from the percentage of viable cells. All the four samples showed more than 80 per cent of viable cells; and the percentage of cell inhibition was recorded less than 15 per cent for the same type of samples. Thus the samples were proved to be highly biocompatible and nontoxic to the users irrespective of the type of finishing method.

5

SUMMARY AND CONCLUSION

Increasing global competition in textiles has generated many challenges for textile researchers across the globe. The rapid growth in technical textiles and their end-uses has created many opportunities for the development of new innovative products in the field of medical textiles. The medical textile industries have always played an important role in the protective aspects of fabrics. The fabrics have long been recognized as a good support medium for the growth of microbes. A microbe on textile causes the unwanted effects to both the wearers and textile itself. The negative factor of the microbes has resulted in the development of innovative and hygienic finishes on textiles.

Anti-microbial textiles with improved functionality find a variety of applications such as infection control and barrier control (Rajendran et al,2016), health and hygiene commodity, especially the clothes worn close to skin and several medical applications, such as infection control and wound healing materials. Many opportunities are available to add value and improve products by incorporating novelty finishes to protect the textiles against microbial infestation. Demand for the hygienic clothing is on the rise and the green minded customers are opting for eco-friendly textile materials treated with medicinal herbs. These herbal textiles are not only permanently effective but also skin compatible and eco-friendly (Sathianarayanan et al,2010).

Designing appropriate structures for the healthcare and medical industries is the need of the hour. The wound dressing is one of the health care medical textile products which require at most attention. The antimicrobial finished wound dressing band aids reduce the growth and transmission of microorganisms. The use of standard herbal formulations for wound dressing product will determine its acceptability and commercial success. Standardized laboratory tests can provide a clear idea about the functionality of the wound dressing product.

Considering all the above factors, the present study on **"Developing Herbal Antimicrobial Finished Cotton Fabric for Wound Dressing"** was carried out to develop an antimicrobial finished cotton wound dressing band aid using polyherbal extract. Herbal components were extracted from natural plant sources and were applied onto cotton fabric samples in various ratios by different finishing methods and analyzed for effectiveness with the following objectives;

- To study the availability of wound dressing in marketand their demand
- To select the yarn for weaving fabric for wound dressing
- To select medicinal herbs
- To optimize herbal extract concentration and determination of polyherbal formation
- To treat the woven fabric with polyherbal extract and test the fabric performance.
- To develop a product and evaluation.

Experimental Procedure

The experimental procedure adopted for the study consisted of four phases as presented below;

- **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.**

Literature survey was carried out to select the herbal plants possessing antimicrobial effect. Plant authentication test was conducted at Tamil Nadu Agricultural University, Coimbatore. The selected herbs were then dried, garbled and ground into dry powder. Information on commercially available wound dressing band aids was studied to understand the size, price, type and properties. Based on this information an interview schedule was prepared and conducted survey among the surgeons, senior doctors and physicians, a total of fifty

members responded and the required data was collected at PSG Institute of Medical Science and Research, Coimbatore.

Based on the information on commercially available wound dressing band aids properties and opinion of the experts, 30s Ne count yarn for the warp and 10s Ne used for the weft were used. The selected yarns were then tested for the following factors as per ASTM standard methods prior to weaving;

- Count Cv% (ASTM D 1907-01)
- Evenness of yarn U% (ASTM D 1425-96)
- Yarn tenacity cN/tex (ASTM D 2256-97)
- Hariness mm (ASTM D 5647-01)
- Moisture Content % (ASTM D 2495-01)
- Yarn thickness mm (ASTM D 1425-96)
- Twist per inch TPI (ASTM D 1422/D 1422M)

The physical property of the yarn of 30sNe and 10sNe were identified based on the ASTM standard procedure. The yarns count CV% were calculated as 3.3% and 2.8%. The evenness of the yarns were identified as 12.2 U% and 12.1 U%. Similarly, the yarns tenacity and yarn hariness were identified as 18 cN/tex,12 cN/tex and 2.3mm,3mm respectively. Moisture content % of the yarns were identified as 8.5% and 7.7% respectively. The yarn thickness were identified as 0.25mm and 0.71mm. Similarly, the twist of the single yarn (TPI) 32.6(TPI), 22.7(TPI) and balance of the twist(TPI) were calculated as and 22.7(TPI) and 8.2(TPI) respectively.

Weaving of fabric with 30's and 10's count single yarns was performed at Kumaraguru College of Technology, TIFAC Core, Coimbatore. Drum winding method was followed for warp winding. 500 grams of yarn was used to weave two meter length and 18" wide fabric. Totally 8 meter fabric was woven for the study. Semi automatic Shuttle loom (Sakamoto) was used and the loom speed was set as 180rpm and the efficiency was determined as 80%. 1 1 plain weave structure was opted for the study. The ends per inch and picks per inch of the yarn were

noted to be 58 and 30 respectively. The cover factor was calculated as 3.86 mm and 6 mm respectively. Tappet shedding was used to weave plain woven fabric as the existing band aids were made of this structure. The woven fabrics were then desized, scoured and bleached.

- **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 herbs *Abutilon indicum, Tridax procumbenz, Cassia fistula and Cassia auriculata* for the study was selected based on their potentiality of antimicrobial nature as studied through the literature survey. The herbs were collected in and around the districts of Coimbatore and Madurai and Theni. Herbal Extraction was done in Soxhlet apparatus and the resultant extract was subjected to Qualitative Phytochemical Analysis to test the presence of Carbohydrates, Tannins, Saponins, Flavonoids, Alkaloids, Quinones, Glycosides, Glycosides, Terpenoids, Phenols, Coumarins, Steroids and Phytosteroids, Phlobatannins and Anthraquinones. Considering the results of phytochemical screening with three different solvents, Methanolic extract was opted for the 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.**

Microbes such as *Candida albicans, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Escherichia coli* and *Aeromonas hydrophila* were selected to conduct antimicrobial assay. The antimicrobial analysis was done using Agar Well Diffusion method. An Agar plate was prepared and the selected microorganisms were swabbed on the agar plate individually and the herbal extracts were loaded and incubated at $37^{o}c$ for 24 hours.

The MIC of the herbal extracts against the selected microbes were analysed by the two fold serial dilution method to identify the activity breaking point of the microbes. Each of the selected herbal extracts was dissolved in the 5% dimethyl sulfoxide to obtain 2000 µg/ml stock solutions and the samples were diluted to the concentration of 1000, 500, 250, 125, 62.5, 31.25 µg/ml. Further about 100µl of 10^5 CFU/ml of the microbes were inoculated in test tubes with nutrient bath and herbal extract samples and incubated at 37°c for 24 hours. The MIC value had been recorded.

As per the results of MIC, microbes such as *Abutilon indicum, Cassia fistula, Cassia auriculata* showed Minimum Inhibitory Concentration at 250µl whereas, *Tridax procumbenz* showed Minimum Inhibitory Concentration at 500µl. This result formed the basis for the combination of polyherbs. The herbal extraction concentration for antimicrobial testing was confirmed based on the Minimum Inhibitory Concentration of the herbal extract.

Poly herbal extraction was prepared at the ratio of 1:1:1:2 using the sources such as *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* respectively. The contents of selected sources were mixed using magnetic stirrer and put in a double cone blender to get the blend thoroughly. Polyherbal extracts of 50 µl, 100µl, 150µl and 200µl were subjected to antimicrobial analysis in which 200µl showed good zone of inhibition. The extract was analysed by *In vitro* wound scratch assay in fibroblast cell line analysis method. The cells were grown in 24 well plates and the extract were applied to the grown cells and monitored for 1, 4, 12, 24 and at 72 hours.

- **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.**

The herbal extracts of *Abutilon indicum, Cassia fistula, Cassia auriculata* and *Tridax procumbenz* were converted into Nanocapsules and Microcapsules. Later the extract was finished on the fabric with Dip and Drying method, and the Nanoencapsule and Microencapsule particles were applied on the fabric by exhaust method. Finally the treated fabrics were subjected to antimicrobial testing by well diffusion method.

Furthermore, SEM and FTIR testing were carried out on the polyherbal finished fabrics. The treated and untreated fabrics were subjected to physical testing such as Fabric weight, Tensile Strength(ASTM -D -5034: 1995), Sinking(AATCC 17-1994),Air Permeability (IS 11056: 1984),Water absorbency (AATCC 79:2007),Vertical Wicking (BS3424) and Water Holding Capacity(DIN 53923). Self adhesive Bandaid was developed. To assess the efficacy of developed band aid, Microbial Filteration Test and Bandaid Toxicity Test were performed.

Findings of the Study

- From the market survey, it was found that the respondents expressed that the herbal antimicrobial band aid for minor cut, burns and scratches should be made with plain woven cotton fabric of yarn count 30's in the warp yarn and 10's in the weft yarn.
- Hydro colloide, Hydrogel, Alginate dressing, Collagen, Foam and Cloth dressings are best for pressure ulcers, painful or nercotic wounds, high amount of drainage and venous ulcers, transplant sites, burns are injuries with large surface areas, absorbes exuades from wound surface and cover up wound respectively. Each wound dressing material was different and was used for different kind of wounds. Breathable, comfortable and antimicrobial properties are most desirable factors for wound dressing as expressed by 92% of respondents respectively. This is followed by easy wearable, effectively speading up healing prevent infection and suitable for

sensitive skin type as mentioned by 88, 84 and 80% of the respondents respectively. Considering the standard size of band aids (3/4 inch width and three inch length) three inch length and two inch width had been adopted for the study.

- Considering the percentage yield of herbal concentrate upon different solvents, Methanolic extract with respect to all the selected herbs had better yield when compared to the other solvents. With regard to minimum inhibitory concentration under serial dilution method methanolic extraction of *Tridax procumbenz* showed the breaking point at 500µg/ml whereas *Cassia fistula, Cassia auriculata* and *Abutilon indicum* show the breaking point at 250 µg/ml. Among the herbal extracts, *Cassia auriculata* (250µg/ml) indicate better zone of inhibition when compared to other herbs.
- In the case of polyherbal formulation 50 µl, 100 µl, 150 µl and 200 µl of extracts were prepared at the ratio of 1:2:1:1 *Abutilon indicum, Tridax procumbenz, Cassia fistula* and *Cassia auriculata* concentration of 80 %, in which 200 µl of poly herbal extract showed better zone of inhibition which was followed by150 µl, 100 µl and 50 µl. The Antimicrobial assessment for polyherbal finished fabric such as Dip and Dry, Microencapsulation and Nanoencapsulation against microbial pathogens were evaluated by AATCC 147 Agar well Diffusion method, and the sample NEF showed the maximum zone of inhibition as 34,33,32,35 and 29 mm against the microbes followed by MEF and DDF.
- The *In vitro* wound scratch assay in fibro blast cell line analysis method proved the development of tissue growth over the scratch ensures the healing property of the herbal extracts.
- Considering the surface morphology of the herbal finished fabric examined through Scanning Electron Microscopy analysis, the range of particle size of herbal extract is observed between 3.328µm of RSD 09 28, similarly the particle size of nanocapsules had been identified as 171.43nm to 345.25nm.

- With reference to the FTIR results the band width spectrum showed the presence of alkaloids, alkyl halides, carbohydrates groups, alkalides, acidhalides, phenolic compounds, carbohydrates, alcohols, alknes, carboxylic, ester acid, ether in various ranges proving the presence of phytochemicals which lay a base for healing property in the polyherbal extract finished fabrics.
- With reference to fabric properties, the result pertaining to weight of MEF fabric showed minimum increase as 175.02 GSM when compared to the C, DDF and NEF samples as recorded to be 174.18, 174.18 and 174.19 GSM respectively. From the analysis of Tensile Strength results obtained in the warp direction, the MEF fabric with 45.52 Kgf showed maximum tensile strength followed by DDF and NEF with 40.86 and 40.20 Kgf respectively. As far as the strength in weft direction of the fabrics, maximum strength was noted in original fabric with 58.27 Kgf, followed by MEF, NEF and DDF fabrics with 39.47 and 35.96 and 35.33 Kgf respectively. The elongation of the samples along the warp direction increased in NEF sample but decreased in the DDF and MEF samples when compared to the original as noted to be 6.53, 9.73 and 7.57 per cent respectively.
- When considering the sinking time in seconds, the herbal finished fabrics had shown reduction in sinking time than the control. The herbal extract finish has enhanced the wettability of the finished fabric. Sample DDF took two seconds whereas MEF and NEF samples have taken only 1 second to sink. The water holding capacity of the finished fabrics had increased for the herbal finished fabrics. DDF finished fabric had the water holding capacity of 70%. This was followed by MEF and NEF finished fabrics with a holding capacity of 66% and 53% respectively. From the analysis of absorbency tests it could be identified that that the control and the MEF fabric took three seconds to absorb a droplet of water. Whereas, DDF and NEF samples had taken two and one seconds respectively. The wickabilty of DDF sample was noted as 0.39 minutes along the warp and weft direction. It has increased gradually to 0.410, 0.430 and 0.440 minutes in C, NEF and MEF samples respectively.

- The air permeability of sample C and MEF is 79.3 and 79.1 c.c/cm.sq./sec respectively and hence it could be concluded that the fabrics have good air permeability.

- With regard to Bacterial Filteration test, the samples tested with *Escherichia coli* ATCC 25922, *Staphylococcus saprophyticus* ATCC 6538, *Aeromonas hydrophila* ATCC 100-2004 and *Pseudomonas aeroginosa* ATCC 100-2004 showed better filteration when compared to the control untreated sample. The Dip and Dry finished fabric show 80% to 85% of bacterial reduction whereas Micro encapsulation finished and Nano encapsulation finished samples showed more or less same bacterial reduction properties as 90% to 95% and 93% to 96%.

- With respect to Fungal Filteration test, the samples tested with *Candida albicans* for showed better filteration when compared to the control untreated sample. The Dip and Dry finished fabric showed 85% of fungal reduction whereas Micro encapsulation finished and Nano encapsulation finished samples showed more or less similar fungal reduction properties as 90% and 96%.

- During the toxicity test analysis, the antibacterial agents finished onto the sample swatches (extracts from dip-dry swatch, micro encapsulation and nano encapsulation finished swatches) did not exhibit any toxicity for the mouse fibroblast cell lines (L_{929}). All the four samples showed more than 80% of viable cells; and the percentage of cell inhibition was recorded less than 15% for the same type of samples. Thus the samples were proved to be highly biocompatible and non toxic to the users.

Conclusion

Indian history of medicinal plants has proved the use of herbs for the cure of small cuts to heavy burns. From the long list of herbs *Abutilon indicum, Tridax procumbenz, Cassia fistula* and *Cassia auriculata* are some of the common herbs that are found in abundant. These herbs are proved to have antimicrobial activity

and produce an efficacious active antimicrobial compound which is eco-friendly. Micro and nano encapsulation of these herbs confirmed the better wound healing property with higher microbial reduction. Poly herbal finished adhesive band aids did not possess any toxicity; therefore these products could be developed and effectively used.

Recommendation

- Similar studies with potential medicinal herbs could be tried to make health care products.

- Cell line assay for combination of medicinal herbs is worth attempting.

Limitations

Due to time limitation the prepared self adhesive band aid was not subjected to animal/human study.

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Appendix I
Commercial Dressings Available in the Market and Their Rating

S.No	Type of bandaids	Available size	Price	Properties
1	Gauze dressing	Begin from 3 meters	10rs-200rs	Antimicrobial gauze, conforming gauze, impregnated gauze, bordered gauze, packing gauze, others (sterile gauze pads, non-sterile gauze pads, stretch gauze bandages, and paraffin gauze)
2	Semi permeable flim dressing	5 x 7 cm, 9 x 10 cm,15 x 15 cm,9 x 25 cm,9 x 35 cm	Range from 7rs - 200rs	Water proof, bacterial proof, transparent pu flim, non adherent absorbency pad, silver layer,
3	Semi permeable foam dressing	4" x 4",6" x 6", 6" x 8"	Ranges from rs115 and more	Duoderm extra thin foam dressing, CGF dressing, Ag hydro fiber dressing.
4	Hydro gel dressing	2" x 2",4" x 4",	Ranges from rs7500 per pack of 10	Hydro gel sheets, amorphous hydro gel, impregnated hydro gels dressings
5	Hydrocolloid dressing	5 x 5cm, 10 x 10cm, 10 x20cm, 15 x 15cm, 20x 20cm, 10 x 15cm	Ranges from rs7500 per pack of 10	With border adhesive finishing, without border adhesive finishing, ConvaTec Duo DERM CGF - Control Gel Formula wound
6	Absorbent and bio-degradable dressing	5 x 5cm, 10 x 10cm, 10 x20cm, 15 x 15cm, 20x 20cm	Range from rs200	Haemostatic agent-surgical sponges, wound dressing, non-woven fabrics for surgical use
7	Bio active wound dressing	5 x 5cm, 10 x 10cm, 10 x20cm	Range from pre piece rs390	Alginate dressing, antimicrobial dressing and hydrocolloids dressings

Appendix I

Different Type of Commercial Wound Dressing

Gauze wound dressing

Semi permeable flim dressing

Semi permeable foam dressing

Hydro gel dressing

Dressing

Hydrocolloid biodegradable wound dressing

Absorbent and Bioactive wound dressing

Appendix - II

Interview Schedule to Collect Information with Respect of Wound Dressing Bandaids

Investigator : **S.Archanaa Preetha (Ph.D Scholar)**
Department of Textiles and Clothing, Faculty of Home ScienceAvinashilingam Institute for Home Science and Higher Education for Women,Coimbatore

1. Name :
2. Address :
3. Designation :
4. What are the type of wound dressing bandages were you use for acute* wounds please tick

List of Bandages		Please Tick	Reasons
Hydro colloid	Burns		
	Wounds that emitting liquids		
	Necroticwounds		
	Pressureulcers		
	Varicose ulcers		
Hydro gel	Leaking wounds		
	Painful or necrotic wounds		
Alginate dressing	High amounts of drainage		
	Burns		
	Venous ulcers		
	Packing wounds		
	Higher state pressure ulcers.		
Collagen	Chronic wounds		
	Pressure sores		
	Transplant sites		
	Surgical wounds		
	Ulcers		
	Burns or injuries with a large surface area		
Foam	Injuriesexhibitodour		
	Absorbs exudates for wound surface.		
	Used on surgical incision sites		
	Burns		
	ulcers		
	IV sites		
Cloth	Cover open wounds		
	Grazes		
	Cuts		
	Areas of delicate skins		

List of Properties Which are Best Suitable for Bandaids Used for Acute Wounds Please Tick

Best Suitable Properties for Wound Dressing	Please Tick	Reasons
Non-breathable		
Breathable		
Easy wearable		
Comfortable		
Self-adhesive		
Suitable for sensitive skin type		
Reduce pain		
Remove dead tissue		
Cooling effect on burning wounds		
Absorb excess liquid		
Containing sodium and seaweed fibres		
Helping to bring the wound edges together		
Aiding the growth of new blood vessels		
Effectively speeding up healing		
Allow water vapour to enter		
Keeping the area moist		
Promoting faster healing		
Antimicrobial property		
prevent infection		
Used to dress all shapes and sizes		
Biodegradable		

***Signature**

Appendix-III

Plant Authendication

The Plant Authentication Report for the Selected Herbs were given below

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी.एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. /2576

दिनांक/Date: 21st December 2018

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimens brought by you for authentication are identified as follows:

(1) *Abutilon indicum* (L.) Sweet – MALVACEAE

(2) *Senna auriculata* (L.) Roxb. – CAESALPINIACEAE

(3) *Tridax procumbens* L. – ASTERACEAE

(4) *Cassia fistula* L. - CAESALPINIACEAE

The identified specimens are returned herewith for preservation in their College/ Department/ Institution Herbarium.

21/12/2018

डॉ सी मरुगन/Dr. C. Murugan
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /
Scientist 'D' & Head of Office

वैज्ञानिक 'डी' एवंकार्यालयअध्यक्ष
SCIENTIST 'D' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयंबत्तूर / Coimbatore - 641 003.

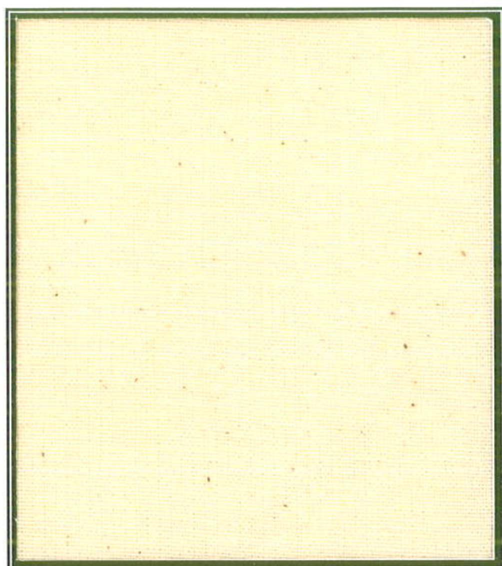
सेवा में / To

Ms. S. Archanaa Preetha
Ph. D. Research Scholar
Department of Textiles and Clothing
Avinashilingam Institute for Home Science
And Higher Education for Women
Coimbatore – 641 043

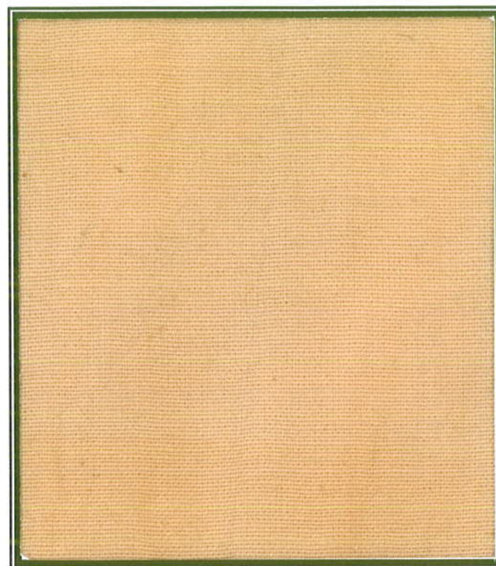
Appendix - IV

Control and Herbal Finished Samples

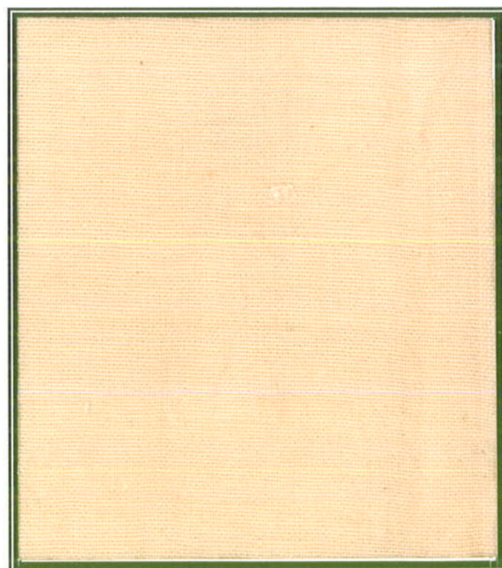
The polyherbal antimicrobial finished fabric samples of C,DDF,MEF and NEF are given C-Control,DDF-Dip and Dry finish, MEF-Microencapsulated finish and NEf-Nanoencapsulated finish



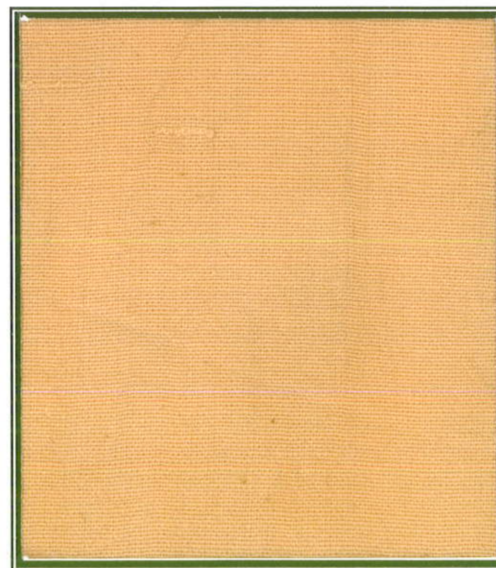
C



DDF



MEF

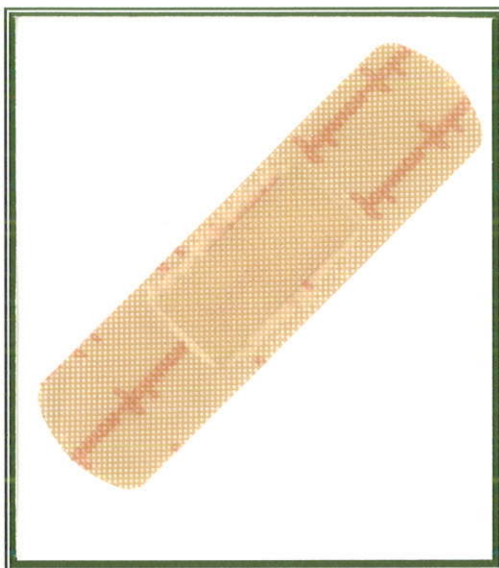


NEF

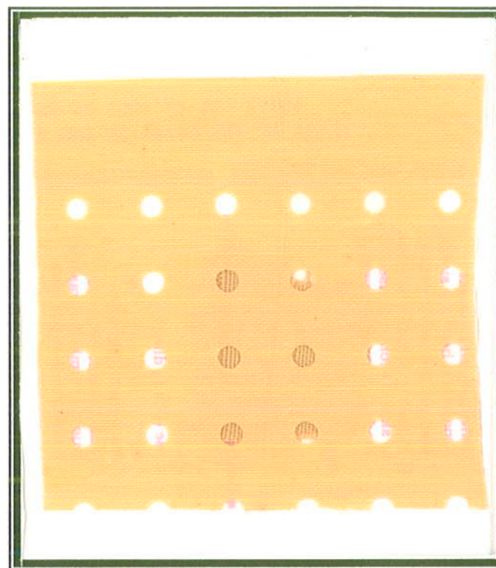
Appendix - V

Self adhesive Bandaid

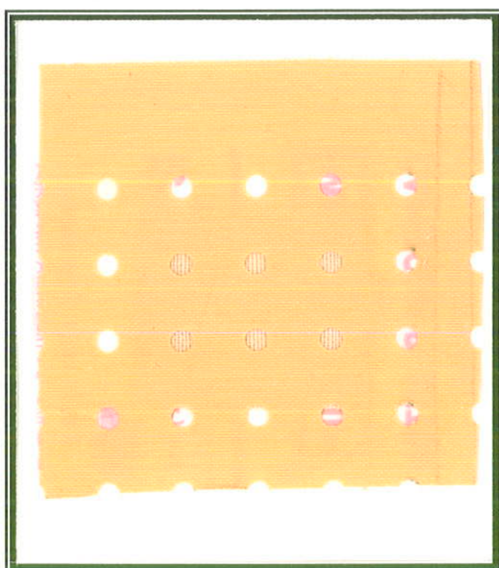
The polyherbal finshed fabric had been developed into selfadhesive bandaids of DDF, MEF and NEF along with the commercial bandaids available in the medicals.



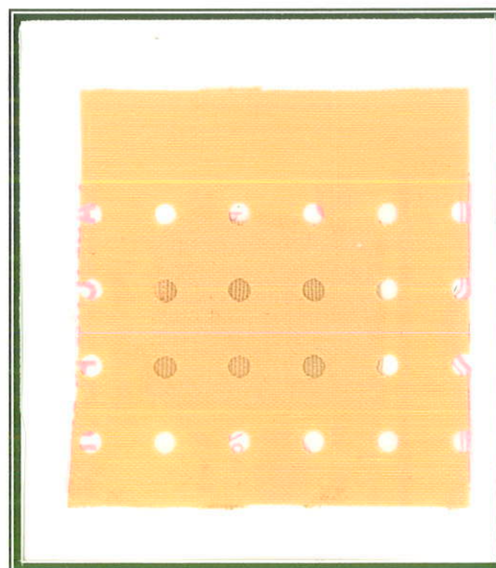
Commercial Bandaid



DDF



MEF



NEF

Papers Published

TITLE	JOURNAL/BOOK	YEAR OF PUBLICATION, COAUTHOR
Natural Herbal Extract for Medical Textile	ACTPAQ-2011,3rd National Conference	2011, Dr.G.Bagyalakshmi
A Study on Phytochemicl Screening and Antimicrobial Property of Tridax Procumbenz	9th NABS National Conference	2016 ,Dr.G.Bagyalakshmi
Efficacy of Traditional Medicinal Plants in Functional Finishing of Textiles	2017,Shanlax International Journal of Arts and Science	2017, Dr.G.Bagyalakshmi
Encapsulation of Polyherbal Extract for the Development and Evaluation of Medical Textiles	IJAR	2017, Dr.G.Bagyalakshmi
Polyherbal Antimicrobial Finish on Textile Material Used in Developing Medicate Products	IJETSR	2017, Dr.G.Bagyalakshmi