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Modification of cotton fabric with Natural antimicrobial agents for Ecofriendly protective textiles

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University of Rajshahi, Rajshahi

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MODIFICATION OF COTTON FABRIC WITH NATURAL ANTIMICROBIAL AGENTS FOR ECOFRIENDLY PROTECTIVE TEXTILES

Ph.D. Thesis

A Dissertation submitted to the Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Bangladesh for the fulfilment of degree of Doctor of Philosophy

By

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June, 2021

DEDICATED

TO

My parents and family members

DECLARATION

I do hereby declare that the entire research work submitted as the thesis entitled "**MODIFICATION OF COTTON FABRIC WITH NATURAL ANTIMICROBIAL AGENTS FOR ECOFRIENDLY PROTECTIVE TEXTILES**" under the supervision of **Professor Dr. Md. Ibrahim H. Mondal, Professor Dr. Md. Rezaul Karim Sheikh** towards the fulfillment for the degree of Doctor of Philosophy in the Department of Applied Chemistry and Chemical Engineering, University of Rajshahi is based on the results of my own investigation and has not ever been submitted before in any form for any other degree at any place. I further declare that the whole work of the submitted thesis paper for the degree of Doctor of Philosophy to the University of Rajshahi is based on my original investigation except references used in the text of the thesis.

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CERTIFICATE OF RESEARCH

This is to certify that the Ph.D. thesis entitled **"MODIFICATION OF COTTON FABRIC WITH NATURAL ANTIMICROBIAL AGENTS FOR ECOFRIENDLY PROTECTIVE TEXTILES"** submitted by **Joykrisna saha,** Roll No.: 01611175504, Registration No.: 0045, Session: 2015-2016, to the Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Bangladesh, in partial fulfillment for the award of the degree of **Doctor of Philosophy** has been completed under our supervision. This is a original record of research work carried out by the candidate.

To the best of our knowledge, the contents of this thesis, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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The Author

ABSTRACT

In recent time's health and hygiene issues have achieved the greatest attention among the awareness people of all over the world**.** Health and hygiene are the primary obligations for human beings to live comfortably and work with maximum safety. The aim of the present work is to develop environment friendly protective textiles using *Aloe vera*, chitosan and sericin on bleached cotton woven fabrics for medical and health care apparel against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. *Aloe vera*, chitosan and silk sericin are natural biopolymer which exhibited different significant biological property. *Aloe vera* extract was prepared from *Aloe vera* leaves through methanol solvent using a rotary evaporator. Chitosan was made from shrimp shell through several steps of alkali and acid treatments and silk sericin powder was obtained from a boiled water solution of silk cocoons through ethanol precipitation.

Extracted *Aloe vera*, chitosan and sericin powder were characterized by Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Energy dispersive spectroscopy (EDS), UV-visible spectrophotometer, X-ray diffraction (XRD). Solubility, degree of deacetylation, antibacterial activity, antioxidant property and UV protection factor were also analyzed. Both quantitative and qualitative methods were used to assay the antimicrobial activity. The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Chitosan was found to have a 90% degree of deacetylation. *Aloe vera* solution absorbed three region of UV radiation. On the other hand silk sericin and chitosan solution only absorbed the UV-C region radiation. FTIR analysis illustrated that *Aloe vera*'s notable broad peak of about 3265 cm-1 was attributed to phenolic -OH stretching, which was linked to the existence of aloe-specific phenolics such as flavonoids and anthraquinones. Chitosan and sericin revealed the N-H bending functional groups. The crystallite diameter of *Aloe vera* was highest and sericin showed the lowest crystallite diameter (2.17nm). The surface morphology of chitosan showed a tiny porous structure. *Aloe vera* sample exhibited a rough and high aggregated appearance. On the other hand, silk sericin displayed a more sponge-like agglomerated arrangement. *Aloe vera* powder was exhibited the highest thermal stability. Among the materials, chitosan solution showed best antibacterial activity and sericin displayed lowest antibacterial activity. In

comparison to other specimen, *Aloe vera* solution had a high scavenging potential for DPPH radicals. The extracted *Aloe vera* showed better UV resistant property.

The pad-dry-cure method was used to apply *Aloe vera* extract, chitosan, and sericin to cotton samples, either alone or in combination, using critic acid as the cross-linking agent. Different physical and mechanical properties such as stiffness, thickness, GSM, whiteness index, tensile strength, crease recovery and abrasion resistance of modified fabrics were determined. The add-on% of chitosan-treated fabric was greater than that of *Aloe vera* and sericin treated fabric, in the same concentration. After treatment crease recovery angle was increased by 12%, 15% and 14% for 2 g/l *Aloe vera*, chitosan and sericin treated cotton fabrics. Again it was found that absorbency sharply increased after treatment of *Aloe vera*, chitosan and sericin compared to critic acid treated fabric which could be attributed to the hydrophilic groups containing in the *Aloe vera*, chitosan and sericin. The tensile strength of 2 g/l *Aloe vera*, chitosan and sericin treated cotton fabric was declined by 22%, 30% and 28% respectively. As the concentration of the finishing agent was increased further, the tensile strength of the treated fabric did not decrease significantly. The whiteness index of the fabric was progressively reduced by treatment with increased *Aloe vera*, chitosan, and sericin concentrations. Soil degradation test proved bio compatibility of the treated samples. The combination of 5g/l chitosan and 5g/l *Aloe vera* applied to cotton fabric demonstrated excellent antimicrobial activity against gram-positive *S. aureus* and gram-negative *E. coli* bacteria at the quantitative examination, which was higher than the 10 g/l Aloe vera treated result. The incorporation of *Aloe vera*, chitosan and sericin on the untreated cotton fabric was investigated by FTIR, XRD and thermal analysis. High-resolution scanning electron microscopy was used to examine the surface morphology of treated and untreated fabrics. Thermal comfort was not considerably changed by finishing treatment as measured by air permeability, water vapor permeability, and thermal conductivity. The UV protection factor of finished cotton fabric was also significantly improved. Thermal comfort qualities were also statistically analyzed.

Keywords: *Aloe vera*, Chitosan, Sericin, Cotton fabric, Antimicrobial, Antioxidant, UV resistant, Thermal comfort

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LIST OF ABBREVIATIONS

AATCC	=	American Association of Textile Chemists Colorists
ASTM	=	American Society for Testing and Materials
B. S	=	British Standard
cm	=	Centimetre
Cm^{-1}	=	Per centimetre
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl
DSC	=	Differential Scanning Calorimetry
E. coli	=	*Escherichia coli*
EDS	=	Energy Dispersive Spectroscopy
FTIR	=	Fourier Transform Infrared
g/l	=	Gram per litre
gm	=	Gram
h	=	Hour
min	=	Minute
ml	=	Mililitre
ml^{-1}	=	Per mililitre
^{o}C	=	Degree Celsius
OD	=	Optical density
OP	=	Oral presentation
PP	=	Poster presentation
RSA	=	Radical scavenging activity
S. aureus	=	*Staphylococcus aureus*
SEM	=	Scanning Electron Microscopy
TGA	=	Thermogravimetric Analysis
UPF	=	Ultra-violet Protection Factor
XRD	=	X-ray Diffraction
ZOI	=	Zone of Inibition
%	=	Percentage

Chapter One
GENERAL INTRODUCTION

1.1 An introduction to protective textiles

Human comfort and safety requires health and hygiene. Consumers' attitudes towards hygiene and their vigorous lifestyle have generated a strong market for protective textiles. Sales of such textiles have encouraged research in anti-microbial clothing. Value-added apparel has changed the world's textile industry scenario. Research shows that consumers want functional clothing. This functional or protective clothing protects the wearer from bacterial contamination, UV radiation, oxidative stress and injuries which could lead to illness or even death. Protective textiles are garments and other fabrics that shield the wearer from hazardous environmental conditions (**Khurshid et al., 2015; Thilagavathi et al., 2008).**

Increased global competition in the textiles industry has posed numerous challenges for textile researchers and manufacturers. In recent years, the population and pollution have compelled researchers to develop new health and hygiene products for the benefit of humanity. The production of textiles with antimicrobial properties is one of the greatest issues for hygiene life style. In this regard, the expansion of antibacterial textile market is growing rapidly **(Gao & Cranston, 2008**). One aspect is the developing market for antibacterial clothing and materials. At present, foremost trend in the textile factory has been the development of high-value fabrics for apparel and technical textiles and other textiles which can protect users from harmful UV rays, microbial activity and free radical effect. The market also demands textiles with improved function such as flame-retardant, crease resistant, antistatic and anti-repellent properties with the help of different chemical finishes (**Ibrahim et al., 2010).**

Bacteria, everywhere around us, available in our surroundings are categorized in two types, one is can be pathogenic and another is or non-pathogenic. Between them, pathogenic bacteria are very harmful especially in hospital as it causes dangerous infection of the surgical patient despite the enormous advancement in the medical and pharmaceutical sectors. Pathogenic bacteria cause human and animal disease. Sick patients put them into the hospital environment and they can affected by cross infection.

The main source of cross-infection in the surgical gowns, masks, theatre drapes, bed sheets and pillowcases (**Ristić et al., 2011; Shahidi et al., 2007**). Americans spend more than above $120 billion a year for the treatment of transferable infections. Conversely, the US government spends $5 billion a year solely on the treatment of pathogens that are immune to antibiotics (**Boucher, 2010**). Recently, the large pharmaceutical companies have not been inventing new antibiotics. Because research for them is time-consuming, expensive and risky. Therefore, contrary to, searching the smart better solutions which are both inexpensive, and yet effective to prevent the spread of pathogens **(Ding & Smith; 2013)**. Therefore anti-microbial clothing can be one such solution.

Both pathogenic and non-pathogenic bacteria grow in textile clothing creating stains, bad odors, discoloration and deterioration. The antimicrobial agent can control, annihilate or repress the development of microorganisms and their negative effects of cross-infection, various diseases, odor, staining and deterioration of textile quality.

Cotton is the most popular fibre in the textile industry. Majority of the people in the World like cotton fabric due to its comfortable attributes as cotton fabric can readily absorb moisture. On the other hand, cotton possess most suitable atmosphere for proliferation of microorganism. This microorganism creates stain, bad odor, and discoloration. So the treatment of cotton fabric with natural antimicrobial agents is the appropriate way to avoid detrimental actions of microorganisms **(Chang et al., 1998; Seventekin & Ucarci, 1993**). Modification of cotton fabric with natural antimicrobial agents is to develop the further desirable properties and overcome the some disadvantages. Modification means a change to something, usually to improve it or make it more acceptable (**Mucha et al., 2002**).

Antimicrobial finished textiles with improved functionalities are suitable for a wide range of applications, especially in numerous medical applications such as infection control, wound dressing and as barrier material. Antimicrobial finished textiles serve two purposes: they protect both the wearer and the textiles themselves. The antimicrobial finish is added to the fabric in such a way that it retains its physical properties, look, and feel. There is no chemical odor left (**Hooda et al., 2013**).

Among anti-microbial agents available in the market for the use on textiles application, many are synthetic and may be harmful to the environment. As a result, a growing number of consumers are deciding for herbal antimicrobial textile finishes. It must be assured that these substances are not only long-lasting but also skin-safe and environmentally friendly (**Sathianarayan et al., 2010**). There are several synthetic antimicrobial agents are available for textile finishing. Common agents include nano-silver, titanium di-oxide, triclosan, quaternary ammonium compounds and so on. But the price of synthetic antimicrobial agents is high and synthetic antimicrobials create environmental hazards (**Thilagavathi & Kannaian, 2010**).

Applications of natural antimicrobial agents are now common in medical and health care textiles. They are environmentally-friendly, safe, skin-friendly, and non-toxic compared to synthetic antimicrobial agents (**Joshi et al., 2009**).

1.2 Cotton

Cotton is a natural fiber made of cellulose polymers that has a wide variety of textile applications. Cellulose is the world's most plentiful sustainable organic resource. The annual amount of cellulose generated is in the billions of tons, indicating its high economic value. Plant cells are made up primarily of cellulose **(Yang, 2008)**.

1.2.1 Brief description on cotton

Cotton is a natural fiber that accounts for just under half of all fiber sold worldwide. Cotton is produced on a plant that is a member of the Hibiscus family. The plant is a leafy, green shrub with pink flowers that develop into cotton bolls as the plant matures. Cotton is grown on every continent except Antarctica. Cotton fiber can be used to make everyday items like jeans, T-shirts, sheets, towels, and hospital gowns. Cotton fiber is used to make yarn and cloth, and the seeds are crushed for oil and animal feed **(Yang, 2008)**.

1.2.2 Chemical structure of cotton

Cotton is mainly composed of cellulose substance also found in other textile fibres. Cellulose is a tightly packed micro fibril of linear chains of linked D-glucose. Two anhydroglucose units are linked together by beta-(1→4) glycoside bonds and create cellubiose repeating unit of the polymer chain. Cellulose is technically a polymer of cellubiose rather than -D-glucose since it is made up of repeating cellubiose units. The

"degree of polymerization" refers to the number of cellubiose repeating units that are joined together to form the cellulose polymer. Carbon (44.4%), hydrogen (6.2%), and oxygen (49.4%) are the three major constituents of cellulose (**Yang, 2008**). The chemical structure of cellulose molecule is shown in **Figure 1.1.**

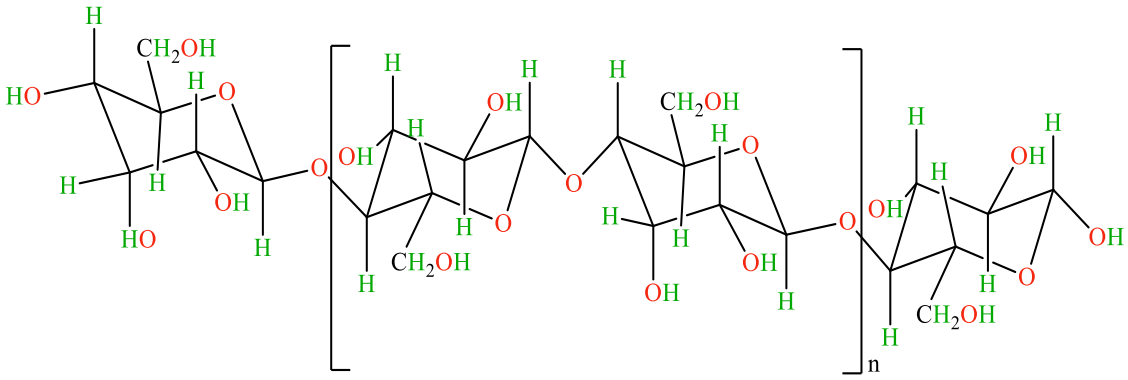


Figure 1.1: Cellulose molecule (Yang, 2008)

1.2.3 Physical and chemical properties of cotton

More than 90% cellulose and 8% moisture exists in cotton fibre. Cotton fibre possesses 65-70% crystalline domain and a 30-35% amorphous domain. The fiber molecules are tightly packed and parallel to one another, as shown by crystallinity. Cotton has a polymerization degree of 9000-15000 on average (**Gao & Tang, 1996**). Cotton is soft, comfortable and wrinkles easily. It absorbs perspiration quickly. Cotton fibre has good durability due to its composition and compatibility with human skin. This explains its universal use in clothing. It has good color retention and is good to print on. Cotton is hypoallergenic and does not cause allergies or skin irritation. As it is a natural product, containing no chemicals. Cotton is the largest material for the overall antimicrobial hospital textiles market. This is because cotton is susceptible to microbial attack, as is it a naturally occurring plant fiber. Cotton is very soft in nature and renders comfort when it is in contact with the skin. Due to comfort property, it is used more as compared to other antimicrobial hospital textiles material. This factor is driving the demand for cotton in the antimicrobial hospital textiles market.

Cotton's tensile strength is heavily influenced by its moisture content. The strength of regenerated cellulose fibers decreases as the moisture content rises. Cotton, on the other hand, tends to get stronger as moisture levels rise. This is thought to be due to intermolecular hydrogen bonding and the degree of crystallinity of the cellulose chains (**Lewin, 2007).**

1.2.4 Chemical reactivity of Cotton

One basic anhydro-D-glucose unit $(C_6H_{10}O_5)_n$ exists one primary and two secondary alcoholic hydroxyl groups, are at numbers 6, 2 and 3 carbon positions respectively. With the use of dyes and finishing chemicals, these groups may undergo substitution reactions. The hydroxyl groups are also responsible for the principal sorption and dye-ability of cellulose materials. Primary hydroxyl groups are more reactive than secondary hydroxyl groups in many reactions (most notably esterification). On the other hand, the secondary hydroxyl group in the C2 position exhibits the highest reactivity in etherification. High water solubility depends on high numbers of hydrogen bonding with hydroxyl groups of adjacent chains (**Zhang, 1997).**

1.3 Microorganisms

Microbes are the tiniest organisms that are invisible to the naked eye. Bacteria, viruses, algae, and fungi are examples of microorganisms. They grow very rapidly under warm and moist conditions.

Dust mites are tiny eight-legged creatures similar to spiders that live in household textiles like sheets, bed linen, pillows, mattresses, and carpets. Dust mites feed on the cells of human skin. In humans, their waste products can cause allergic reactions and respiratory problems.

Unicellular microorganisms are available in our surroundings and may be categorized into two types: pathogenic and non-pathogenic. Pathogenic microorganisms can cause cross-infection as well as disease. Non-pathogenic microorganisms develop stains on fabric and damage the performance properties of the fabric (**Gupta & Laha, 2007).** Microbial infestation poses dangers to both living and non-living matter. Microorganisms wreak havoc on textile raw materials and manufacturing chemicals, as well as wet mill operations, roll or bulk products in storage, and finished goods in storage and transportation. Some of the negative effects of bad microbes include offensive odors from garments such as gloves, underwear, socks, the spread of diseases, staining, and deterioration of textiles.

1.3.1 Factors influencing the growth of microbes in textiles

Payne & Kudner **(1996)** stated that cotton textiles have an optimal breathing atmosphere for bacteria, yeast and fungi in close proximity to the human body. All the requirements, including nutrients, water, oxygen and warmth, needed for the growth of these species are fulfilled.

Nutrients

Microorganisms may get nutrients from dirt, dust, perspiration, and some cloth finishes. Salts, amino acids, carboxylic acids and other essential nutrients are found in perspiration. A possible carbon source is often dead skin cells or oils secreted by the skin. Cellulose itself is not a nutrient, but certain bacteria develop extracellular cellulolytic enzymes that convert cellulose into readily metabolized glucose. This has the dual effect of fostering further growth while also causing the fiber to degrade.

Water

Cotton fibres have a hydrophilic and porous structure. Enough water would be available to sustain fungal growth in a humid climate. Bacteria need more water and a moist fabric to thrive **(Nelson et al., 1991**).

Oxygen

The atmosphere provides a ready source of oxygen.

Warmth

McNeil et al. (**1963**) noted that most fungi and bacteria will grow at ambient temperatures of 20-40°C. Certain bacteria grow in slightly warmer conditions of clothing or bedding that is kept close to the skin. These bacteria, such as *Staphylococcus aureus, Staphylococcus epidermidis, and Cornybacterium* species are most commonly found on the skin. Synthetic fabrics such as polyester and nylon, according to **Mairal & Patel (2002**), do not have suitable living environments for microorganisms. The fibres have a lower water retention capacity and are more resistant to enzyme degradation. However, these fibres will also support some microbial growth.

1.4 Bacteria

Bacteria, a type of microbes, are tiny creatures that individually are too small to be seen with the naked eye. But, they can have a major impact on human life by causing disease.

They can cause a local disease like a skin infection or systemic disease, if they can transfer through the pores to the blood or lymph system. They need to metabolize nutrients to survive. According to **White et al. (2002)** they will multiply from one organism to over one billion in just 18 hours if growth conditions are favorable.

1.4.1 Chemical composition of bacteria

Bacteria are single cell creatures that appear in three forms: spherical (cocci), rod (bacilli), and spiral (spirillum). They can be arranged in pairs, clusters, or chains. Bacteria comprise a cytoplasmic membrane and a rigid layer. The cytoplasmic membrane is the internal structure which comprises of a ribosome and a nucleoid. Ribosome translates genetic messages to proteins and the nucleoid contains DNA of the cell. The rigid layer, also called the surface layer, consists of the capsule, cell wall, and plasma membrane. The capsule protects the cell wall and maintains the overall shape of the cell. For the movement of ions, nutrients, and waste, the plasma membrane is used. Pilus and flagellum are two kinds of appendages present on certain bacteria. Pilus helps bacteria to bind to other cells, while flagellum allows the organism to move around **(Kumari, 2007)**. **Figure 1.2** describes the anatomy of bacterial cell structure**.**

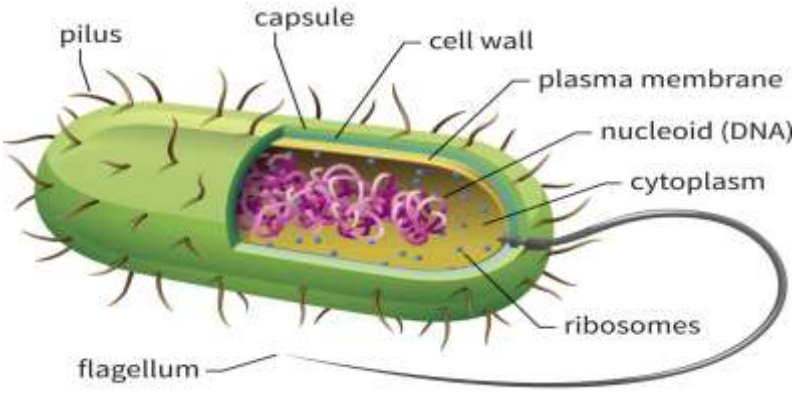


Figure 1.2: The anatomy of bacterial cell structure

(Source: https://www.proprofs.com/quizschool/story.php?title=mtc1mtyzmqarg5)

1.4.2 Types of bacteria

Bacteria are categorized as gram-positive or gram-negative based on the composition and function of their cell walls, as determined by a staining technique known as gram-stain. If the bacteria remain purple color after the procedure, they are gram-positive, while they are gram-negative if no stain occurs.

1.4.2.1 Gram-positive bacteria

Gram-positive bacterium contains peptidoglycan and lower lipid content. 90 percent of the cell wall contains peptidoglycan which is composed of amino acids and sugar. One example of gram-positive bacteria is *S. aureus* that appears in pairs, short chains, or grape-like clusters. It varies from 0.5 to 1.0 μm long. The temperature for growth ranges from 35 to 40°C. *Staphylococcus aureus* is a type of bacteria that is resistant to certain antibiotics and may be responsible for nosocomial infection. In the hospital, *S. aureus* is responsible for 19 percent of total surgical infections. **Figure 1.3** portrays *S. aureus* bacteria. Despite most advances in the medical field, nosocomial or iatrogenic infections are still clinically noteworthy problems. In an injured person, *S. aureus* may also cause boils, skin infections, pneumonia, and meningitis, particularly (**Kumari, 2007; Krasner, 2010)**. It is also responsible for scalp skin and toxic shock syndromes. This highlights the value of wearing antimicrobial garments in hospitals.

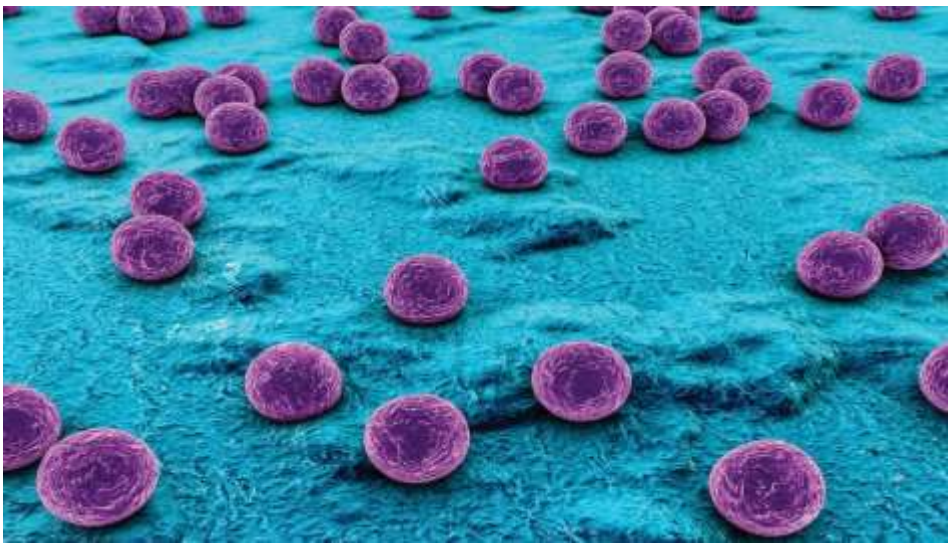


Figure 1.3: Staphylococcus aureus, Gram-positive bacteria

(Source:https://www.biomerieux-industry.com/pharma-healthcare/resources/pharma-microorganisms-library/2020-03-02-prevention-and-control)

1.4.2.2 Gram-negative bacteria

Gram-negative bacteria are similar to gram-positive bacteria, except that they have an extra layer of outer membranes connected to their peptidoglycan layer by lipoproteins. Lipopolysaccharide and porin make up the outer layer. Porin is used to move molecules of low molecular weight.

Porin is used to transport low molecular weight substances. Escherichia coli, one example of gram-negative bacteria. *E. coli* is shaped like a bacillus and lives in the intestines of humans. This can be transmitted through handling consuming raw foods. Extreme diarrhea, especially in infants, and kidney damage are the symptoms of *E. coli*. **Figure 1.4** displays *E. coli* bacteria. Gram-negative bacteria like *Klebsiella pneumoniae* are another example. It has the form of a bacillus and comes in single, pair, or short chains. Urinary tract infection, septicemia, and pneumonia are all caused by K. pneumoniae. The bacteria can be spread via the fecal-oral path, the mouth and throat, air to the lungs, and, most importantly, hospital personnel's hands. Fever, trouble breathing, chest pain, and bloody stool are all signs of K. pneumonia (**Kumari, 2007; Krasner, 2010**).

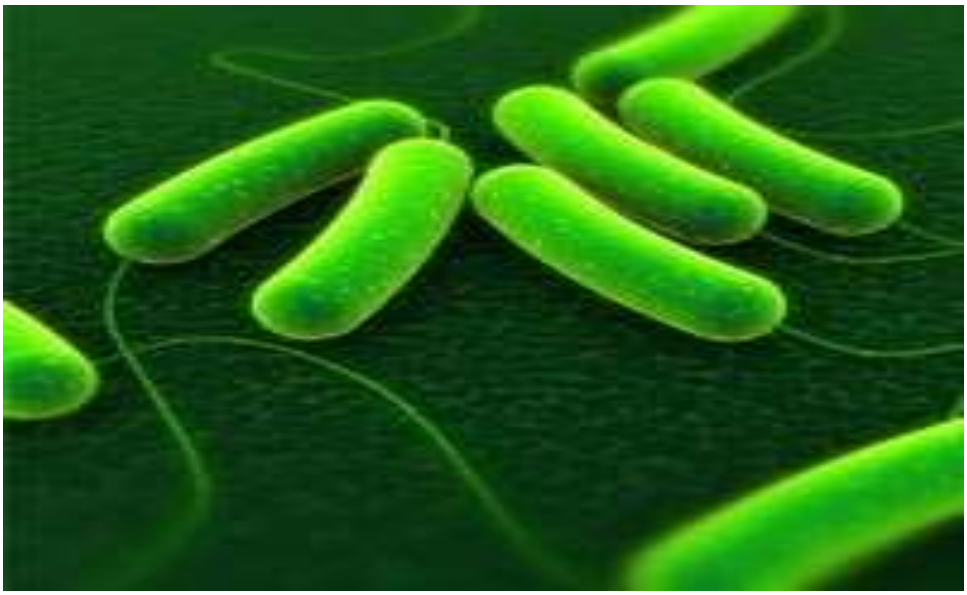


Figure 1.4: Escherichia coli Gram-positive bacteria

(Source: https://www.thermofisher.com/blog/food/fact-sheet-on-escherichia-coli)

Gram-negative bacteria have a thicker cell wall than gram-positive bacteria, making them more difficult to kill. Gram-negative bacteria have a cell wall peptidoglycan content of 10% and a high lipid content. Gram-positive bacteria, on the other hand, have a cell wall peptidoglycan content of 90% and a low lipid content (**Kumari, 2007; Krasner, 2010). Figure 1.5** depicts the cell walls of gram-positive and gram-negative bacteria**.**

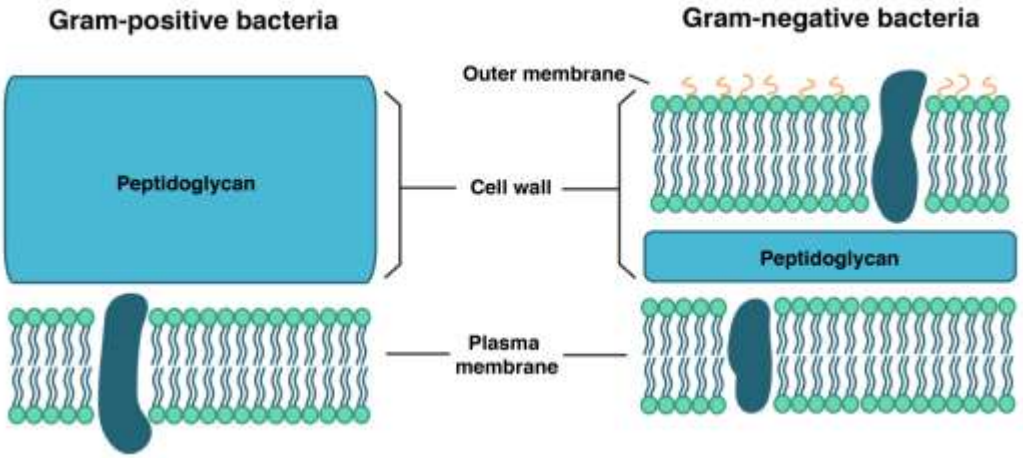


Figure 1.5: Cell wall of gram-positive and gram-negative bacteria (26. Kumari, 2007; 27. Krasner, 2010)

1.4.3 Identification technique of bacteria (Gram staining method)

The most significant technique in bacterial identification is the gram staining process. Using an inoculation needle, a bacterial cell is taken and placed on a glass slide for spreading. Second, the bacterial cell is fixed on the surface of the microscope slide by heating. Thirdly, a dye of crystal violet is added to the bacteria and left on for one minute. After that, the slide is rinsed with purified water to remove any residual crystal violet. Two drops of iodine solution (mordant) are added in the fourth step and retained for 60 seconds to form a crystal violet-iodine (CV-I) complex. All the cells are turn into blue. Gram-positive and gram-negative cells are separated by the decolorization step. The blue dye complex is derived to a greater extent from lipid-rich, thin-walled gram-negative bacteria than from lipid-poor, thick-walled gram-positive bacteria using an organic solvent such as acetone or ethanol. The gram-negative bacteria appear colorless and gram-positive bacteria remain blue. Eventually, safranin is introduced to the stain and allowed to remain for one minute. Gram-negative bacteria that have been decolorized with safranin now look pink, whereas gram-positive bacteria remain blue (**Bonin, 2005). Figure 1.6** illustrates the gram staining procedure.

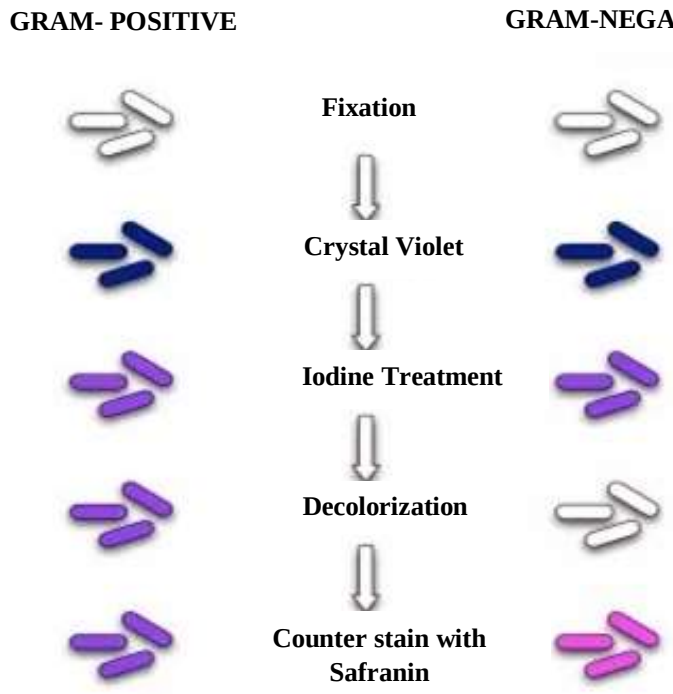


Figure 1.6: Procedure of gram staining method (Bonin, 2005)

1.5 Antimicrobial textiles

1.5.1 Necessity of antimicrobial textiles

Antibacterial materials are useful not only in hospital environments, but also in everyday circumstances (**Erdem & Yurudu, 2008**). Antimicrobial finishes have many potential uses. Lately, people are very serious about having a healthier life style. They are also conscious of the harmful effects of microorganisms on textiles **(Holm, 2002**).

In the health care sector, antimicrobial textiles are very much essential as doctors and nurses are easily contaminated by microorganisms. And unprotected textiles are significant contributors to the spread of microorganisms with transmission of infection and diseases (**Appidi et al., 2010**). The natural antimicrobial agents are not only eco-friendly, but also renewable. There are various medicinal plants which exhibit excellent antimicrobial properties (**Bhoomika et al, 2007).**

By forming a physical shield, antimicrobial finishes on fabrics will limit the transmission of microorganisms to the wearer. It protects the skin from infections caused by microorganisms. Microbial growth in textile materials, especially bacteria

growth, can lead to degradation of fabric properties, the creation of foul odors, skin irritation, and cross-infection. Antimicrobial finishes perform the following roles:

- To discourage cross-infection by pathogenic microorganisms.
- To avoid microbe infestation.
- To stop microbes from forming odors by stopping their metabolism.
- To prevent stains, discoloration, and degradation of quality in textile products.

The propagation rate of microorganisms is very high. Cell division doubles the number of cells within 20 minutes. Moisture, humidity and nutrients determine the propagation rate of microorganisms. Antimicrobial-finished fabrics can act as barriers against the growth of various microorganisms and therefore, contribute to deodorizing. Antimicrobial finishes are divided into two categories: bactericides (which kill bacteria) and bacteriostats (which prevent bacteria from multiplying) (**Toshniwal et al, 2009**).

Textiles with antimicrobial finishes are more functional and valuable like yarns, fabrics, and finished products by inhibiting the micro-organisms that cause odor and fiber degradation (**Thiry, 2010).**

1.5.2 Origin of antimicrobial textiles

The use of antimicrobial textiles began during the Second World War. At that time, cotton fabrics were used extensively for purposes such as webbing, military dress, tent, tarpaulins and truck covers. But within a very short period, cotton fabrics became damaged by microbial attack. This problem was visible especially in the South Pacific region, where most of the fighting took place under jungle-like conditions. Cotton duck and other military materials were processed with a combination of chlorinated waxes, iron, and antimony salts in the early 1940s, stiffening them while giving them an unusual odor. After the Second World War, copper ammonium fluoride, hydroxyquinoline salts and chlorinated phenols were applied to cotton fabrics (**Purwar & Joshi, 2004).**

1.5.3 Demand of antimicrobial textiles

The number of antibiotic-resistant microorganisms is rising, along with the occurrence of infections from these microorganisms. This concern has created a boom of interest in healthy living, which, in turn, has influenced the psychology of consumers of every

field. The healthcare industry is the biggest consumer of antimicrobial textiles. Manufacturers are keen on developing products that can combat hospital-acquired (iatrogenic) infections. Use of antimicrobial textiles prevents the spread of such infections in hospitals. The growing prevalence of acute diseases such as chickenpox and diphtheria and even chronic diseases like tuberculosis, and others have created an increasing incidence of cross-infection which has, in turn, spurred demand for antimicrobial clothing. Finally, the widespread improvement of health care services has increased the need for antimicrobial textiles in the healthcare industry.

The direct and indirect costs of iatrogenic disease in the United States are estimated to be around USD 97–147 billion annually, according to a survey by the CDC (Centers for Disease Control and Prevention). In 2011, nearly 75,000 people died as a result of these illnesses. All of these factors are driving the market for antimicrobial textiles in the healthcare industry.

During the forecast period, the global antimicrobial textile market is projected to rise at a compound annual growth rate (CAGR) of 5.4 percent, from USD 9.5 billion in 2019 to USD 12.3 billion in 2024.

The adaption of best practices by healthcare workers to deter iatrogenic disease has resulted in a rise in demand for safer and preventive materials to be used in healthcare facilities, which is expected to drive the antimicrobial market growth during the forecast period **(Antimicrobial textiles market, 2019**)

1.5.4 Requirements of antimicrobial textiles

The public's concern regarding antibacterial finishing's possible effect on the environment and biological systems is increasing. An ideal textile antibacterial finishing should not only destroy unwanted microorganisms and close the spread of diseases but also fulfill four other traits necessary for any antimicrobial finishes. According to **Ramachandran et al. (2004).** To begin with, it must be effective against a wide variety of bacterial and fungal organisms while still being low in toxicity to users, i.e., it must not cause toxicity, allergy, or discomfort to the customer. Before being sold, antimicrobial-treated textiles must undergo compatibility checks (cytotoxicity, inflammation, and sensitization). Second, the finish must be durable enough to handle

laundering, dry washing, and hot pressing. Since textile items are washed repeatedly during their lives, this is the most challenging task. The finishing should not negatively affect the quality (e.g. physical strength, handle, comfort) or appearance of the textile. Finally, the finishing should be cost-effective and contain no chemicals that are toxic to the producer, the customer, the production staff, or the environment.

1.5.5 Benefits of natural antimicrobial finishes in textiles

According to previous studies, there is a growing global interest in antimicrobials derived from natural sources because of the following advantages: (**Dawson, 2009; Masoud et al., 2014)**

- Natural antimicrobials can be produced from low-cost renewable materials with tremendous potential.
- Natural antimicrobials are more safer for atmosphere than synthetic antimicrobials in two ways:
    - All synthesis processes in natural materials are carried out by nature, with no contamination of the atmosphere.
    - Since these products are quickly biodegradable and do not generate hazardous waste water upon deteriorated in the atmosphere, waste water treatment is not needed before discharging it into the environment.
- The majority of natural antimicrobial components are harvested from wild and self-growing plants with no added expense for cultivation.
- The majority of natural antimicrobial components have no harmful effects on beneficial non-target microorganisms.
- The majority of natural antimicrobials have no adverse effects on humans.
- Modern characterization methods have made extraction, purification, and application of natural antimicrobials simpler and more possible.

1.5.6 Types of antimicrobials textiles

The antimicrobial finishes have been classified into leaching and non-leaching. Antibacterial activity can be classified in two ways: bactericidal and bacteria static

Bactericidal finishes produce destruction of microorganisms. Bacteria static finishes indicate produce inhibition of bacterial growth without significant destruction of the microorganisms **(Edward Menezes, 2002).**

1.5.6.1 Leaching and non-leaching types antimicrobial finish

Leaching means that antimicrobial agents come out from the textile surface to kill the bacteria. This type of finish is not really durable as it leaves the surface of the textile. Non-leaching finishes kill or inhibit the bacteria on the surface of the textile. This type of finish is durable and safe as well as less harmful for human skin or likely to cause skin irritation.

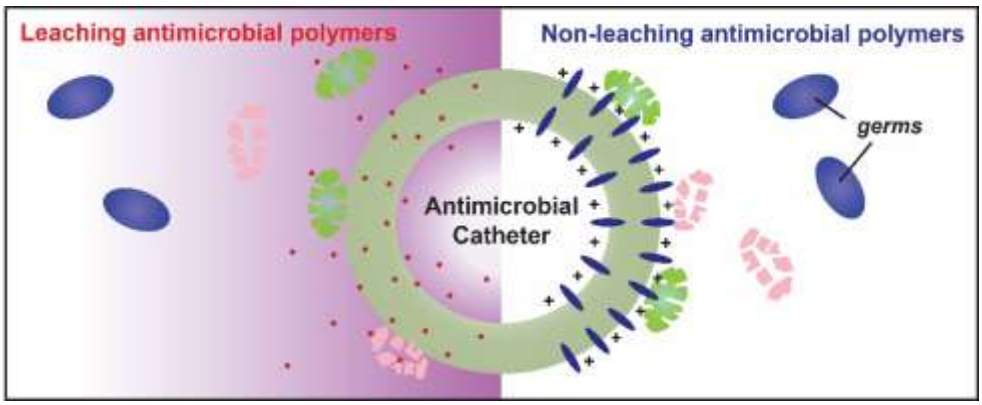


Figure 1.7: Mode of action of leaching and non-leaching antimicrobial polymer (Bruenke et al, 2016)

Leaching and non-leaching type's antimicrobial polymer is shown in **Figure 1.7.** In **Figure 1.7** leaching antimicrobial polymers, indicated by red dots, are released into the area surrounding the textiles, causing chemical interactions with the microbes (green). The finishing additive forms a concentration gradient (pink), which can contribute to the development of resistant pathogens in sub-lethal concentrations of the additive. Non-leaching antimicrobial polymers have immobilized antimicrobial agents (blue) which generate a positively-charged surfaces. In this case, direct contact is needed between microbes and material surface to have any impact on the microbes.

1.5.6.2 Active and passive types antimicrobial finish

Antimicrobial active terms include materials with passive effects and materials with active effects. Bioactive compounds are not found in passive materials. The lotus effect or micro- domain structured like textile surfaces prevent the microbial colonization. To put it another way, the bacterial cells themselves are unaffected; instead, they are

discouraged from adhering to the fiber's surface. This has a negative influence on the living conditions of microorganisms (anti-adhesive effect). During metabolism, active finishes include antimicrobial substances that work on microorganisms on the cell membrane or inside the core material (genome). The majority of bioactive substances, with the exception of antibiotics have varying effects depending upon where the antimicrobial activity occurs, either in or on the cell **(Russell & Chopra, 1996; Beumer et al., 2000)**. Waterproof fabric with a water contact angle greater than 150° is the best example of a passive substance. Many natural surfaces, such as butterfly wings, cabbage and lotus leaves, elephant ears, Indian cress, and so on, have hydrophobic and self-cleaning properties. Fluorocarbon polymers, aluminum and zirconium derivatives, waxes, metal complexes, or silicone-based polymers, such as poly (dimethyl siloxane), are used to produce this type of hydrophobic surface.

1.6 Action mechanism of antimicrobial agents

Antimicrobial agents either inhibit the growth (static) or kill (cidal) the microorganisms. They damage the cell wall or alter cell membrane permeability, denature proteins, inhibit enzyme activity or inhibit lipid synthesis, all of which are essential for cell survival.

The bactericidal process depends upon the substance used. Some common examples include preventing the bacteria from reproducing, damaging their cell walls, denaturing their proteins, blocking their enzymes and generally making it impossible for the bacterial cell to survive and replicate to progress colonization of the garment. **Figure 1.8** depicts one type of antimicrobial action by one agent. It is possible, at the chemical level, that polycationic compounds in the agent target the cytoplasm within the microbe in a six-step process as follows (**Gao & Cranston, 2008; Heine et al, 2007; Vigo, 2001**):

(1) Adsorption onto the microbial cell surface,

(2) Diffusion through the cell wall,

(3) Binding to the cytoplasmic membrane,

(4) Disruption of the cytoplasmic membrane,

(5) Release of cytoplasmic constituents such as K+ ion, DNA, and RNA.

(6) The death of the cell.

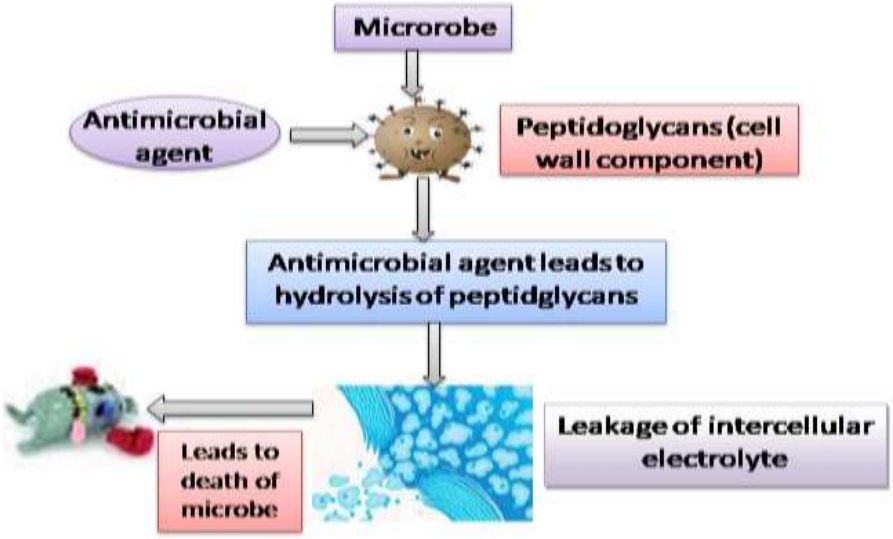


Figure 1.8: Mechanism of microbial cell disruption by antimicrobial agents (Gao & Cranston, 200; Heine et al, 2007; Vigo, 2001)

1.7 Necessity of antioxidant enriched textiles

1.7.1 Role of antioxidant agent

An antioxidant is a molecule that stops other molecules from oxidation. Oxidation is a chemical reaction that creates free radicals, which can induce chain reactions that injure cells. Antioxidants including thiols and ascorbic acid (vitamin C) prohibit these chain reactions. Polyphenols have antioxidant effects in vitro, but due to widespread metabolism, they do not always act as antioxidants in vivo. The catechol group serves as an electron acceptor in several polyphenols and is hence responsible for the polyphenol's antioxidant function**.** To sum up, antioxidant acts as electron donors which reacted with free radicals to convert them to more stable products and terminate the radical chain reaction (**Csepregi et al., 2016)**. The role of antioxidant is shown in the **Figure 1.9.**

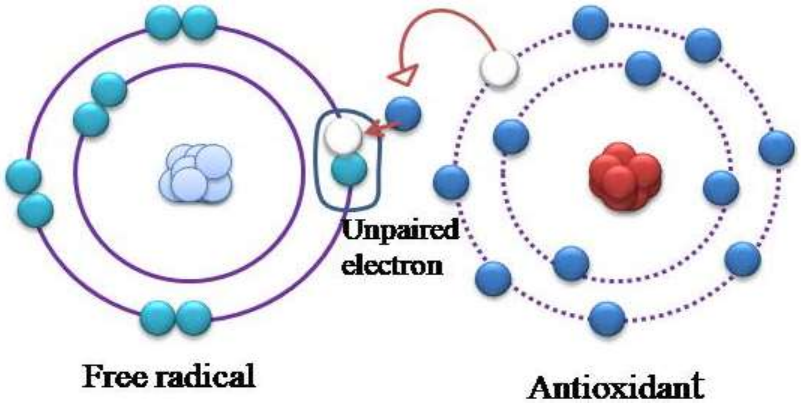


Figure 1.9: Role of the antioxidant agent

(Source:https://www.stapleton-spence.com/nutrition/antioxidants-and-free-radicals/)

1.7.2 Sources of reactive oxygen species (ROS) and its effects

Metabolism is a chemical reaction that enables humans and other animals to keep their body function. Smaller molecules are reorganized into larger molecules, and larger molecules are broken down into smaller molecules, is called metabolism. In this chemical reaction, some molecules lose an electron, and the electron will become a free radical or a reactive oxygen species (ROS). Reactive oxygen species are caused by, UV radiation, smoking, pollution, a lack of sleep, inadequate diet, cell metabolism, and stress.

Reactive oxygen species (ROS) are electrically charged, unstable molecules that can interfere with other molecules (such as DNA) and cause damage. Antioxidants usually donate electrons to free radicals, neutralizing them. Reactive oxygen species are oxygen-containing molecules with an uneven number of electrons. Large chain reactions in our bodies can be caused by reactive oxygen species. These reactions are called oxidation reactions. When there are more reactive oxygen species in our bodies, damage to fatty tissue, DNA, lipids and proteins will occur. Major part of our body are made up of protein, lipids, and DNA. As a result of the damage, a wide variety of diseases such as high blood pressure, diabetes, and heart disease may evolve (**Milatovic et al., 2016; Krumova & Cosa, 2016). Figure 1.10** depicts the origins of reactive oxygen species and their implications.

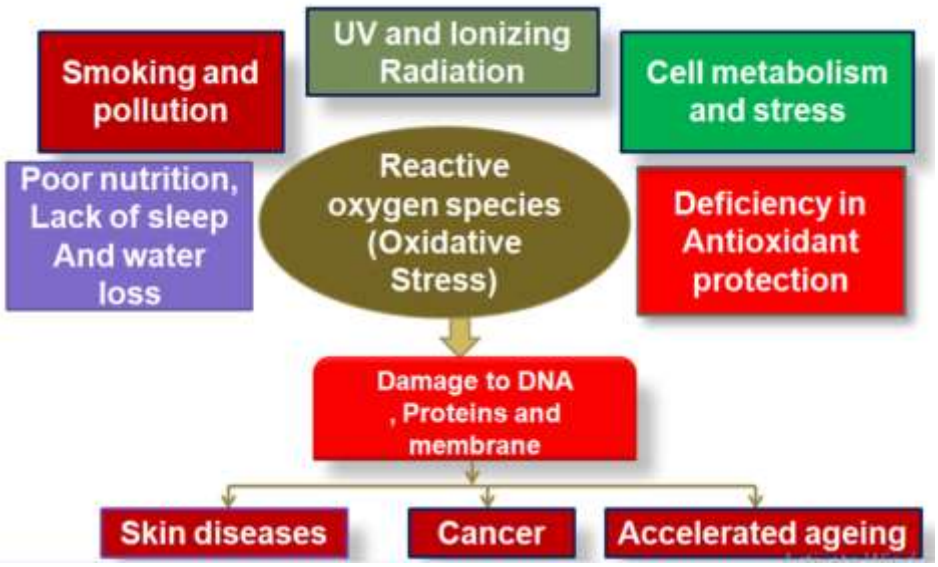


Figure 1.10: The sources of reactive oxygen species (ROS) and its effects (Milatovic et al., 2016; Krumova & Cosa, 2016)

1.7.3 Antioxidant finished textiles

Recently, natural antioxidant agents have gained considerable attention because natural antioxidant agents are safe, non-toxic and environment-friendly. Antioxidant finishing of textile materials with natural agents has emerged as a new trend in the development of bioactive fabrics for multi-functional applications, particularly medical applications. If the antioxidant agent used in garment finishing, it will be very helpful for the wearer. It is expected that antioxidant finished clothing to minimize or stabilize the free radicals of the outer side of the body. In this way, natural antioxidant inhibits to develop chronic diseases in our body. **Ghaheh et al. (2017)** examined the functionalization of cotton fabrics with protein-based nanoparticles incorporating vitamin E (α-tocopherol). The ABTS ((2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging technique was used to measure the antioxidant efficacy of vitamin E coated samples. When compared to non-coated fabrics, the findings indicate that all of the coated samples had antioxidant activity. The samples coated with nanoparticles containing the largest amount of vitamin E had the highest antioxidant values. Photosynthesis produces free radicals on human skin. Antioxidants have the ability to neutralize free radicals. Atoms or molecules with an unpaired electron in their outer shell are called free radicals. These free radicals form as a byproduct of cell breathing's attempt to snare an electron from other structures, resulting in cell membrane harm. Through donating an electron to the cell membrane, antioxidants (such as vitamin E) protect it. **Figure 1.11** depicts the transfer of vitamin E (an antioxidant) to human skin through textiles.

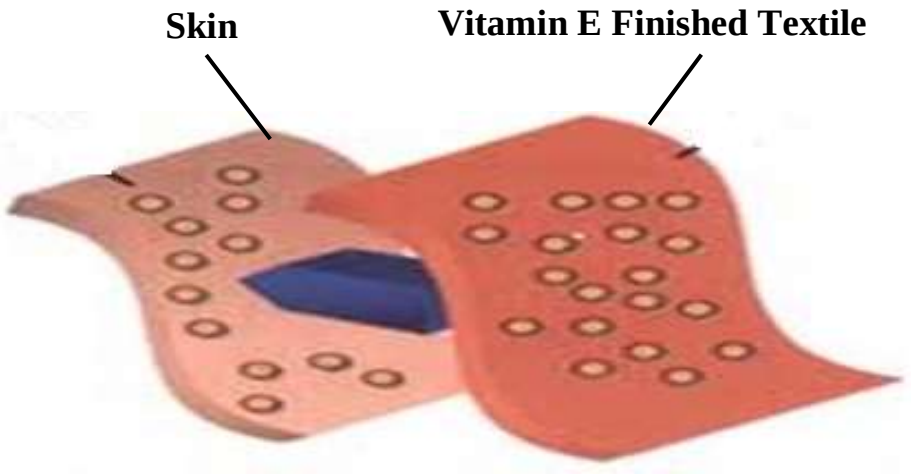


Figure 1.11: Transfer of vitamin E to skin (Ghaheh et al., 2017)

1.8 Necessity of UV protective textiles

1.8.1 UV radiation

The ultraviolet light is electromagnetic radiation with a wavelength between visible light and X-rays which ranges from 40 nm to 400nm and energies from 3 eV to 124eV. Further ultra violet light are categorized into UV-A (320 to 400 nm), UV-B (290 to 320 nm), and UV-C (200 to 290 nm). UV-B rays exhibited shorter wavelength compare to UV-A rays, but more dangerous than UV-A rays. Unlike the penetration depth of UV-A rays are more than UV-B rays on the human skin (**Sarkar, 2004**). The penetration intensity of UV-A and UV-B in the human body is shown in **Figure 1.12**.

Historically, the ozone layer has absorbed the UV-C range. Yet now, owing to the depletion of the ozone layer, UV rays come to earth easily. The UV-A rays are associated with skin wrinkling and human ageing. UV-B rays are mainly responsible for causing sunburn and dangerous skin cancer (**Grifoni, 2011)**. UV-A rays are present throughout the year and it can penetrate clouds and glass. UV-B ray intensity varies depending on the season, place, and time.

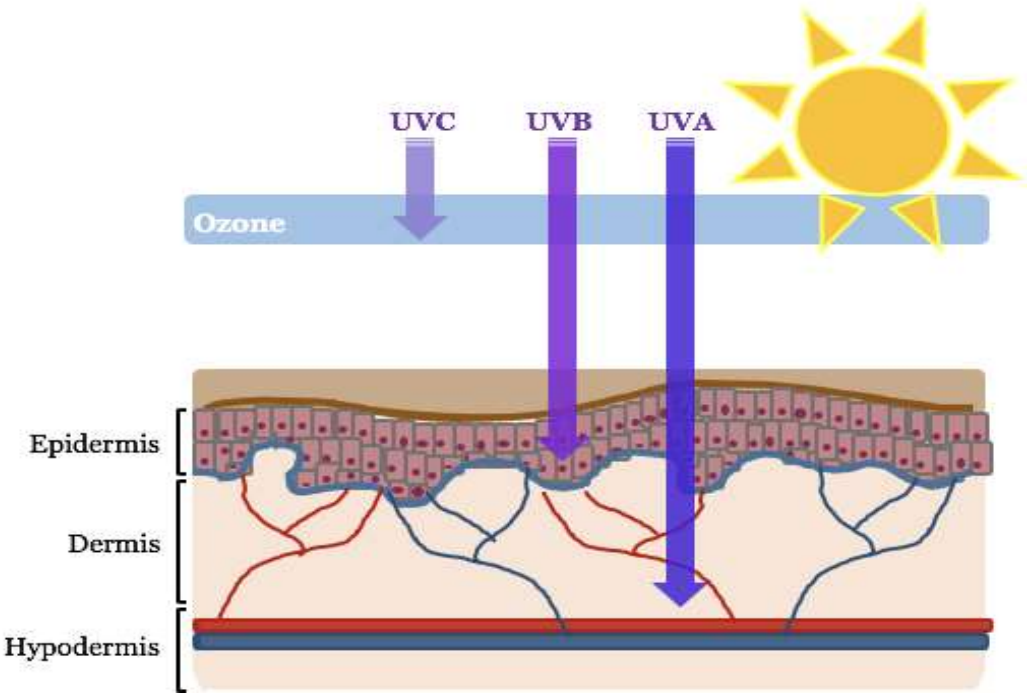


Figure 1.12: Penetration intensity of UV ray into the layers of the skin

(Source: https://www.mondoscience.com/blog/2017/4/22/sunlight-and-your-skin)

1.8.2 Effect of UV radiation on human being

A minimum level of sunlight is good for human health. It helps to increase blood circulation, metabolism, and production of vitamin D and can kill pathogens. Excess of sunlight, however, may lead to various problems such as premature ageing, skin burn, tan, eye damage, DNA damage, skin cancer etc. Approximately 10% of sun's energy is in the form of UV radiation. **Figure 1.13** depicts a summary of the effects of UV irradiation on skin.

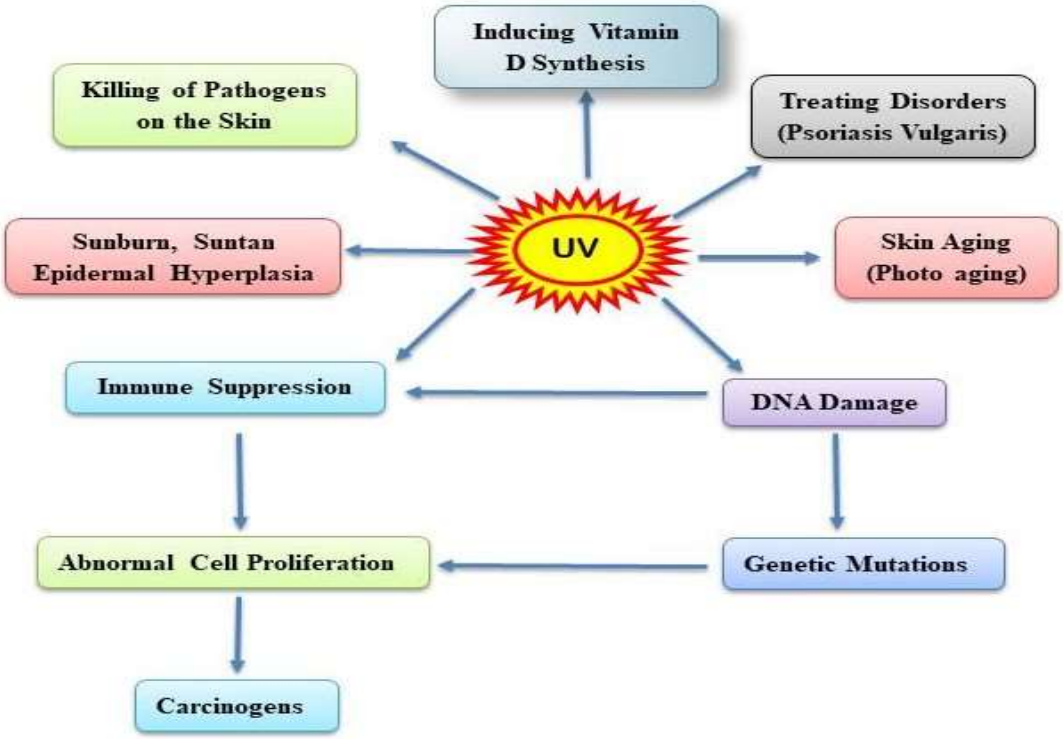


Figure 1.13: Effects of UV irradiation on human being
(Source: www.healthyfellow.com)

1.8.3 Effect of UV radiation on textile materials

UV radiation is one of the most common sources of degradation of textile materials, which is caused by excitations in certain areas of the polymer molecule and a gradual loss of integrity. The degree of degradation depends on the nature of the fibres. Sun light and other environmental conditions may have an effect on textile materials. UV rays cause photo oxidation, which allows elasticity and tensile strength to decrease and can alter the degree of crystallinity in the fibre's component. Textile dyes' polymer chains are often broken down by UV radiation. As a result, dyed textile materials fade very quickly (**Mallik & Arora, 2003**). **Figure 1.14** illustrates the causes that induce fading in textile dyed fabrics.

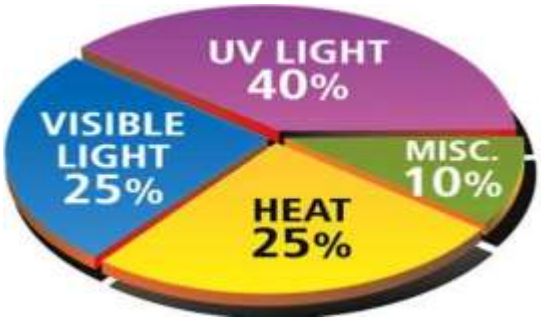


Figure 1.14: Fading source of textile dyed materials

(Source: https://www.naturalux.com/NaturaLux_Lighting_Filters_Fading.htm)

Textiles and clothing serve as the primary interface between the human body and the Sun. Untreated textiles can reflect, absorb or scatter UV light, but they don't provide complete sun protection in most cases. For this reason, UV-resistant finishing on textiles and clothing is necessary. In the presence of the UV-resistant finishing, UV rays do not create chemical reactions in clothing or textiles and resist textile dye polymer degradation. As a result, UV-resistant finishing will be helpful to retain the color fastness of dyed cloth, tensile strength, crystallinity and preventing of fading and elasticity.

1.9 Natural Antimicrobial agents

Antibacterial agents are a class of materials that combat bacteria that cause disease. Thus, bacteria's pathogenic impact in biological settings can be reduced by destroying or reducing their metabolic activity. The term antimicrobial refers to a substance that destroys or regulates microbes.

1.9.1 *Aloe vera*

Naturally occurring antibacterial materials have gained popularity over time. Natural antibacterial are common because they have fewer adverse effects on human health. *Aloe vera* is one of the most widely used antimicrobial plants on the planet. *Aloe vera* has shown to have significant therapeutic benefits for humans. It is referred to as a "healing herb" because of its wound-healing, UV defense, antioxidant, and antimicrobial properties. For thousands of years, *Aloe vera* has been used as a popular herbal medicinal plant. The *Aloe vera* plant was thought to be a natural panacea by ancient Greek scientists. Aloe was dubbed "the wonder of heaven" by the American Indians. **(De Rodrıguez et al, 2005**). *Aloe vera (Aloe barbadensis, Miller)* is a Liliaceae plant that is known as the “Lily of the Desert”. The external green rind of the *Aloe vera* leaf, which includes the vascular bundles, and the inner colorless parenchyma, which

contains the aloe gel, are the two main sections of the *Aloe vera* leaf (**Ni & Tizard, 2004)**. It is primarily found in dry areas of Africa, Asia, Europe, and North America. In Bangladesh, it is found abundantly in Rajshahi, Natore, Rangpur, and Dinajpur.

1.9.1.1 Active components of *Aloe vera*

About 75 nutrients and 200 active compounds are contained in aloe leaf, including 20 minerals, 18 amino acids, and 12 vitamins *Aloe vera* gel is almost entirely made up of water, with just 1% solid matter. *Aloe vera* gel consists of a range of compound: saccharides (mannose, glucomannan, acemannan.), vitamins (B1, B2), phenolics (anthraquinones, flavonoids), enzymes (amylase, carboxypeptidase), low molecular weight substances (cholesterol, salicylic acid) and so on **(Choi & chung, 2003).** Polysaccharides such as acemannan or acetylated mannan are abundant in aloe leaf gels. Acemannan is a long chain polymer of β (1 → 4) linked galactomannan saccharides (**Ni et al. 2004; Dagne et al. 2000)** as shown in **Figure 1.15**. Many of the beneficial effects of Aloe leaf gels have been attributed to acemannan and related polysaccharides, such as antibacterial, antifungal, antioxidant, anti-inflammation, anti-cancer, anti-diabetic, anti-allergic, immuno-stimulation, and antitumor properties.

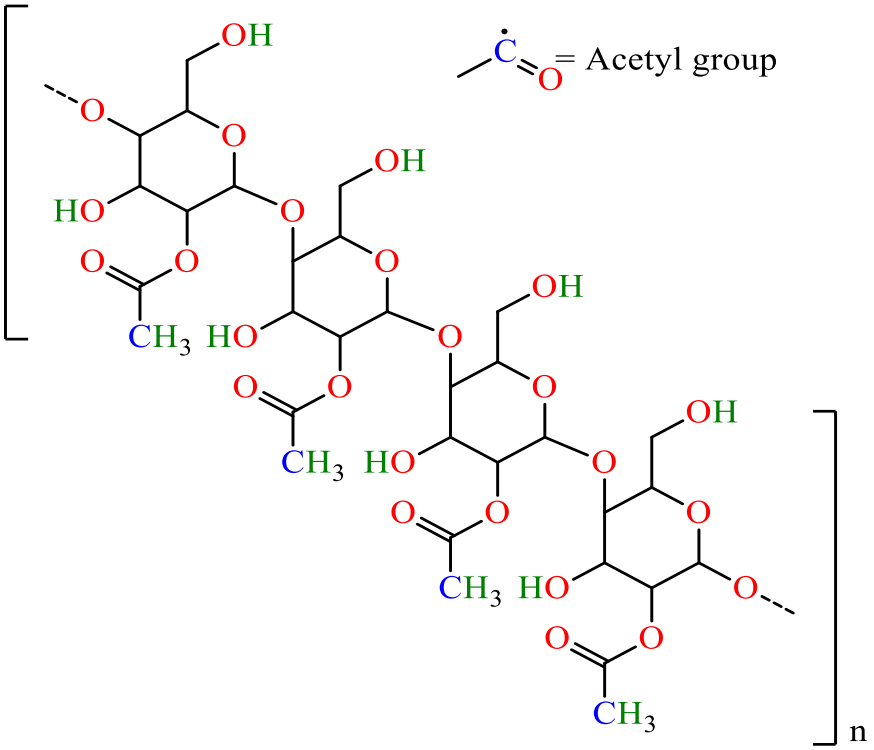


Figure 1.15: The structure of acelytedmannan or acemannan (a major polysaccharide component of A. vera leaves) consists of a polymer of β (1 → 4) linked galactomannan sugars

1.9.1.2 Antibacterial, antioxidant and UV absorbance properties of *Aloe vera*

There are several solvents available to extract *Aloe vera*: ethanol, methanol, chloroform, petroleum ether, aqueous extraction and so on. Among the solvents methanol extraction produces the best antibacterial activity (**Das & Srrivastav, 2015**). *Aloe vera* has antibacterial properties that are effective against both gram-positive and gram-negative bacteria (**Habeeb et al., 2007**).

Aqueous extract of *Aloe vera* exhibits a number of antioxidant components like phenols, flavonoids, ascorbic acid, β-carotene and α- tocopherol **(Raksha et al., 2014**). *Aloe vera* extracts were found to be effective at scavenging 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radicals **(Sultana et al., 2009**). Azoxymethane (AOM), which can cause oxidative stress and the production of noxious substances in the liver, is reduced by *Aloe vera* extract **(Anilkumar et al., 2010**).

Ray et al. (**2012**), found UV spectra of *Aloe vera* absorption peaks at 270 nm (UV-C region), 290 nm (UV-B region) and 350 nm (UV-A region) in a spectrophotometer. The phenolic group of *Aloe vera* was described by **Ozsoy et al. (2008)** at an absorption band between 320 and 380 nm. Long-term exposure to UV-B spectrum radiation (315–280 nm) can have a variety of negative effects on the human skin, immune system, and can even cause skin cancer. Overexposure to UV radiation increases the risk of malignant melanoma by causing DNA damage. In 92 percent of melanoma cases, UV radiation is a factor **(Davies et al., 2002**).

1.9.1.3 Antibacterial, antioxidant and UV absorbance properties of *Aloe vera* treated textiles

Aloe vera-treated cotton had excellent antimicrobial activity against Staphylococcus aureus (*S. aureus*) and Escherichia coli (*E. coli*) according to **Ibrahim et al. (2017).** It's suspected that *Aloe vera* bleeds various components from the treated cloth, which are responsible for microbe inhibition and death. The antibacterial efficacy of aloe anthraquinone was tested against *E. coli* and *S. aureus* after it was applied to cotton fabric. *C. albicans* has also been subjected to antifungal effectiveness testing. The Aloe anthraquinone-treated fabric had better antibacterial capabilities than the control group against both *E. coli* and *S. aureus* bacteria. Treated fabric showed over 91 percent bacterial suppression. Moreover, for *C. albicans*, fungus decrease was observed to be up

to 69 percent. *C. albicans* had a lower rate of inhibition than *E. coli* and *S. aureus* bacteria. Because of the cationic nature of Aloe anthraquinone, it adsorbs the anions of the bacterial cell wall and easily fractures the peptide polysaccharides. Further, the fungal cell wall is comprised of amylase, which is different from the walls of bacteria **(Xu & Deng, 2011**)**.** The amount of bacterial reduction in *Aloe vera* finished fabric varied depending on the *Aloe vera* concentration. As the concentration of the solution was increased, the rate of bacterial colony reduction increased. Fabric treated with 5 g/l of *Aloe vera* had a high level of anti-microbial activity (**Jothi, 2009)**. *Aloe vera*-treated fabric retained more than 70% of its original antibacterial activity even after 20 washing (**Das et al., 2019**)**.** Cotton fabric coated with Aloe vera was tested for antibacterial and antifungal qualities **(Ghayempour et al., 2016)**. The bacterial and fungal reduction percentages of the treated fabric were found to be 75, 80 and 81%, against *E. coli, S. aureus* and C. *albicans*, respectively. The antibacterial and antifungal activities of Aloe vera extract may be due to the components acemannan, anthraquinone, and salicylic acid.

The UV transmittance value of the Aloe anthraquinone treated cotton fabric is very poor when compared to the untreated sample, indicating that it has strong anti-ultraviolet properties. Assume that the UV radiation is fully absorbed by the Aloe anthraquinone that has been applied to the fabric surface. Modified cotton fabric had an ultraviolet protection factor (UPF) of around 57, while untreated cotton fabric had a UPF of around 13 **(Yu & Deng, 2011).**

1.9.2 Chitosan

After cellulose, chitosan is the most available biopolymer. Chitosan is a linear chain of β-(1, 4) linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D glucopyranose derived from chitin by N-deacetylation. **Figure 1.16** illustrates its chemical configuration (**Seo et al., 2007**). Chitin is present in shrimp shells waste which is available in Bangladesh. The raw material of chitosan is unprocessed shrimp shells. Originally the head and skin materials of crude shrimp possess low economic value and are treated as bio-waste or sold to animal feed manufacturers. Large amounts of shrimp bio-waste are found in shrimp processing zone, approximately 45-55% of the weight of raw shrimp. However, this bio-waste can be converted some value-added products such as chitosan (**Islam & Bhuyan, 2016)**.

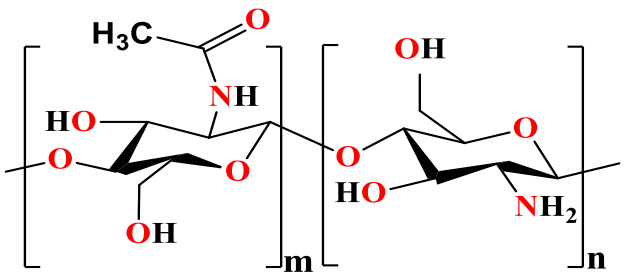


Figure 1.16: Chemical structure of partial deacetylated chitosan (Seo et al., 2007)

1.9.2.1 Chemical reactivity of chitosan

There are three reactive groups in chitosan. The primary and secondary hydroxyl groups are present in positions (C-6) and (C-3), respectively, and each monomer has a free amine group in position C-2 (C6H11O4N). This natural biopolymer is made up of two sugars: glucosamine and acetyl glucosamine, which are also found in mammalian tissues. Chitosan is a chemical counterpart of cellulose, with an acetamido (-NHCOCH3) or amino groups (-NH2) replacing the hydroxyl (-OH) at the C-2 site of cellulose. In contrast to other popular polysaccharides, chitosan contains nitrogen as well as carbon and hydrogen (**Yen et al., 2009).**

Chitosan has achieved considerable attention in many applications due to its excellent biocompatibility and biodegradability property. Most of the present day polymers are synthetic materials. Synthetic polymers have poorer biocompatibility and biodegradability than those of natural polymers like chitin, chitosan, cellulose and their derivatives. Microorganisms in the soil and in the water can decompose chitosan. As a result, chitosan is environmentally friendly. Chitin is a linear chain of acetyl glucosamine groups, and chitosan is made by eliminating the bulk of the acetyl groups (CH3-CO). Chitosan is readily soluble in most diluted acids due to the lack of acetyl groups. The degree of solubility in chitosan is determined by the number of free amino groups present. The amount of free amino groups present in chitosan determines the degree of deacetylation. The antimicrobial and other functional properties of chitosan are also determined by the degree of deacetylation (**Li et al., 1992**).

1.9.2.2 Antibacterial, antioxidant and UV absorbance properties of chitosan

In most cases, chitosan is dissolved in a 1% acetic acid solution. Different levels of concentration of the solution are used to determine the antibacterial activity against

gram-positive and gram-negative bacteria. The antibacterial activity of chitosan is effective in inhibiting growth of pathogenic bacteria, qualifying it as a "broad-spectrum antibacterial agent **(No et al, 2002).** The antimicrobial activity of chitosan is influenced by its molecular weight, degree of deacetylation, concentration of chitosan, type of bacterium and bacterial inoculum size (**Chen et al, 1996; Fernades et al, 200**8). Chitosan exhibited stronger effects for gram-positive bacteria than for gram-negative bacteria (**Coma et al, 2003; Dutta et al, 2009).**

Chitosan also exhibited antioxidant properties. This naturally renewable resource demonstrated its ability to interact with free radicals through ionic interactions with its amino groups. Several in vitro and in vivo studies have shown that chitosan's antioxidant activity is relative to its molecular weight (**Mahdy et al, 2013**). Antioxidant activity was higher in chitosan with a lower molecular weight (MW). Furthermore, chitosan's low solubility is linked to its high MW, which has an effect on the polymer's applications.

Historically, UV rays have been almost completely absorbed by the ozone layer. Due to the ozone layer's depletion, UV radiation can now easily reach the planet. Sunburn, premature skin aging, allergies, and skin cancer are all consequences of this. UV rays are dangerous to human beings. When UV rays contact human skin, an oxidation reaction occurs. As a result, free radicals are generated and, consequently, different diseases can result.

Chitosan absorbs at 220-2255 nm UV spectra **(Tyagi et al., 1996**). The presence of surviving amide linkages in chitosan causes absorption in this region. Chitosan has enough amide linkages to preserve this property since it is only partially deacetylated chitin.

1.9.2.3 Antibacterial, antioxidant and UV absorbance properties of chitosan treated textiles

Numerous investigations have demonstrated the effectiveness of chitosan as an antibacterial finishing agent (**Shin et al., 2001; Mondal & Saha, 2019; Dhiman & Chakraborty, 2015)**. Chitosan, when combined with a poly carboxylic-acids-combination finish, gives cotton fabric good antibacterial properties. The zone of inhibition for samples treated with chitosan and poly carboxylic acids is found to be 21 – 25 mm for *E.coli* and 24 – 27 mm for *S. aureus* (**Sunder et al., 2014).** The cotton

fabrics treated with chitosan have a wide range of antibacterial activity against gram-positive and gram-negative bacteria and fungi (**El-Tahlawy et al., 2005).**

Chitosan belongs to the non-leaching or bound type antimicrobial agents, in which the antimicrobial agent is covalently bound to the fiber surface and can destroy microbes that come into contact with the fiber **(Gao & Cranston, 2008). Figure 1.17** depicts chitosan's mechanism of action on a textile substrate.

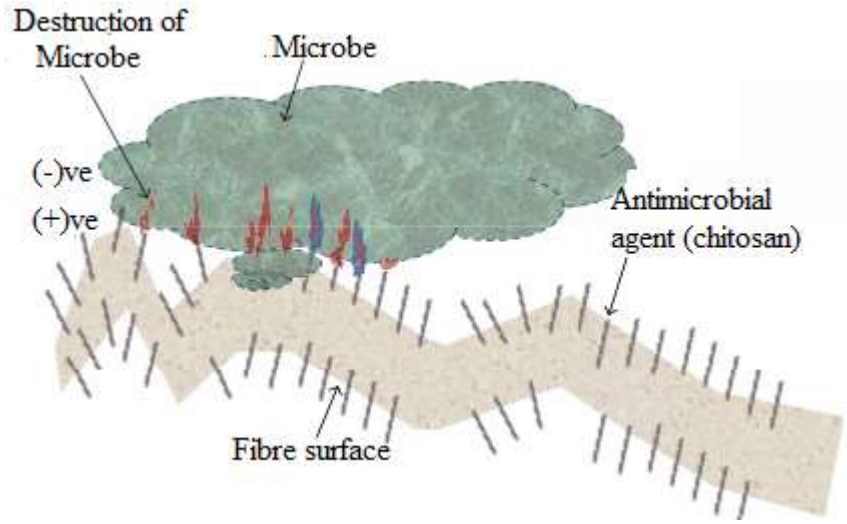


Figure 1.17: Action mechanism of chitosan (Lim & Hudson, 2003; Young et al., 1982)

According to studies, the positively charged chitosan interacts with negatively charged residues in fungi or bacteria's cell walls. The interaction induces internal substance leakage by altering cell permeability **(Lim & Hudson, 2003; Young et al., 1982).** Other research claims that the formation of a polymeric substance around the bacterial cell prevents nutrients from entering the cell (**Helander et al, 2001**).

1.9.3 Silk sericin

Sericin, a primary ingredient of silk fiber that makes for around a quarter of the weight of raw silk, is known as silk gum. And it holds a number of other ingredients such as waxes, fats, and pigments. Sericin is a yellow-colored, brittle and inelastic substance (**Zhou et al., 2012**).

The silk cocoon comprises three layers of silk sericin. The outer layer, middle layer, and inner layer each comprise 15%, 10.5 percent, and 4.5 percent sericin, as seen in **Figure 1.18** (**Cao & Zehang, 2015).** In cold water, sericin is insoluble. However, sericin is

soluble in hot water so heat breaks down the long protein molecules into smaller fractions that are quickly hydrolyzed and dispersed. (**Zhang, 2002; Kundu, 2008**).

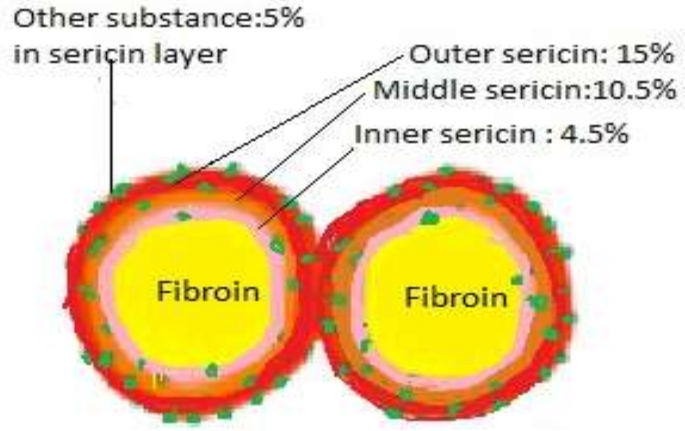


Figure 1.18: Three layers sericin of silk cocoon (Cao & Zehang, 2015)

Dry cocoons are estimated to be produced in the sum of 400,000 tons per year around the world. At least 50,000 tons (12.5 percent) of sericin was discharged into wastewater after degumming (**Kim, 2007**).

Chemical oxygen demand (COD) and biological oxygen demand (BOD) in the water supply are increased when effluent is discharged into wastewater from industry. As a result, the wastewater released by the silk industry pollutes the water environment **(Fabiani et al., 1996**). If sericin can be recovered from waste water, it can be used to develop value-added products, benefiting both the economy and the environment **(Vaithanomsat & Kitpreechavanich, 2008)**. Sericin can be extracted from silk by detaching it from the fibroin. Several techniques are currently used to remove sericin from fibroin. Degumming with soap and alkali is a very good way to fully remove sericin from fibroin. But this method is not effective because the separation of soap and alkali from sericin is very difficult. As a result, sericin purification becomes more complex (**Yamada et al., 2001**).

Other acids, such as citric, tartaric, or succinic acid, can also be used to remove sericin. Certain acids dissolve proteins, and since sericin is proteinous in nature, it may be destroyed during extraction (**Khan et al., 2010**). On the other hand, extraction in urea solution with 2-mercaptoethanol is preferable because it does less harm to sericin (**Takasu et al., 2002).** However, this treatment is expensive and time-consuming because sericin

must first be purified, which is achieved by dialysis. Enzymatic degumming for sericin extraction has also been anticipated. However, it is inefficient and does not always work (**Arami et al., 2007; Gulrajani et al., 2000; Freddi et al., 2003**). The extraction of sericin using an autoclave system is a very simple technique. In this method, a silk cocoon is heated in hot distilled water, adding no other chemicals. The amount of sericin extracted is dependent on the time and temperature of extraction. But this extraction process is excellent for protecting the atmosphere and reducing costs.

1.9.3.1 Chemical composition and properties of sericin

Protein is the main component of silk sericin. Sericin's protein content accounts for nearly 90% of its total weight. There are 18 different amino acids in sericin. Serine, aspartic acid, and glycine are three of the 18 amino acids that contribute to sericin's physiochemical and functional properties. The 18 amino acids also contain 70% hydrophilic amino acids, which are responsible for sericin's strong solubility and water absorbability. On the other hand, aromatic amino acids make up just 6.6 percentage of the 18 amino acids according to UV spectrum (**Wu et al., 2007**). The chemical structure of sericin is illustrated in **Figure 1.19 (Morrison & Boyd, 1992).**

Sericin is a mixture of proteins with different molecular properties. It may have molecular weights ranging from 10 to over 400 kDa, based on extraction techniques, temperature, pH, and processing time. Sericin with molecular weight less than 20 kDa is soluble in cold water and can be recovered during the early stages of raw silk processing. Sericin with a molecular weight greater than 20 kDa is insoluble in cold water and cannot be recovered **(Aramwit et al., 2012**).

Sericin

Where R = $-CH_3$ or $-CH_2C_6H_4OH$

Figure 1.19: Chemical structure of sericin (Morrison & Boyd, 1992)

1.9.3.2 Antibacterial, antioxidant and UV absorbance properties of sericin

Proteins normally have two UV absorbance peaks, one between 215 and 240 nm and the other between 260 and 290 nm. Aromatic amino acids including tryptophan, tyrosine, and phenylalanine absorb UV radiation in the 260–290 nm range (**Gupta et al, 2014**). **Kato et al (1998)** discovered that sericin may inhibit tyrosinase (polyphenol oxidase) activity and prevent lipid peroxidation. Sericin also showed antioxidant activity. The results indicated that sericin is a valuable cosmetic ingredient since it prevents tyrosinase activity, which is responsible for biosynthesis of skin melanin. Furthermore, **Siqin et al (2003)** discovered that sericin decreases oxidative stress and prevents ultraviolet radiation. After acute serum deficiency**, Masakazu et al (2003)** discovered that sericin can inhibit cell death and stimulate cellular growth**.** Additionally, sericin has also been shown to be effective as a biomaterial, biomedical application and functional membranes (**Zhang, 2002**).

1.9.3.3 Antibacterial, antioxidant and UV absorbance properties of sericin treated textiles

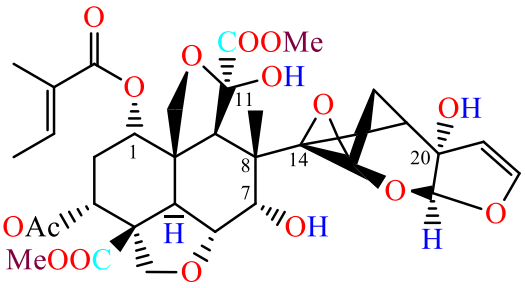
The sericin-coated fabric showed a bactericidal activity against test organisms *E. coli* and *S. aureus* (**Rajendran et al., 2012**). PET's moisture recovery (MR) and radical scavenging activity (RSA) were shown to be improved when sericin was applied. The 0.25 percent shade basic dyed samples had higher MR and RSA values than the 2 percent shade dyed samples. Basic colored samples treated with sericin had antibacterial action against *S. aureus*. An increase in dye concentration from 0.25 to 2% resulted in an increase in zone diameter. In samples dyed with 1–2% shade, the highest value of ZOI (16.5–17 mm) was observed. The diffusion of active dye in the agar medium caused a leaching effect in the zone surrounding the sample (**Chaudhary et al., 2017**).

1.10 Textile performance of some other important natural antimicrobial agents

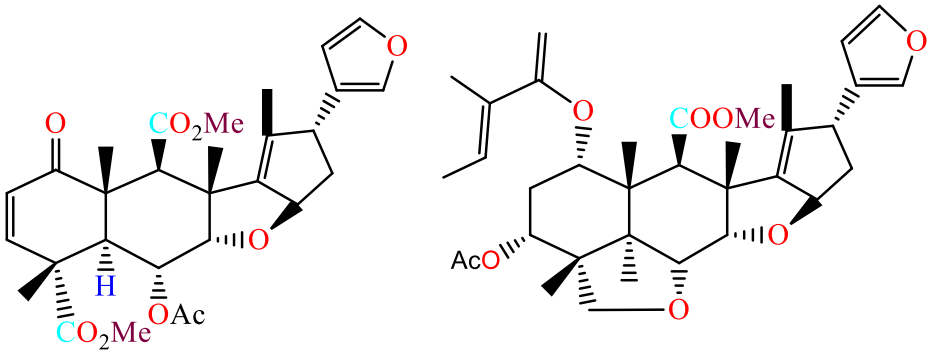
1.10.1 Neem

The plant family Meliaceae contains neem (Azadirachta indica), an evergreen tree native to India. It has been listed as one of the most promising sources of insecticides, antimicrobials, and medicinal compounds. These bio-active ingredients are known to have anti-allergenic, anti-dermatic, anti-fungal, anti-inflammatory, anti-bacterial and other biological activities **(Ogbuewu et al, 2011**). From ancient times in India, neem

has been utilized as a traditional medicine to treat a variety of human illnesses. Traditional Ayurvedic medicine uses around 700 herbal medicines based on neem. Neem has also gained a lot of attention worldwide for its potential use as a natural insecticide and for its usage in other therapautic formulations, in countries inclusive of China, USA, France, Germany, Italy, etc. Although the active compounds in neem can be found in many parts of the tree, the seed, bark, leaves, and roots are the most commonly employed for extraction. More than 300 unusually vibrant chemicals have been discovered in various areas of the neem tree. The most important limonoids are *azadirachtin, salanninand nimbin* as illutrated in **Figure 1.20** (**Schmuttere, 1995**).



AZADIRACHTIN



NIMBIN SALANNIN

Figure 1.20: Active limonoids in neem extracts (Schmuttere, 1995)

Neem (*Azadirachta indica*) and Mexican daisy have been applied to cotton through a variety of methods, such as direct application, pad-dry-cure, micro-encapsulation, resin crosslinking and their combinations. All procedures produced excellent antibacterial properties with acceptable wash durability, with the exception of direct application of extracts, which had low wash fastness (**Thilagavathi et al., 2007**).

Antibacterial characteristics were added to the polyester/cotton mix fabrics using a seed from the Neem tree (Azadirachta indica). Antimicrobial activity against gram-

positive and gram-negative bacteria was assessed using quantitative analysis. Up to five machine washes, antimicrobial activity against gram-positive bacteria remained constant, but reduced after that. When compared to Proteus vulgaris gram-negative bacteria, the antibacterial activity was stronger against gram-positive bacteria (**Joshi et al., 2007**). **Figure 1.21(a)** illustrates the amount of bacterial colonies of untreated cotton/polyester blend fabric and completed fabric with neem seed extract (5 % w/v) against Bacillus subtilis as seen in **Figure 1.21(b)**. The treated textiles suppressed the development of gram-positive bacteria (Bacillus subtilis) by more than 90%, compared to the untreated sample.

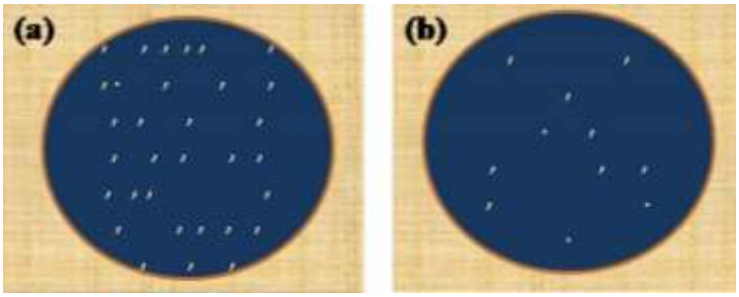


Figure 1.21: Bacterial colony (a) untreated and (b) neem seed extract (5%w/v) treated 52:48 cotton/PET blends against *B. Subtilis* bacteria (Joshi et al., 2007)

1.10.2 Tulsi (Ocimum sanctum L)

Tulsi *(Ocimum sanctum* L) is native to Asia and to central and western Africa. It is one of the *Lamiacae.* Tulsi has long been used for medicinal purpose (**Prakash & Gupta, 2005**). Tulsi is an antioxidant (**Shah & Verma, 2012)** as well as an antimicrobial (**Agarwal et al, 2010**). Antimicrobial activity of Tulsi (Ocimum sanctum) extract treated cotton fabrics revealed a zone of inhibition ranging from 9.9 mm to 12.5mm for gram-positive and from 4.9 mm to 6.6 mm for gram-negative bacteria for different extraction method (**Sathianarayanan et al, 2009**). Methanol, petroleum, ethanol, ether and water were used to extract the tulsi. Among the extraction, methanolic extract of tulsi showed the best antimicrobial activity. Cotton fabrics treated with tulsi nano particle illustrated outstanding antibacterial property with extraordinary wash durability than methanol extract tulsi treated cotton fabric against hospital pathogenic bacteria (**Rajendran et al, 2013**).

1.10.3 Curcumin (*Curcuma longa L.)*

Curcumin (*Curcuma longa L.)*, a yellow pigment, is known to have biological properties. Its common name turmeric. *Curcumin* has a long history of both antimicrobial use and also as an insect repellant **(Rudrappa & Bais, 2008**).The chemical formulae of curcumin is 1.7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, as shown in **Figure 1.22 (Labban, 2014). Lawhavinit et al (2010)**–proved the antibacterial properties of hexane and methanol extracts of curcumin**.**

Ammayappan & Moses (2009) found that curcumin inhibits the microbial growth in cotton, wool, and rabbit's fur**.** Curcumin-finished wool showed 45% and 30% inhibition rates against *Staphylococcus aureus* and *Escherichia coli*, respectively, after 30 cycles of home laundering **(Han & Yang, 2005).** Curcumin was found to be more efficient for *S. aureus* than *E. coli* bacteria. Antimicrobial property of curcumin treated wool fabrics was superior than cotton fabrics interms of laundering (**Reddy et al, 2013).**

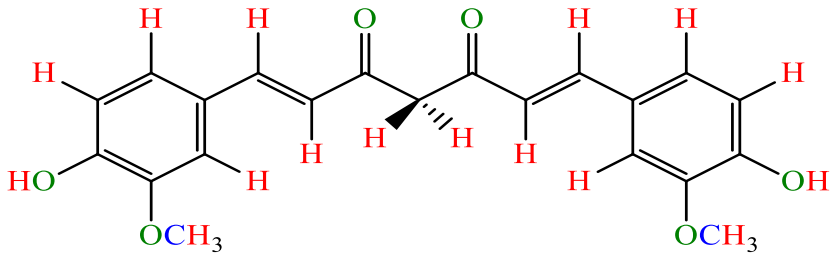
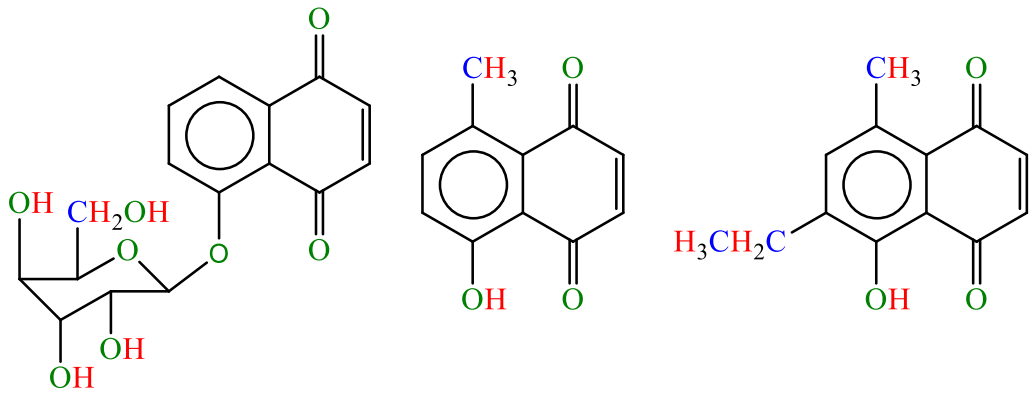


Figure 1.22: Chemical structure of curcumin (Labban, 2014).

1.10.4 Walnut *(Juglan regia*)

The green husk of the walnut (**Juglans regia L**.) is a waste product. If it is not properly managed, this garbage might pollute the environment. However, it could be a valuable source of natural compounds, such as juglone and phenolic compounds **(Fernandez-Agullo et al, 2013)**. Juglone, also called 5-hydroxy-1, 4-naphthoquinone, is a brown pigment that occurs naturally in leaves, roots, husks, and the bark of plants**.** Walnut shells extract contained phenolic and naphthoquinone compounds possess distinct antibacterial activities, antioxidant as well as coloring agent **(Oliveira et al, 2008; Pereira et al, 2006).** The chemical structure of the extracted dye from the walnut shells with different solvents are shown in **Figure 1.23** (**Mirjalili & Karimi, 2013).** A brown color dye can be achieved by using aqueous walnut extract **(Mirjalili et al., 2011).** When compared to a TiO_2-only treated sample, green walnut shells and titanium dioxide-treated cotton fabric had superior antibacterial and antifungal activities (**Nazari, 2019**).



(a) (b) (c)

Figure 1.23: Considering the results from IR and NMR spectra, Chemical structures of the extracted dye from the walnut shells with different solvents:(a) hexane/ petroleum ether (90:10,v/v), Rf=0.32,(b) petroleum ether/diethyl ether(50:50,v/v), Rf = 0.68, and (c) petroleum ether/diethyl ether(55:45,v/v), Rf = 0.64 (131. Mirjalili & Karimi, 2013)

1.10.5 Terminalia chebula (Terminalia chebula)

Terminalia chebula has long been employed in Indian outdated medicine (**Dash, 1991**). The plant is effective against different bacteria (**Bag et al, 2009**). The methanol extract of *Terminalia chebula treated* textile material exhibited worthy antimicrobial activity against the widespread pathogenic bacterial strain **(Rathinamoorthy & Thilagavathi, 2013)**.The terminalia chebula treated rib (R) interlock (I) and plain (P) fabric illustrated antibacterial property against the eight odor causing bacterial strains like *Corynebacterium Sp* (MTCC 8730), *Micrococcus luteus* (ATCC 49732), *Escherichia coli* (MTCC 1687) and *Staphylococcus aureus* (MTCC 737). Among the odor causing bacteria, zone of inhibition was found 44, 48 and 43 mm for rib (R), interlock (I) and plain (P) fabric respectively against *Staphylococcus aureus* gram-positive bacteria. Again zone of inhibition was found 39, 42 and 36 mm for Rib (R), interlock (I) and plain (P) fabric respectively against *Escherichia coli* gram-negative bacteria. Interlock fabric had the highest antimicrobial property. Treated fabric showed a higher zone of inhibition against *Staphylococcus aureus* gram-positive bacteria than *Escherichia coli* gram-negative bacteria. The active components of Terminalia chebula extracts, such as tannins, saponins, and phenols, were detected on the treated cloth surface, according to FTIR tests. These active ingredients are thought to be responsible for antibacterial action (**Rathinamoorthy & Thilagavathi, 2014). Figure 1.24** shows a symbolic example of the treated fabric's zone of inhibition against two bacteria.

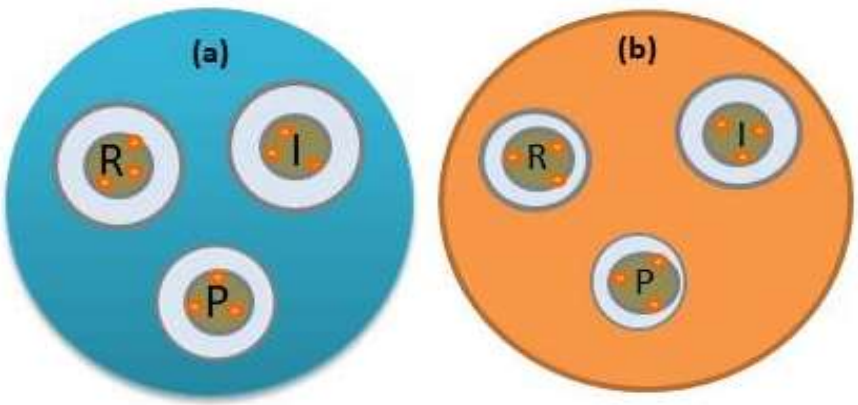


Figure 1.24: Zone of inhibition against the odor forming bacterial strains (a) *Staphylococcus aureus* and (b) *Escherichia coli* (Rathinamoorthy & Thilagavathi, 2014)

1.10.6 Natural Dyes

Generally, natural dyes produce extraordinary unusual, captivating, and soothing shades on textiles. Since most natural dyes have innate antimicrobial properties, they would have a high medicinal efficacy. The bark (e.g. Purple bark, Sappan wood, Shillicorai, Khair, Red, and Sandalwood), the leaf (e.g. Indigo, Henna, and Sandalwood), and other parts of the plant are used to produce natural dyes that contain coloring materials like tannin, flavonoids, quinonoids, etc. Natural dyes can also be present in microorganisms like fungi, algae, and bacteria. These dyes can be used as not only a rich and diverse source of dyestuff, but also they are safe, environmentally friendly, and low-cost treatments **(Samanta & Agarwal, 2009; Samanta et al, 2011).**

Gupta et al (**2004**) and **Singh et al** (**2005**) examined the antimicrobial properties of many natural dyes against gram-positive and gram-negative bacteria. They discovered that the antimicrobial potency of a natural dye varies based on whether it is in solution or retained in a cloth substrate. As a result, the antimicrobial activity of textiles impregnated with these natural dyes is decreased because the dyes' absorption on the textile substrate is below the minimum inhibitory concentration. The antimicrobial activity of those dyes is also affected by their chemical structure, especiall the functional groups present. The presence of tannins is liable for anti-microbial activity of maximum of those herbal dyes.

Antimicrobial properties of tannin-based natural dyes (*Quercus infectoria* and *Acacia catechu*), and anthraquinone-based dyes (*Rumex maritimus* and *Rubia cordifolia*) were tested against four microbes (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*,

Bacillus subtilis and *Proteus vulgaris*). Antimicrobial activity was found in all of the natural dyes against one or more microorganisms. *All bacteria are killed by Quercus infectoria.* The dye *Acacia catechu* is also effective against all microbes except *Klebsiella* pneumoniae. Only Klebsiella pneumoniae is resistant to the dyes Rubia cordifolia and Rumex maritimus (**Gupta et al., 2005**).

Tannins are naturally occurring polyphenols that are water soluble and present in many plant and tree species, accumulating up to 10% by dry weight in sections such as bark, wood, leaf, roots, or fruits. Tannins have antimicrobial effects against a a wide range of bacteria and fungi. **Figure 1.25** represents the molecular composition of tannins **(Lü et al., 2004**).

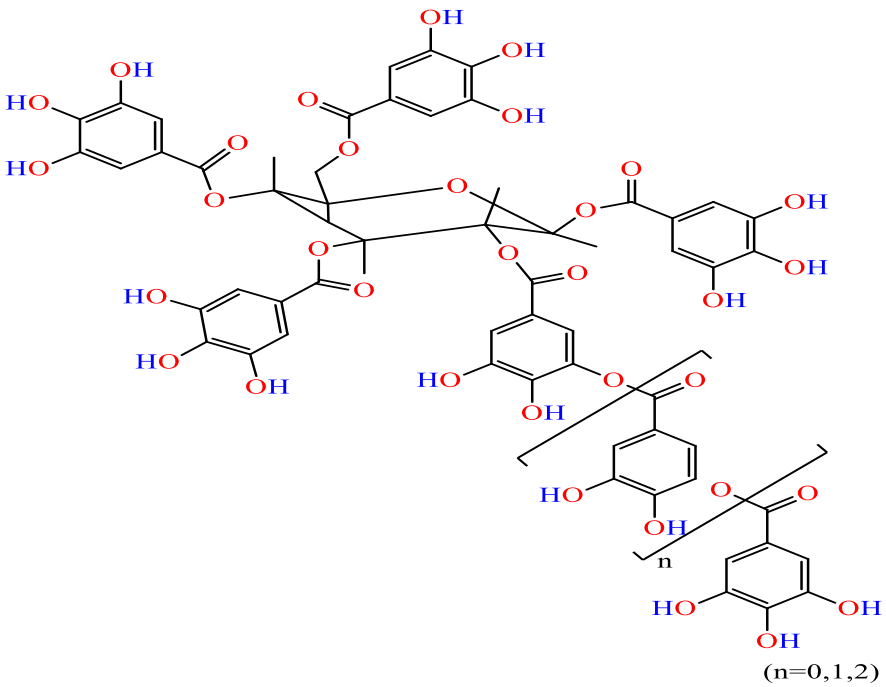


Figure 1.25: The chemical structure of tannins (Lü et al., 2004)

1.11 Existing commercial antimicrobial agents

Different commercial antimicrobial agents are available in the market, such as 3 - trimethoxy silyl propyl dimethyl octadecyl ammonium chloride, poly (hexamethylene) biguanide hydrochloride (PHMB) Tinosan AMI 10 and NW200. The fabric finished with 3-trimethoxy silyl propyl dimethyl octadecyl ammonium chloride has the highest antimicrobial function.. We may speculate that antibacterial behavior was accomplished by physically and ionically destroying the microbes' cell membranes **(Holme, 2002)**. Antimicrobial agents containing poly (hexamethylene) biguanide hydrochloride

(PHMB) are especially suitable for cotton and cellulosic textiles They will, however, be used on cotton-polyester-nylon blends in some cases. Tinosan AMI 10 and NW200 contains as an active antimicrobial, Ciba Irgasan DP300, which is generically-named "triclosan", has been used for over 30 years successfully in skin and body care products (**Cox, 1999**) and it is effective against most gram-positive and gram-negative bacteria, as well as some fungi and yeasts (**Regos et al, 1979**).

1.12 Testing issue of antimicrobial textiles

1.12.1 Efficacy testing

It is essential for consumer loyalty, marketing, and registration in the United States that the textiles have a proven antimicrobial effect. The efficacy of antimicrobial textiles can be evaluated in this way using a combination of systematic approaches and experimental setups. Antibacterial function is assessed using both qualitative and quantitative test methods. Known qualitative test methods include the parallel streak test (**AATCC147, 2004**) and the agar plate diffusion test (**DIN EN ISO20645, 2004**). A quantitative test for antibacterial textiles is defined by the shake flask test **(ASTM E2149, 2013)** and the plating process **(AATCC-100, 2004)**. Semi quantitative test method **(152. AATCC 30, 2004**) is used to assess the antifungal activity, mildew and rot resistance of textile materials.

1.12.2 Durability testing of antimicrobial textiles

Antimicrobial textiles should retain their potency for as long as possible. Antimicrobial textiles' durability is determined by the antimicrobial's application process, stability/consumption, and antimicrobial concentration **(Lorenz et al, 2012; 154. Ranganath & Sarkar, 2014)**. The reduction of antimicrobial agent concentration on the textile results obviously in loss of effectiveness. Antimicrobial efficacy must be studied in the laboratory after various washing cycles and storage intervals in order to assess the durability of antimicrobial fabrics. The antimicrobial effectiveness is tested both before and after laundry. Antibacterial clothing has a short lifespan if its antimicrobial activity drops drastically. In terms of wash durability testing, a suitable washing technique must be determined based on the textile's future application, such as work wear (**1DIN EN ISO 15797, 2004**) or apparel for leisure time (**DIN EN ISO 6330, 2013)**.

1.12.3 Safety testing

As most anti-microbial textiles are primarily in direct contact to the human skin, it is very important that the human skin will not be harmed or irritated **(Kramer et al., 2006)**. Since antimicrobials can attack bacterial cells, they must be guaranteed not to attack human cells. This is why there must be proof that antimicrobial textiles are not harmful to humans. There are many methods available for determining biological skin protection. In vitro cytotoxicity **(DIN EN ISO10993-5, 2009)**, skin inflammation (**DIN EN ISO10993-10, 2010**), and the influence of resident skin flora are all determined through these studies. However, before these experiments can be carried out, it must be established that the antimicrobial used on the cloth is safe. In the basis of a risk assessment and a measure for genotoxicity, carcinogenicity, and reproductive toxicity, this must be explained (**DIN EN ISO 10993-3, 2014)**.

1.13 Regulations of antimicrobial textiles

1.13.1 Regulations for European markets

The European Chemicals Agency would list all antimicrobial (biocidal) agents used in antimicrobial textiles in Europe (ECHA). With the exception of medicinal devices, antimicrobial textiles must be licensed under the Biocidal Substance Regulation (BPR) No. 528/2012. **(Das europäische parlament und der rat der europäischen union, 2012**) and No. 34/2014 **(Das europäische parlament und der rat der europäischen union, 2014),** but only if the anti-microbial efficacy of the textile is advertised. As a result, textiles that promise to eliminate odor are exempt from registration. According to BPR, biocidal products are compounds or compositions that contain active substances which engage harmful organisms chemically or biologically. The registration of an antimicrobial textile is valid for ten years, but it is possible to renew it for another ten years. If the antimicrobial fiber is licensed with BPR, special marking is required for consumers and distributors to be notified about biocidal substances. It is not permitted to trivialize the antimicrobial textile's biocidal function in advertisement. Non-European exporters and producers, who export treated articles into European markets, must ensure that the anti-microbial agents contained in the anti-microbial textiles are approved for use in Europe before exporting them to European markets.

1.13.2 Regulations for US markets

Antimicrobial agents must be licensed under Environmental Protection Agency (**EPA**) guidelines in the United States, whereas antimicrobial textiles containing pesticides, with the exception of medical products, must comply with the Federal Insecticide, Fungicide, and Rodenticide Act **(FIFRA)** (**United States Code Title 7, 2012).** According to the EPA, anti-microbial pesticides are substances or mixtures of substances used to destroy or inhibit the growth of harmful microorganisms. Regarding anti-microbial textiles, a distinction is made between public health and non-public health anti-microbial pesticide textile (**United States Environmental Protection Agency, https://www.epa.gov/agriculture**). Human-infectious microorganisms are said to be protected by public health textiles. Registration and efficacy test data are needed for such products. As the number of test microorganisms on the textile is reduced by 99.9% when compared to a control textile, the efficacy is proven. Antimicrobial textiles for public health are labeled with terms like "fights germs" or "protects against bacteria." The product must be labeled with the textile's function, such as "control of odor-causing germs" or "control of microorganisms that cause spoilage, deterioration, or fouling."

1.14 Application of antimicrobial textiles

Patient-specific, general patient safety, and procedure-specific medical textile devices can be divided into three groups. Sponge, pads, and burn sheets are among the patient-specific products. Patient management products include under pads, adult diapers, and wipes. Sterilization wrap, surgical gowns, drapes, and sanitary pads are in the third group of procedure-specific items. Generally speaking, medical textiles such as surgical gowns, drapes, linens, upholsteries and uniforms for doctors, nurses and patient dresses, should all have biocidal properties. Because most medical textiles come into direct contact with the skin and are worn for long periods of time, human protection is a major concern, especially in items that have a biocidal function. Antimicrobial textiles also used for producing artificial lung, vascular graft, wound dressing, artificial ligament, baby diaper are illustrated in the **Figure 1.26** (**Desai, 2007; Walker, 1999).**

Figure 1.26: Application of antimicrobial textiles (Desai, 2007; Walker, 1999)

1.15 Application of UV protective and antioxidant enriched textiles

Mainly UV protective and antioxidant enriched textiles are used as skin care textiles or cosmetotextiles. Cosmetotextiles currently offered on the market claim to be moisturizing, perfumed, cellulite reducing and body slimming. Face-caring fibrous products are engineered to move an active agent for cosmetic purposes as they come into contact with the skin. The aim is to integrate bioactive agents into functional textiles so that the skin is eventually rejuvenated as a result of natural body activity. **(Fisher, 2002; Czajka, 2005; Anon, 2005; Kan & Yuen, 2005).** The function of cosmetotextiles on human body is shown in the **Fig 1.27.**

Figure 1.27: Function of cosmetotextiles on the human body (Fisher, 2002; Czajka, 2005; Anon, 2005; Kan & Yuen, 2005)

Cheng et al (2010) used a commercially available cosmetic textile agent incorporating *Aloe vera* for skin-care benefits in the microencapsulation method to create cosmetic textiles. In a cytotoxicity test, the cosmetic textile agent treated textile materials did not destroy any cells, meaning that it was non-cytotoxic to the fibroblast cell line (NIH-3T3). Furthermore, no formaldehyde was detected in the cosmetic textiles. Figure 1.28 depicts microcapsulated cosmetotextiles.

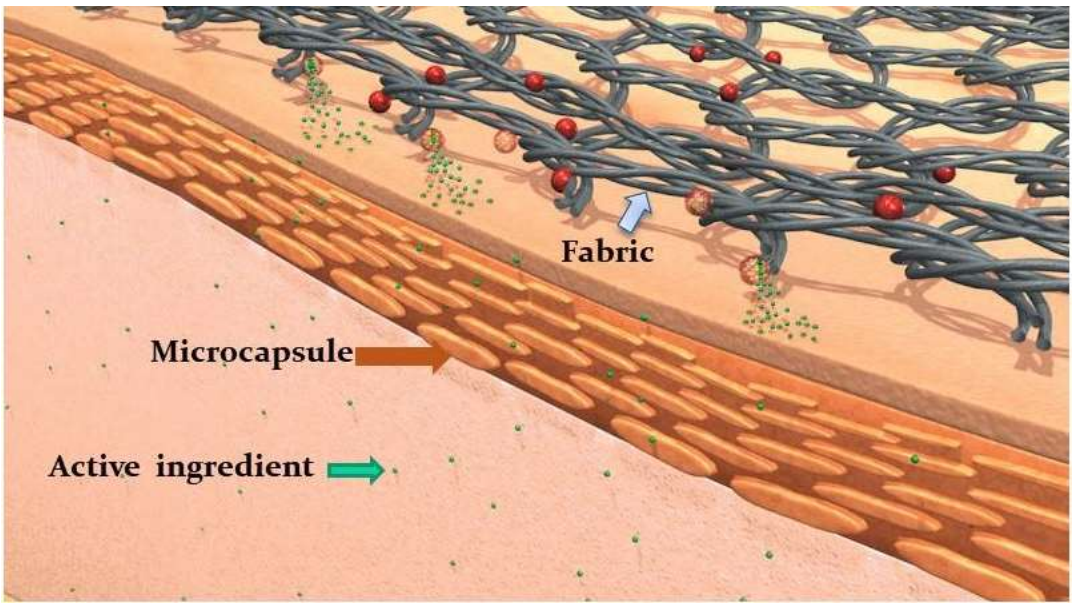


Figure 1.28: Microcapsulated cosmetotextiles

(Source: https://www.innovationintextiles.com/)

1.16 Comfort properties of textiles

When a natural plant extract, natural polymer, or solvent is added to cotton, the surface properties as well as the fabric's comfort properties are altered **(Ibrahim et al, 2010; Jj & Vk, 2014**).

Comfort is a complicated concept. It's difficult to understand, but pain can be easily explained using words like prickle, scratch, and hot and cold sensation (**Li, 2001**). We cannot define it chemically but the wearer knows it when he/she feels it. 'A state of physical ease and relief from pain and distress,' according to one generally accepted concept of comfort **(Dictionary, 1989)**. Comfort is often described as "a pleasurable state of physiological, psychological, and physical equilibrium between a human being and his or her surroundings" **(Hatch, 1993)**. Fabric comfort is a multi-dimensional phenomenon that involves physical relations between the human body, the fabric, and the surrounding world.

1.16.1 Types of comfort

Psychological comfort, tactile comfort and thermal comfort are the three primary types of clothing comfort. Aesthetic appeal, which involves color, luster, shape, height, suit, fashion versatility, and so on, is usually linked to psychological comfort. Tactile comfort is related to the surface and mechanical properties of the cloth. The fabric's air permeability, as well as its permeability to water and heat, can be used to evaluate thermal comfort **(Yoon & Buckley, 1984, Behera et al, 1997)**. Sensorial comfort and non-sensorial comfort are the two broad categories that define comfort. Sensorial comfort is a sense of clothing comfort that is based on the sensory response of nerve endings to external stimuli including temperature, pressure, pain etc, producing neuro-physiological impulses which are sent to the brain. These signals are responded to by adjusting the blood flow, sweating rate or heat production by shivering. The brain interprets these sensory signals to form subjective sensation experiences, which are organized as follows:

i) Tactile Sensations: prickly, tickling, rough, scratchy, itchy, sticky

ii) Moisture sensations: clammy, damp, wet, sticky, sultry, non-absorbent, clingy.

iii) Thermal sensations: cold, chill, cool, warm, hot.

iv) Pressure (body fit) sensation: snug, loose, lightweight, heavy, soft, stiff.

Heat transfer by conduction, convection, and radiation, as well as moisture transfer by absorption, sorption, wicking, and evaporation, are examples of non-sensorial comfort mechanisms. It also includes mechanical interactions in the form of pressure friction and dynamic irregular contract. Non-sensorial comfort is not only comprised of thermal and moisture transmission but also includes air pressure resistance, water repellence and water resistance (**De et al., 2005**). Classification of clothing comfort is shown in **Figure 1.29.**

The human body produces heat on a continuous basis as a result of its physiological processes. The heat is dissipated from the body by convection, radiation, evaporation and perspiration. The heat generated should be maintained constantly within and outside the body. During rest, most surplus body heat is lost by conduction and radiation, whereas during physical activity, the dominant means of losing excess body heat is by evaporation of perspiration. When the temperature increases, the sweating system is activated and removes the heat through heat waves and perspiration. The fabrics shield the human body from extreme sun, wind, water, and a number of harmful agents. At the same time, it also permits effective transmission of moist vapor from inside to outside, into the atmosphere. If the clothing's breathability is sufficient to compensate for body heat, the need to generate perspiration would be reduced.

Breathability is sometimes confused with wind penetration and substance wick-ability. The word 'breathable' means that the cloth is actively ventilated, which it is not. Breathable fabrics allow water vapor to diffuse through them while preventing liquid water from entering. Breathability is one such factor that decides the design of apparel and some specific technical products. The pores in breathable fabrics are 20,000 times smaller than a drop of liquid water, but 700 times bigger than a water molecule. Fire-fighting, racer suits and in other occupations requiring more metabolic activity. As result the body perspires extensively. Additionally, the garment must be able to convey a sufficient volume of water vapor while still shielding the body from external heat and pressure. Waterproofing is another such property that stops water molecules from going into cloth structures. It is used in special-purpose items. Water-repellent fabrics have properties such as waterproofing and breathability.

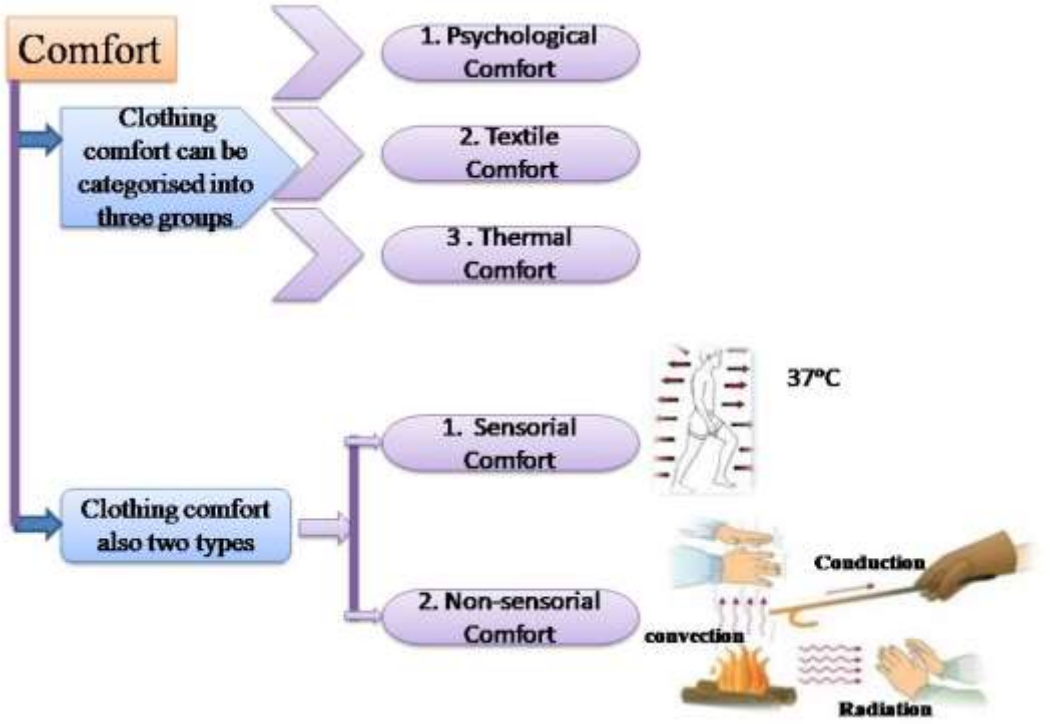


Figure 1.29: Classification of clothing comfort (177. Yoon & Buckley, 1984, 178. Behera et al, 1997).

1.16.2 Effect of finishing treatments on thermal comfort

There are a variety of finishing treatments available, such as chemical, mechanical, plasma therapy, and radiation procedure, to alter the texture and properties of textiles in order to increase the value of the fabric and garments**.** Thermal comfort is mainly determined by three factors: air permeability, thermal conductivity and water vapor permeability**.**

Thermal conductivity is an important element in assessing the comfort level of the clothing. The wearer would not feel good if excess body heat is not released sufficiently by treated cloth. If sweat is not sufficiently evaporated, more sweat is secreted, which can lead to skin rashes, fever, and bacterial growth on the skin (**Budd, 1981**).

The water vapor permeability rate of textile fabrics has a big impact on how comfortable a garment is. Water vapor would be impacted in the body if the water vapor permeability rate is poor. As a result, it creates favorable conditions for bacterial and fungal growth. The garment will also feel heavy and uncomfortable. When moderate work is performed, metabolic heat is generated by the body and there is a temperature

rise of about 3ºC in 30 minutes. This rise could be completed in 10 minutes if heavy work is undertaken and impermeable clothing is worn **(Day & Sturgeon, 1986**).

Air permeability rate also influences the comfort level of garments. When the amount of air permeability decreases then the clothes feel hot and smothering. The air permeability rate of *Aloe vera*- and chitosan-treated fabrics make the fabrics feel comfortable because the difference in air permeability rate between treated and untreated samples is small. This is not true of all treatments. Water vapour permeability increased when a silane-coupling agent, a fluoropolymer wax, was applied to cotton fabric. This made the fabric hydrophobic. As a result, thermal comfort was reduced. In contrast, alkali treatment and oxygen plasma application enhances the hydrophilic property of polyester fabric and thus improves its comfort level. In addition mercerizing, singeing, bio polishing, Antibacterial, UV protection, flame retardant, water repellent, waterproof, antistatic finishes, applied to textile fabric, influence the comfort level of the garment.

1.17 Environmental impact of the present research Work

In recent times environment friendly or ecofriendly is the big issue in the textile sectors. The word "eco" is an abbreviation for "ecology," which refers to the global relationship between animals and their ecosystems, or humans and their homes. The term 'eco-friendly' refers to a positive association between humans and their surroundings (**Atilgan, 2007**).

The environmentally sustainable agents not only help to efficiently mitigate the negative impact of microbial growth on textile materials, but they also comply with government regulations to protect the environment **(Joshi et al., 2009)**. Textiles are one of the major manufacturing industries where water, electricity, and chemicals are used. Huge amount of pollutant is discarded from these textiles. These pollutants are very much harmful for environment. For this reason, Eco-friendly processing trends are becoming more and more popular. Government agencies are placing more restrictions on the control of the quality of effluents. Under these circumstances, producers are looking for eco -friendly alternative chemicals for finishing (**Etters, 1999**). Top fabric manufacturers, eco-friendly scientists, and researchers are working together to find new fabrics that are safe for the environment, wildlife, and humans. Eco-textiles is a mission that has resulted in the development of modern technologies to produce fabrics in an environmentally sustainable way (**Veni & Mani, 2012**).

Many infectious diseases have been treated with herbal remedies throughout the history of mankind. Recently, natural antimicrobial agents have recently gained popularity due to their eco-friendly and nutritious properties **(Rathinamoorthy et al., 2011).**

The raw material of chitin is shrimp shells. During the processing of shrimp, only the meat is taken. After taking the shrimp's meat, its head, shell and tail are discarded and considered solid waste. Shrimp wastes are typically treated as rubbish, and their treatment necessities either additional funds or additional manpower (**Nowsad, 2005**). Around 40-50 percent of the shrimp is lost in the head, shell, appendages, and tail. The shrimp processing industry in Bangladesh dumps 30,000 tons of shrimp waste per year (**BFFEA, 2008**). Despite the fact that these wastes are biodegradable, they take a long time to decompose. Meanwhile, they not only emit foul odors but also attract pathogenic insects and bacteria, resulting in an unsanitary environment where they have been discarded.

Silk is a natural protein biopolymer which contains 20-25% sericin, derived from the silkworm *Bombyx mori*. This sericin is discarded into wastewater as an effluent during the degumming process in the silk industry. This increases BOD and COD as well as contaminates the water. Sericin is highly hydrophilic, with strong polar side chains such as hydroxyl, carboxyl and amino groups. By recovering the sericin during the degumming process, it will make degumming an eco-friendly process, making the clothes with which sericin is treated eco-friendly.

1.18 Economic impacts of the present research work

Shrimp shells contain a huge amount of chitin (8-10%). Chitin is converted into chitosan through deacetylation, providing an expensive ingredient used in many foods, cosmetics and pharmaceutical products **(Suparno & Poernomo, 1992**). Recently, chitosan has achieved popularity among textile producers due to its dye-absorbing capacity, antimicrobial activity, antioxidant power and UV-resistant property

Bangladesh imports approximately 100-120 tons of chitin and chitosan each year, mostly for food and medicine (**BFFEA, 2008**). Production of chitin within the country can reduce the present dependency on imports for this valuable raw material. If reasonable care is taken, it is possible to produce both chitin and chitosan within the existing process line of the shrimp processing plants. The products can either be

marketed locally or exported. So it can be said that chitosan production and application will be helpful for the economy and well as to reduce unemployment.

In our country, nearly 500 tons of silk are consumed each year and, when degumming is done then approximately 80 tons of silk sericin is discarded into waste water (20% of the contents of silk is sericin). If we used as this sericin as afinishing agent in textiles for industry and other cosmetic and pharmaceutical uses, this will increase our GDP. Furthermore, foreign currency can be earned by exporting the silk sericin powder which will contribute to our national economy and consequently provide employment. The price of silk sericin is 5,000 to 8000 Taka per kilogram subject to quality and application. Approximately the price of 80 tons of silk sericin will be 60 crore (6,000,000,000) Taka. Used in Bangladesh as a finishing agent,, this sericin can produce at least 400 tonnes of valuable protective medical textiles per year.

1.19 Research gap in the current literature

Many literature have reported that *Aloe vera*, chitosan and sericin treated antibacterial textiles. But insufficient literature was found about their use in UV resistant and antioxidant enriched textiles. On the other hand combind application of chitosan-*Aloe vera* and chitosan-sericin on cotton was not found in previously-published work. Little number of works had been done about thermal comfort.

Thus, the present study is devoted to the antibacterial, antioxidant, UV resistant, thermal comfort properties and their statistical analysis of *Aloe vera*, chitosan and sericin on cotton fabric. This will be a novel work about protective textile finishing as well as other comfort-related properties of clothing finished with these substances.

1.20 Objectives of the work

In this research *Aloe vera,* chitosan and silk sericin have been chosen because chitosan and silk sericin are now considered waste materials. On the other hand, *Aloe vera* is very cheap and available. In addition, these three materials are non-toxic, biologically-safe and environmentally-friendly.

The main aim of the present work is to develop bacteria resistant, UV resistant and oxidation protective textiles using *Aloe vera,* chitosan and silk sericin natural antimicrobial agents, applied on 100% cotton bleached woven fabric. Cotton fabric is

chosen in this research as cotton fabric is most suitable for medical textiles. But cotton is most favorable environment for bacterial growth which creates staining, discoloration, degradation of textile apparel and also generates the bad odor. In addition to bacterial growth in apparel also responsible for various diseases on human body. The objectives of the research were:

- To extract antibacterial, antioxidant and UV resistivity finishing agents like *Aloe vera*, chitosan and sericin from three natural sources (*Aloe vera*, Shrimp shell and Silk cocoon).
- To characterize the different functional properties (antibacterial, antioxidant, UV resistivity) and instrumental analysis (FTIR, UV-vis, XRD, EDX, SEM, DSC and TGA) of *Aloe vera*, chitosan and sericin.
- To apply *Aloe vera*, chitosan and sericin on cotton woven fabric separately and combindly by pad-dry-cure method.
- To investigate the antibacterial, antioxidant and UV resistant properties of *Aloe vera*, chitosan and sericin finished cotton woven fabric
- To characterize physical properties, mechanical properties, thermal comfort properties and instrumental analysis (FTIR, XRD, SEM and TGA) of *Aloe vera*, chitosan and sericin finished cotton woven fabric.
- To investigate the biodegradable property and statistical analysis of thermal comfort properties of *Aloe vera*, chitosan and sericin finished cotton woven fabrics.

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Chapter Two
MATERIALS AND METHOD

2.1 Materials

Aloe vera was purchased from the local market. Shrimp shell waste was collected for chitosan production from Khulna shrimp processing area. The silk cocoons of *Bombyx mori* were obtained from the Bangladesh Sericulture Research Training Institute (BSRTI), Rajshahi. The *Aloe vera* plant, shrimp shell and silk cocoon are shown in **Figure 2.1(a, b, c)** respectively. 100% cotton plain woven fabric was collect from Square Textile Ltd, Bangladesh. Fabric construction was 40×40/133×72. Methanol, sodium hydroxide, hydrochloric acid, sodium hypochlorite, acetic acid etc, all required chemicals were procured from BDH, Sigma-Aldrich, and Merck, used without extra purification.

Figure 2.1: (a) *Aloe vera* plant, (b) Shrimp shell, (c) Silk cocoon

Table 2.1: List of main apparatuses and instruments employed in the present investigation

Sl. No.	Name Instruments
1	Vortex mixer
2	Oven, regulated at 200^{o}C
3	pH Meter
4	Fourier Transform Infrared Spectrophotometer (FTIR)
5	Scanning Electron Microscope (SEM)
6	X-Ray Diffractometer (XRD)
7	Thermogravimetric Analyzer (TGA)
8	UV- Spectrophotometer
9	Energy Dispersive Spectrophotometer (EDS)
10	Water bath
11	Magnetic stirrer
12	Crusher
13	Suction pump
14	Electric balance
15	Incubator
16	Laminar Air Flow
17	Autoclave
18	Universal strength tester
19	Crease recovery tester
20	Abrasion resistance tester
21	Thermal conductivity tester
22	Air permeability tester
23	Water vapor permeability tester
24	Thickness gauge
25	GSM cutter

2.1.1 Instruments used in present investigation

Centrifuge machine

Vortex- Mixer

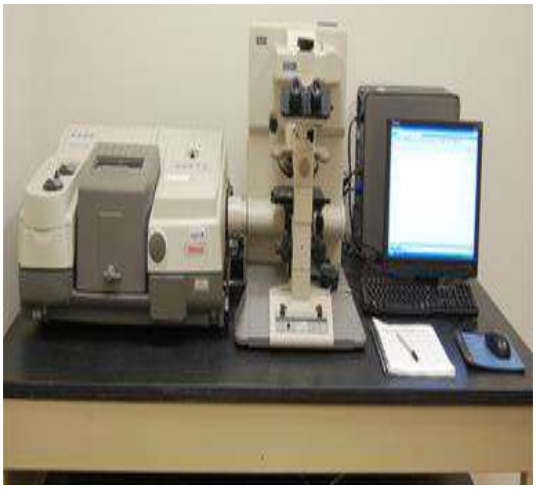
Electronic balance

Magnetic stirrer

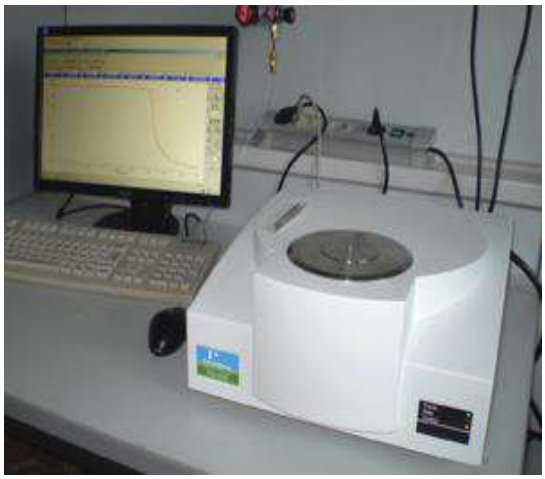
Water bath

Forced convection oven

pH meter



FTIR spectrophotometer



Thermogravimetric analyzer

Scanning Electron Microscope

UV-Visible Spectrophotometer

Laminar Air Flow

Figure 2.2: Instruments used in the present investigation

2.2 Extraction of *Aloe vera*

At the beginning, *Aloe vera* leaves were cleaned with pure water. At the point when a leaf of *Aloe vera* is cut, an orange yellow sap trickles from the open end. Cautiously expel the inward gel while staying away from the yellow sap (latex). The gel was gathered from leaves into a perfect utensil. The gel was dehydrated in an air circulating oven at 75°C for 72 hours. 5gm grinded powder was saturated with methanol (1:20) for 5 a week at room temperature. The bottles were covered with aluminum foil and placed in a dark room throughout this period. The solutions were filtered using filter paper after one week. The solvent (methanol) was then isolated with the help of a rotary evaporator (**Ghayempour et al., 2016**). The extraction method of *Aloe vera* was shown in **Figure 2.3.**

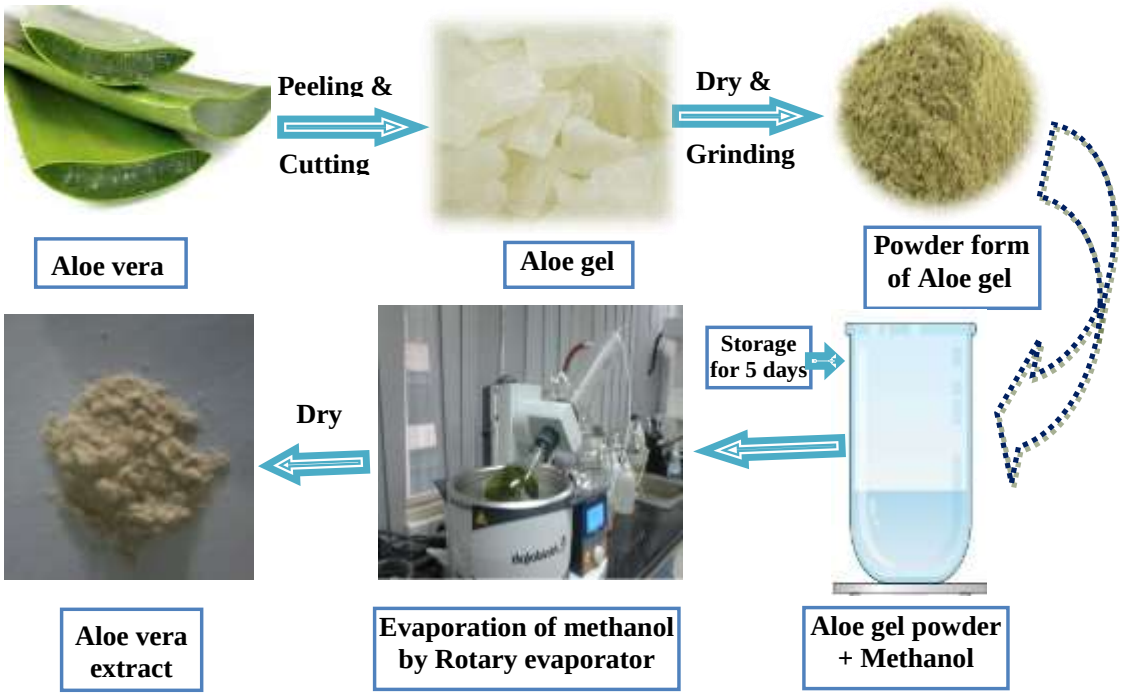


Figure 2.3: Schematic diagram of *Aloe vera* extraction process

2.3 Chitosan preparation process

The shrimp shells were cleaned in hot deionized water and dried in an oven dryer for 1h at 105 degrees Celsius. The dried shrimp shells were compressed into fine fragments using a crushing method. The deproteinisation, demineralization and decolourization process were made complete using 1M NaOH, 1M HCl and 0.15% sodium hypochlorite bleaching agent in that order and produced water-insoluble chitin. At last the water-insoluble chitin was transferred into chitosan through deacetylation. Deacetylation was

accomplished by high concentrated sodium hydroxide solution (**Alam et al., 2008**). Preparation process of chitosan is given away in **Figure 2.4.**

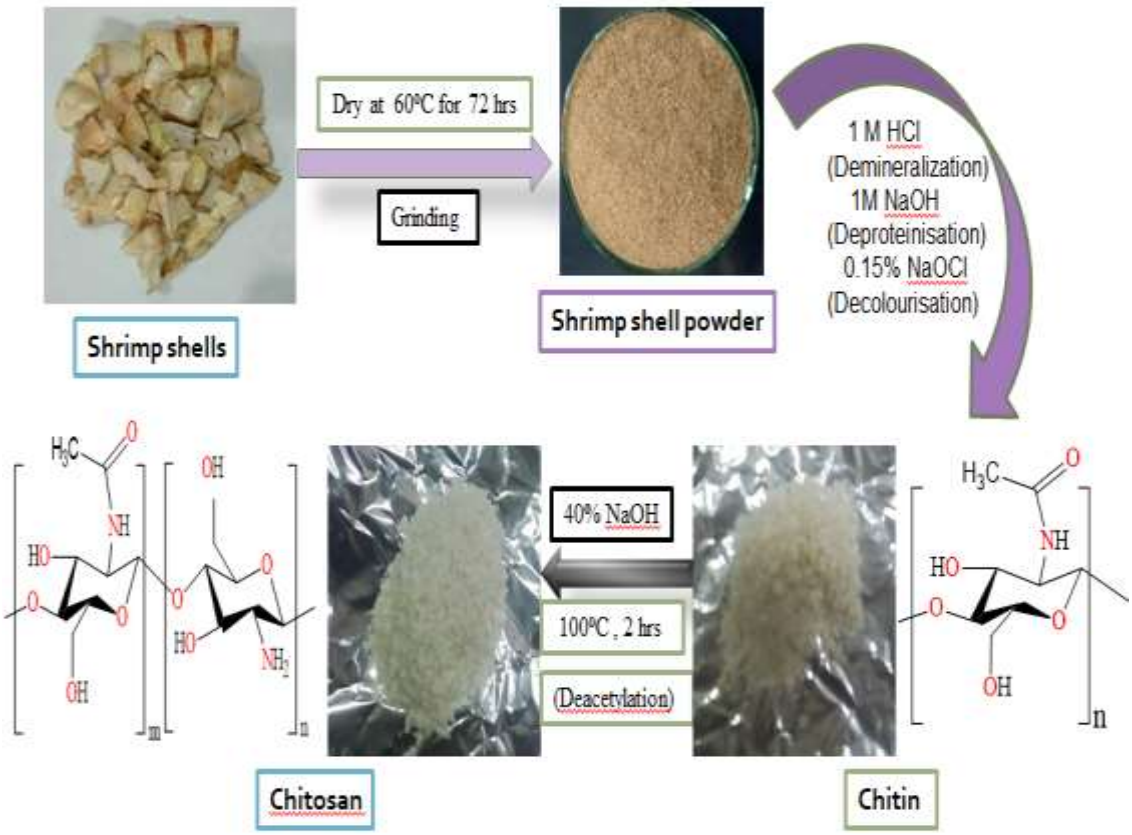


Figure 2.4: Chitosan preparation process

2.4 Silk sericin extraction

Silk sericin extraction was carried out by autoclave machine using only water (**Khalifaa et al., 2012**). 100 grams of silk cocoons is cut into small pieces for this process. Cut pieces of silk cocoon are placed in a breaker and distilled water was added to them. And silk cocoon was autoclaved for 30 min at 120°C.After autoclaving, filter paper was used to separate the silk fibroin from the combined sericin and fibroin solution. Then ethanol is added to the sericin solution for precipitation. The ratio of ethanol and sericin solution was 3:1. Sericin was collected by using Buchner funnel. Eventually, sericin was dried in an oven at 100°C. **Figure 2.5** depicts the silk sericin extraction procedure.

Figure 2.5: Schematic diagram of extraction process of sericin from silk cocoon

2.5 Modification of cotton woven fabric with *Aloe vera*, chitosan and sericin

Aloe vera, chitosan, and sericin were applied to cotton woven fabric using the Bușilă process, with only minor modifications (**Bușilă et al., 2015**).The required quantities of *Aloe vera*, chitosan, and sericin were added to the fabric using the pad-dry-cure method using a padding machine at two bar pressure. Fabric to liquor ratio of 1:15 was used in this process. Padded fabric was dried and cured at 80˚C and 150˚C respectively. The fabric was then conditioned for 6 hours at 27 degrees Celsius and 65 percent relative humidity. **Figure 2.6** illustrates the Pad dry cure process.

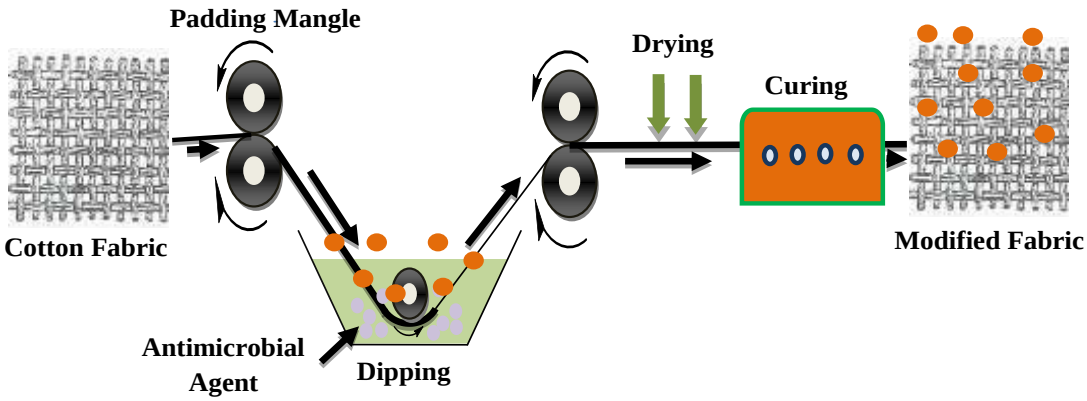


Figure 2.6: Pad dry cure method

2.6 Measurement of different structural and functional properties

2.6.1 Estimation of solubility

Solubility is one of the most important parameter of antibacterial agent as add on percentage on fabric depends on solubility of raw material. Solubility of raw material is measured either visual inspection or filtration or instrumental analysis. Solubility of *Aloe vera* and sericin was evaluated by visual inspection. In a 1 percent acetic acid solution, chitosan powder was dissolved. The following equation was used to determine the solubility of the chitosan solution (**Rastogi et al., 2001).**

$$\text{Solubility \%} = \frac{W1-W2}{W1} \times 100 \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

W_1 is the initial chitosan weight and W_2 is the residue chitosan weight after filtration

2.6.2 Determination of degree of deacetylation

A potentiometric titration procedure was used to assess the degree of deacetylation (DD) of chitosan. After dissolving 0.2 g of chitosan in 10 ml of 0.30 M HCl, it was diluted to 50 ml with ultrapure water and titrated with 0.10 M NaOH. The discrepancy between two inflection points is used to calculate the absorbed volume of NaOH solution, which refers to the quantity of amine groups in chitosan (**Tolaimate et al., 2000).**The degree of deaceylation was determined using the formulae below.

$$DD\% = \frac{2.03(V_2 - V_1)}{m + 0.0042(V_2 - V_1)} \cdots\cdots\cdots\cdots\cdots\cdots (2)$$

Where, m is the weight of the sample, V_1, V_2 are the volume of sodium hydroxide solution corresponding to the deflection points, 2.03 is the coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 is the coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

2.6.3 UV absorption measurement

Aloe vera and sericin were dissolved in distilled water. On the other hand, chitosan has been dissolved in 1% acetic acid solution. For getting sharp peak, very dilute solution of specimen has been taken. The UV absorption spectrum of the solution was measured with a Shimadzu 1650 UV–visible Spectrophotometer (Japan).

2.6.4 Fourier-transform infrared spectrophotometer (FTIR)

Fourier-transform infrared spectroscopy is a method for defining the forms of chemical bonds in a molecule by generating a molecular "fingerprint" in the form of an infrared absorption spectrum. The chemical bonds can be either organic or inorganic and it can give important information about the structure of organic molecule. It may be used to define compounds and investigate the sample structure. The interaction between functional groups on various components in polymer blends and composites are also determined by FTIR analysis (**Pavia et al., 1979**).

FTIR spectroscopic measurements were carried out in Central Science Laboratory, University of Rajshahi, Bangladesh (Spectrum-100, Perkin Elmer, USA). The samples and KBr (potassium bromide) were dried at 105℃ for 10 hours. The samples were combined with potassium bromide (KBr) using mortar and pestle to make powder in the mass ratio of 1:100 (1 mg sample powder and 100 mg KBr). The mixed sample was again dried for 10 hours at 105℃(**Mondal, 2013**). The powdered mixture was then compressed in a metal holder under a pressure to form a pellet. After that, the pellet was put in the direction of an infrared beam with a wave number of 400-4000 cm-1. The transmission mode was used for infrared spectroscopy measurements. **Figure 2.7** indicates the sample preparation for FTIR analysis.

Figure 2.7: Sample preparation for FTIR

2.6.5 X-Ray diffraction (XRD) analysis

XRD analysis was done with an X-ray diffractometer (Model: Empyrean, PAN analytical, Netherlands). The necessary specifications for sample analysis in the XRD instrument are as follows: X-ray source Cu –Kα radiation, wavelength $\lambda = 1.5406$ Å, voltage 45 kV, and Electric current 40 mA. The samples were positioned into the cavity of the aluminum sample holder. The sample holder was placed into the x-ray

goniometer **(Eedara, 2018).** The measurement was carried out at a scanning rate of 8°/min in 2θrange of 5º–80°. X-ray diffraction (XRD) patterns of *Aloe vera*, chitosan, sericin, cotton and modified cotton fabric were recorded.

The'd' spacing is determined by using Bragg's law.

According to the Bragg's law, $n\lambda = 2dSin\theta$

$$0r,\ d = \frac{n\lambda}{2Sin\theta} \cdots\cdots\cdots\cdots\cdots (3)$$

Where, n=1 (number/order of diffraction), X-ray wave length, $\lambda = 0.15406$ nm 0r 1.5406 Å is fixed and d is the inter plane spacing, θ is the peak position in radians ,

The peak width at half maximum (FWHM) as shown **in Figure 2.8** in the XRD was used to determine the crystal diameter as per the following Deby-Scherrer formula (**Jenkin & Snyder, 1996**)

$$D = \frac{K\lambda}{\beta Cos\theta} \text{ ..... } (4)$$

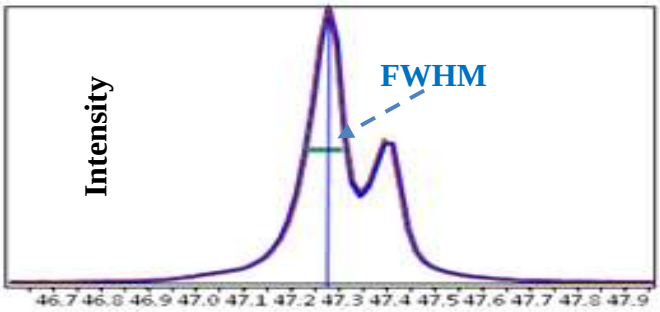


Figure 2.8: Full width at half maximum (FWHM) peak-height of the X-ray pattern

Where, K= 0.9 is the Scherrer constant; the X-ray wave length, $\lambda = 0.15406$ nm; β the peak width of half maximum; and θ, the Braggs diffraction angle. β is the full width of the X-ray pattern line at half peak-height in radians

The crystalline index (CI) of the samples can be calculated from the maximum intensity peak at crystalline region and lowest intensity peak at amorphous diffraction of the XRD pattern. The crystalline index (CI) of the samples was calculated by the following equation (**Ye & Farriol, 2005)**

$$\textbf{C. I} = \frac{I_{cr} - I_{am}}{I_{cr}} \times 100 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots (5)$$

Where, Icr is the maximum intensity of the crystalline region of the material, and Iam is the amorphous diffraction of the XRD pattern.

2.6.6 Scanning electron microscopy (SEM)

The scanning electron microscope is used to study surface morphology of the polymeric material. The images of the sample surface is taken by a high energy electron beam of scanning electron microscope. The electrons interact with the atoms that make up the sample, producing signals that contain information about the samples surface topography, composition and other properties such as electrical conductivity. In SEM, proper sample preparation is necessary prior to characterization **(Hunt & Jame, 1997).**

At first Fabric samples were placed in the plasma chamber and gold/palladium coating was given. After successful coating was done, the samples were placed in the chamber of the SEM and chamber door was closed. Proper vacuum was maintained inside the chamber. When the preset vacuum was reached, emitting electron beamsstarted. The magnification and resolution was decided as per the best viewing conditions on the screen of the monitor. Samples were tested at a magnification of 5100x and were scanned at 5 kV. Scanning electron microscopy (**Model- Phenom G2 pro, Netherland**) was performed to investigate the surface morphology and thus to confirm the binding to the fabric of antibacterial agents and their alignment on the fabric sample

2.6.7 Energy dispersive spectroscopy (EDS/EDX)

Energy dispersive X-ray analysis, referred to as EDX/EDS, is an X-ray technique used to identify the elemental composition of materials. The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the sample being analyzed**.**

2.6.8 Thermogravimetric analysis (TGA, DTA and DTG)

In thermal analysis three wings are involved like thermogravimetric analysis (TGA), derivative thermogravimetric (DTG) and differential scanning calorimeter (DSC)/ differential thermal analysis (DTA).Thermal analysis is a most important tool to measures the changes in physical and chemical properties of samples like absorption,

sublimation, vaporization, oxidation, reduction and decomposition against the temperature with constant heating rate. The weight loss of the sample as a function of temperature was continuously reported. The DTG curve is the first derivative of the TGA curve. The rate of change of mass with respect to time or temperature is determined by the derivative thermogravimetric (DTG) curve. Derivative thermogravimetric (DTG) curve is defined as dw/dt. Exothermic, endothermic, and glass transition points of samples are determined using differential thermal analysis (DTA) as a function of heating temperature. A simultaneous thermal analyzer was used to conduct thermal analysis of *Aloe vera*, chitosan, and sericin samples (STA 8000, Perkin Elmer, USA). Under a steady flow of nitrogen atmosphere, a heating rate of 10°C/min and a temperature range of 30–600 °C were used for thermal analysis.

2.6.9 Antibacterial activity test for *Aloe vera*, chitosan and sericin solution and *Aloe vera*, chitosan and sericin treated fabric by qualitative method

Zone of inhibition of *Aloe vera*, chitosan and sericin solution and *Aloe vera*, chitosan and sericin treated fabric was determined by disc diffusion method **(AATCC TM: 147-2004, 2010).** A nutrient agar medium of 250 mL was prepared and autoclaved. Under aseptic conditions, the autoclaved nutrient agar medium was poured into a sterile petri dish. The petri dish was inoculated with a 20µl culture of *S. aureus* bacteria. Whatman paper is punched into discs, which are then autoclaved. Discs were dipped different concentration of *Aloe vera*, chitosan and sericin solution. On the other hand, nearly 1mm diameter sample disc was cut from the *Aloe vera*, chitosan and sericin treated fabric. The paper discs and fabric discs were placed on the inoculated petridish. The petri dish was incubated for 24 hours at 37°C. The region of inhibition was discovered after 24 hours. The zone of inhibition was determined using the formulae below. Schematic diagram of the antibacterial testing procedure is shown in the **Figure 2.9**.

$$W = \frac{T - D}{2} \dots\dots\dots\dots\dots\dots\dots (6)$$

Where, W is the width of the clear zone of inhibition in millimeters, T is the total diameter of the test specimen and the clear zone in millimeters, and D is the diameter of the test specimen in millimeters.

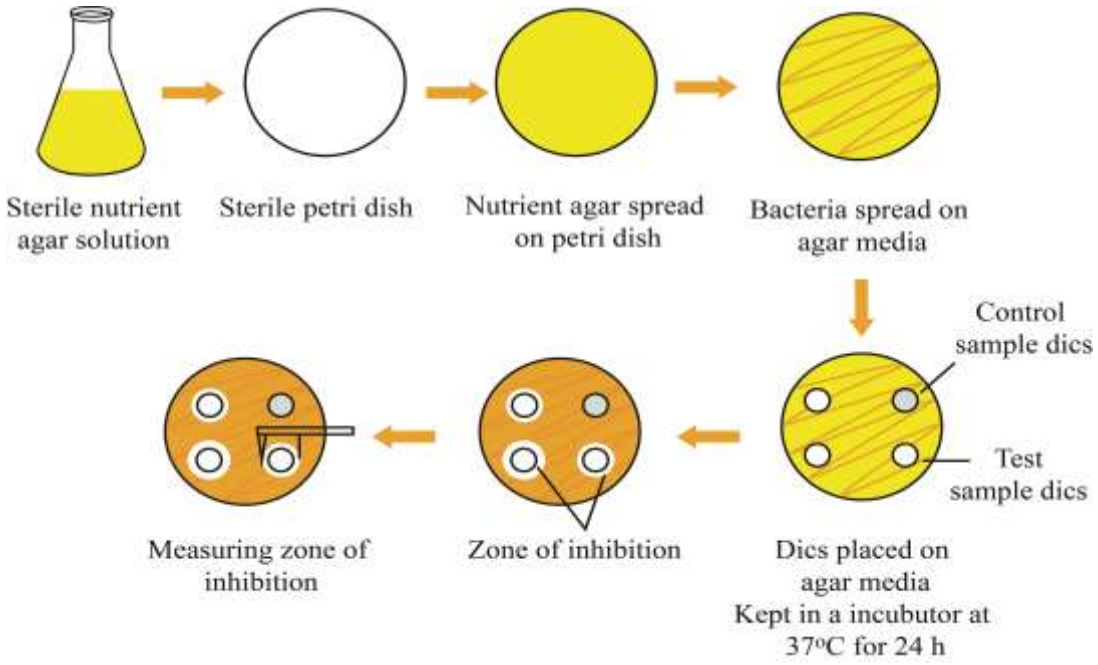


Figure 2.9: Schematic diagram of disc diffusion antimicrobial test method

2.6.10 Antimicrobial activity evaluated by optical density

Antibacterial activity was tested against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Bacterial growth was determined according to the **Ai et al. (2019)** method. The bacterial growth was determined by optical density (OD) which was measured using UV spectrophotometer at 600 nm. At first the bacterial solution was diluted to absorbance of 0.02. Diluted bacterial solution was added into the *Aloe vera*, chitosan and sericin solution. Then, the bacteria and specimen mixed solution was cultured at 37°C for 2, 4, 6, 8 and 10 hours. And take absorbance after fixed interval to plot the growth curve of the bacteria. For each separate experiment, the assay was carried out in triplicate. The transmitted light decreases as the bacterial cell population increases, while the absorbance rises. The bacterial reduction percent was measured using the equation below.

$$\text{Bacterial reduction } \% = \frac{A-B}{A} \times 100 \cdots\cdots\cdots\cdots\cdots\cdots (7)$$

Where, A= Control absorbance, B= Control + sample absorbance

2.6.11 Quantitative antibacterial test of treated and untreated fabric

The AATCC-100 standard was used to assess the bacterial population of treated and untreated samples **(AATCC TM: 100-2004, 2010).** A 2"2" research sample was cut and placed in five 50 ml conical flasks, each containing 20 ml of nutrient broth and 20 µl

microbial culture, as per the norm. All the flasks were kept in a shaker incubator with 200 rpm at 37º C for 24 hours. The sterilized purified water was used to dilute the incubated test culture at five times. In a nutrient agar petri dish, 20 µl of each dilution was spread. All the inoculated plates (both untreated and treated samples) were incubated for 24 hours at 37 ºC. The surviving cells were counted after 24 hours. The percentage reduction was calculated using the following equation: The following equation was used to measure the bacterial reduction percentage.

$$\text{Bacterial colony reduction (\%)} = \frac{(B-A)}{B} \times 100 \quad \text{..... (8)}$$

Where, A is the number of surviving cells of treated samples and B is the number of surviving cells of the untreated sample.

2.6.12 Wash durability test

The treated fabric was subjected to washing by industrial machine and the antibacterial activity of the washed sample was evaluated by the AATCC-124 and AATCC 100 test standards respectively **(AATCC 124-2004, 2010; AATCC TM: 100-2004, 2010)**,

2.6.13 Antioxidant activity of sample solution

The 2.2-diphenyl-1-picryl-hydrazil (DPPH) reagent was used to test antioxidant activity. DPPH is a stable free radical which characteristic absorption peak showed at 517 nm. A system defined by **Wu et al. (2007)** was used to investigate the free radical scavenging effects of *Aloe vera*, chitosan, and sericin with slight modification. The sample solution was formulated in five separate amounts of 2,4,6,8, and 10 mg/ml. In methanol, 0.004% DPPH solution was packed, and 3 ml of it was combined with 1 ml of sample solution and vortexed. To complete the reaction, the mixture solution was kept in the dark for 25 minutes at room temperature (25ºC). However, the reaction mixtures were centrifuged at 6000 rpm for 5 minutes. Consequently, the absorbance of the decolorized solution and control sample was measured at 517 nm against a blank sample using a UV visible spectrophotometer. The following equation was used to calculate the free radical scavenging operation.

$$\text{Radical scavenging activity (RSA) \%} = \left(1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}}\right) \times 100 \cdots\cdots\cdots\cdots\cdots\cdots \text{ (9)}$$

Where, control absorbance means methanol solution of DPPH and sample absorbance means decolorized solution. In case of sample absorbance measurement, blank sample is prepared by 3ml pure methanol without DPPH mixed with 1ml *Aloe vera* or chitosan or sericin sample solution for each respective concentration. **Figure 2.10** depicts a schematic illustration of the antioxidant monitoring method.

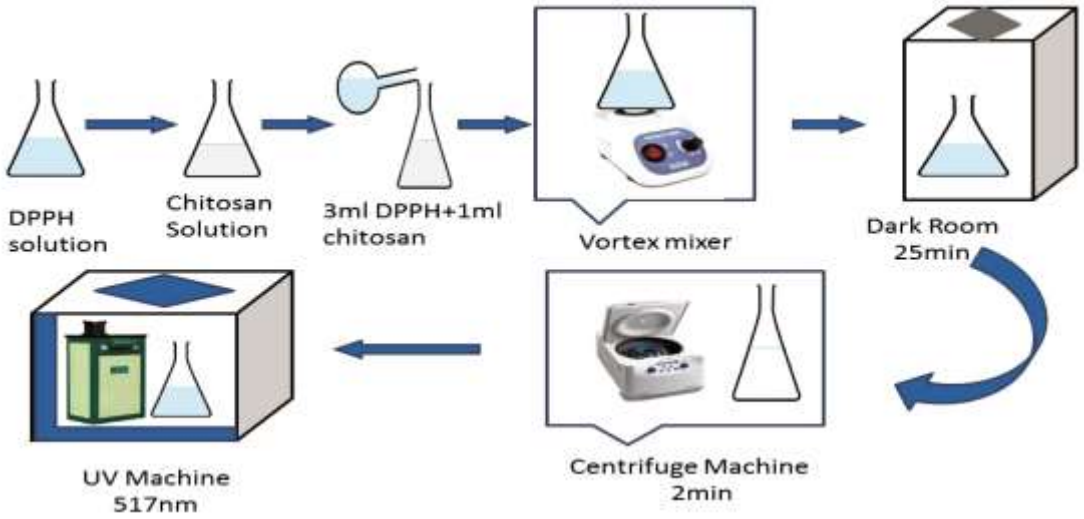


Figure 2.10: Schematic diagram of the antioxidant activity testing procedure

2.6.14 Antioxidant activity of treated fabric

Antioxidant property was estimated by assessing the free radical scavenging activity (RSA) of *Aloe vera*, chitosan and sericin treated cotton textiles. Free radical scavenging activity was observed as per **Wu et al. (2007)** method with barely any alterations. Three observations were taken and average for each sample in this investigation. 1″ x 1″ *Aloe vera*, chitosan, or sericin-treated cloth was immersed in 0.004% DPPH radical in a methanol solution and vortexed. The reaction mixtures were centrifuged at 6000 rpm for 5 minutes after responding for 25 minutes at 25°C (room temperature) in the dark. At 517 nm, the decolorized solution was analyzed. The blind control sample containing 3.5 ml DPPH and untreated sample. The following equation was used to measure the free radical scavenging operation.

$$\text{Radical scavenging activity (RSA) \%} = \left(1 - \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{blind}}}\right) \times 100 \cdots\cdots\cdots\cdots\cdots\cdots (10)$$

The absorbance of the treated sample is $_{\text{ODsample}}$, and the absorbance of the blind control is $_{\text{ODblind.}}$ OD stands for optical density.

2.6.15 Measurement of UV protection factor (UPF)

According to the AATCC test procedure (**AATCC183-2004, 2010**), the UPF was determined using the following equation.

$$UPF = \frac{\sum_{280}^{400} E_\lambda . S_\lambda . \Delta_\lambda}{\sum_{280}^{400} E_\lambda . S_\lambda . \Delta_\lambda . T_\lambda} \quad \text{..... (11)}$$

Where, E_λ stands for erythermal spectral effectiveness, S_λ for solar spectral irradiancies, λ for wavelength in nm, T_λ stands for spectral transmission of the specimen and Δ_λ is the measured wavelength intervals in nm. The AATCC testing manual provides the value of E_λ and S_λ. On the other hand, T_λ was measured by UV spectrophotometer (Shimadzu 1650, Japan).

2.6.16 Weight add-on percentage

The fabric was treated with *Aloe vera*, chitosan, and sericin. The weight add-on was determined by below formula (**Ammayappan & Moses, 2009)**.

$$\text{Weight add on (\%)} = \frac{W_2 - W_1}{W_1} \times 100 \quad \text{..... (12)}$$

Where, W_1 and W_2 are the weight of untreated and treated fabrics.

2.6.17 Fabric thickness

The principle of measurement of fabric thickness is expressed in B.S. 2544:1954 by James Heal's thickness gauge **(BS 2544:1954, 1963)**. The thickness of a fabric gives information about its warmth, heaviness or stiffness in use. Besides, a cloth traps a lot of air between the inter-fibre gaps. This air acts as an excellent thermal insulator. In practice thickness measurements are rarely used. Instead, commercially fabric weight per unit area is used as an indicator of thickness.

2.6.18 Determination of weight of fabric (grams per square meter)

The samples for weighing were randomly cut equal to the size of the template (10cm x 10cm) from the fabric. The samples were weighed individually in the weighing balance. The readings were converted to the weight in grams of the fabric per square meter. The weight of the fabric per unit area was calculated according to **ASTM-D 3776-96 (2002).**

2.6.19 Tensile test

The Grab test was used to assess the samples' tensile strength (**ASTM D5034-95, 2001**). In the warp direction, the test sample was split into 4-inch x 6 inch. The top and bottom jaws were used to clamp the samples. The machine was starting after the pre-tension had been optimized. Its cell load was 3000 Newton and machine works on constant rate of extension (CRE) of 100 mm/min, after a certain time it was break.

2.6.20 Whiteness index

According to International Commission on Illumination (CIE), the whiteness index value was evaluated for the treated and untreated samples by the AATCC test method **(AATCC 110-2005, 2007)**. The whiteness index was measured by Data color spectrophotometer (Model No. TM 650, USA).

2.6.21 Absorbency test by vertical wicking test

In the absence of external forces, the transport of liquids through fibrous assemblies is driven by capillary forces. The wicking rate is dependent on the capillary dimensions of the fibrous assembly and the viscosity of the liquid. The absorbency of *Aloe vera,* chitosan and sericin treated fabric was observed by wicking method. (**Harnett & Mehta, 1984**).

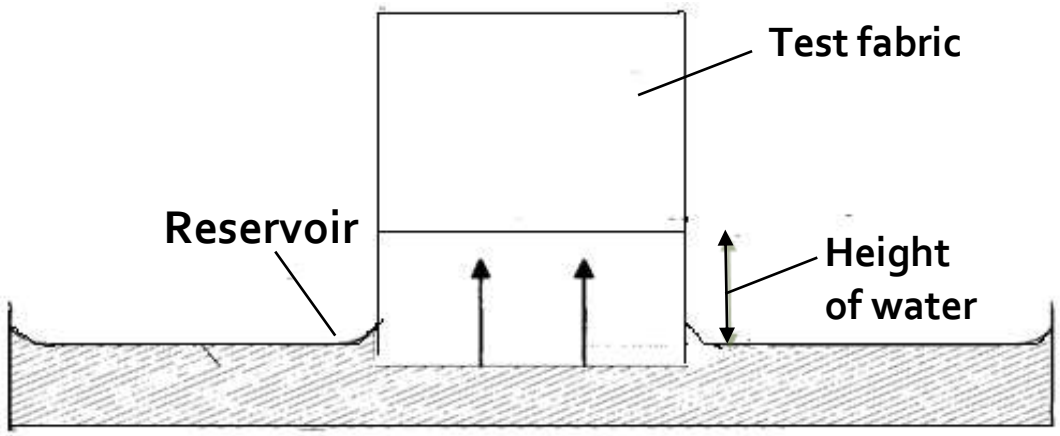


Figure 2.11: Wicking test method

As seen in **Figure 2.11**, a strip of cloth is suspended vertically with its lower edge in a pool of purified water. The rate of rise of the leading edge of the water is then monitored. A dye is applied to the water to detect the location of the water line. The calculated height of increase in a given period is used to determine the test fabric's

wicking potential. The test does not take into account the mass of the water that is taken up. This will depend on the height the water has risen only.

2.6.22 Crease recovery

Different finishing agent was applied on the fabric in order to improve their crease resistance. This test was created to assess the effectiveness of certain finishes. The test involves folding a small cloth specimen in half and placing it under a load for a set amount of time to shape a crease, then allowing it to recover for another set amount of time to determine the angle of the crease. Using the Shirley crease recovery tester, the fabric crease recovery angle was calculated according to the technique stated in **AATCC 66-2008 (2010).**

2.6.23 Abrasion resistance

A typical fabric is used to abrade the fabric under inspection. Visual appearance or mass loss of the specimen was used to determine abrasion resistance. Four specimens each 38mm in diameter are cut using the appropriate cutter. After that, they're placed in specimen holders with a circle of standard foam behind the fabric being checked. The abrasion resistance was measured using a Martindale Abrasion Tester **(ASTM D4966-98, 1989).**

2.6.24 Measurement of fabric stiffness

Stiffness is a special property of fabric. It is a significant element in the analysis of cloth handle and drape. This measure tests a fabric's bending stiffness by bending a strip of the fabric at a fixed angle to its own weight. Shirley cloth stiffness tester used a fabric bending length to assess stiffness. **Figure 4.12** illustrates the Shirley cloth stiffness tester. A form of the cantilever stiffness tester is frequently used for the measurement of a fabric's stiffness. Since it is an easy test to carry out. A horizontal strip of cloth is clamped at one end and the remainder is allowed to hang under its own weight. As a part of this, the cloth strips bends. As the end of the fabric strip touches the stiffness tester scale, the bending length is calculated using the scale. The extent of the bent is estimated in centimeters.

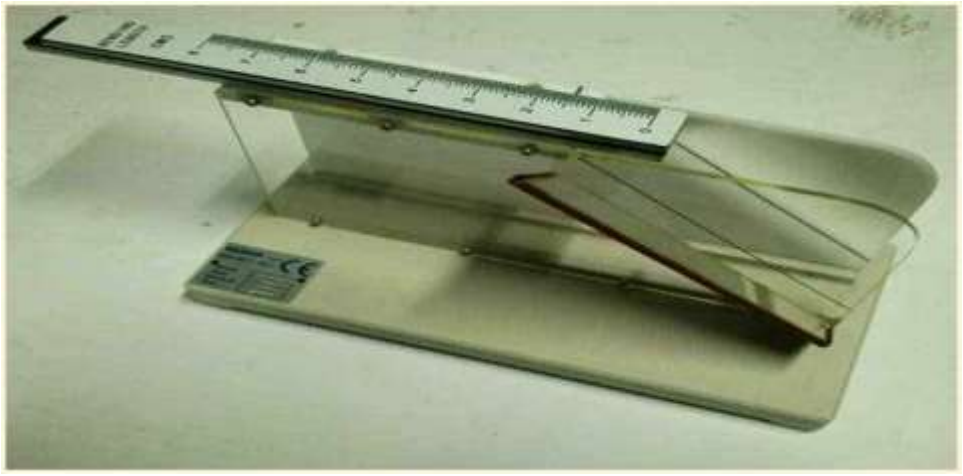


Figure 2.12: Shirley stiffness tester

The bending length is determined by the fabric's weight and is therefore an essential part of a fabric's drape as it is hanging under its own weight. Each test specimen measures 25 mm in width and 200 mm in length. Higher bending length indicates the more stiffness.

On the other hand, flexural rigidity is calculated by multiplying the materials weight per unit area by the cube of its bending length. The letter 'G' stands for flexural rigidity. Flexural rigidity was calculated by the following equation.

$G = WC^3 x\ 10^3$ mg-cm…… (13)

Where, C = Bending length, W = Cloth weight in grams per square cm

2.6.25 Thermal conductivity

Thermal conductivity (K) is the ability of the substance to conduct heat. The following formula was used to quantify thermal conductivity using a Lee disc apparatus (**Vigneswaran et al., 2009**).

$$\text{Thermal conductivity, } K = \frac{\mathbf{msd} \times \frac{\mathbf{d\theta}}{\mathbf{dt}}}{\mathbf{A}(\mathbf{T_1} - \mathbf{T_2})} \quad \text{..... (14)}$$

Where, K is the sample's thermal conductivity, m is the mass of the brass disk, S is the disc's specific heat, d is the specimen's thickness in mm, $\frac{d\theta}{dt}$ is the rate of cooling, A is the cross-sectional area of the specimen, and T_1 is the highest steady temperature and T_2 is the lowest steady temperature.

2.6.26 Thermal resistance

The term "thermal resistance" refers to the resistance to heat flow. Thermal resistance is inversely proportional to thermal conductivity. The following equation was used to measure it **(BS 4745, 1971).**

Thermal resistance, $R = \frac{h}{\lambda}$ ..... (15)

Where, h is thickness of the sample and λ is thermal conductivity.

2.6.27 Water vapor permeability

Fabrics' water vapor permeability is an essential property where clothing systems intended to be worn during vigorous activity. During periods of high activity, the human body cools itself by sweating and evaporating. The main interest is, fabrics with a polymer layer that makes the fabric little bit waterproof while still allowing some water vapor to pass through. **Figure 2.13** depicts the water vapor permeability testing system. Water vapor permeability was assessed by the following formula **(BS 7209, 1990).**

Water vapor permeability = $\frac{24M}{At}$ gm/m^2/day ..... (16)

Where, M is the mass loss in grams, A is the exposed test specimen's area in m^2, and t is the time between successive assembly weighing in hours.

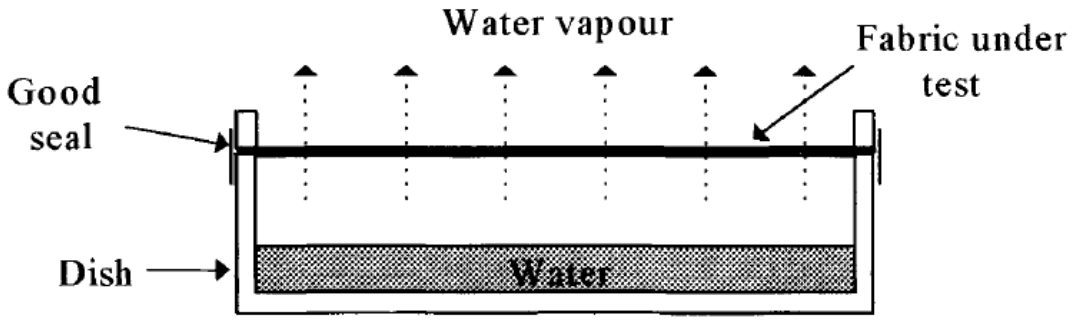


Figure 2.13: Water vapor permeability test method

2.6.28 Air permeability

The air permeability of a fabric is the volume of air passed per second through one square centimeter of the fabric at a pressure of one centimeter of water. The air

permeability was measured using the air flow process (**BS 5636, 1990)**. Three samples were tested in each group and expressed as $cm^3/cm^2/s$. Sample size was 4×4 cm^2.

2.6.29 Soil degradation test

Soil degradation test was carried out according to the Swain method with some modification. Treated and untreated samples were kept under soil of pots in six inches deep. Each pots filled with approximately 750 gm soil and 250 gm cow dung. 100 ml water was poured into the pots at regular time intervals. The degradation of the samples was determined by weight loss after 30 days. The samples were carefully taken out from the soil and gently cleaned with water before being dried in the sun. The following equation was used to calculate the weight loss (**Swain et al., 2015**).

$$\text{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{..... (17)}$$

Where, W_1 is the initial weight and W_2 is the after-burial weight.

2.6.30 Statistical analysis

Correlation coefficient was calculated according to the Karl Pearson's formulae **(Gupta & Kapoor, 2002a)**.Karl Pearson's coefficient of correlation is a widely used statistical tool for determining the degree of relationship between linearly related variables using a numerical representation. The correlation coefficient is denoted by the letter "r."

$$r\,(x, y) = \frac{\frac{1}{n}\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\frac{1}{n}\sum(x-\bar{x})^2 \times \frac{1}{n}\sum(y-\bar{y})^2}} \quad \text{..... (18)}$$

Where, $\bar{x} = Mean\ of\ x\ variable, \bar{y} = Mean\ of\ y\ variable,$

On the other hand, hypothesis test is carried out by **chi-square** (χ^2) test method at 5% level of significance **(Gupta & Kapoor, 2002b**). A **chi-square** (χ^2) statistic is a test that measures how a model compares to actual observed data. The formulae of **chi-square** (χ^2) is given below.

$$\chi 2 = \frac{\sum(f_o - f_e)^2}{f_e} \quad \text{..... (19)}$$

Where, f_o = Observed frequencies, f_e= Expected frequencies

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Chapter Three
RESULTS AND DISCUSSION

3.1 Raw materials Characterization

3.1.1 Solubility

Generally, *Aloe vera* extract is partial soluble in deionized water. When *Aloe vera* extraction is done in presence of methanol. Then methanolic *Aloe vera* extract is easily soluble in deionized water. The appearance of *Aloe vera* solution is like as brown yellowish.

Chitosan solubility is determined by the degree of deacetylation. **Peter (1995)** discovered that chitin with a deacetylation level of more than 50% can be defined as chitosan, which is soluble in acetic acid at 1%. Higher degree of deacetylation of chitosan ensures the higher solubility as reactive site expand when degree of deacetylation increased. The dominating reactive groups of chitosan are considered as - NH_2 and –OH. The solubility % was measured according to equation numbe1. The solubility of chitosan was 88% where initial chitosan weight was 0.25g and after filtration, chitosan weight was 0.03g. Since chitosan has a higher proportion of protonated free amino groups compare to chitin, which attract ionic compounds and cause it to dissolve easily in acetic acid. Apart from that, chitosan with insufficient protonated amino groups due to a lack of deacetylation degree (DDA) was poorly soluble in acidic medium.

In cold water, sericin is insoluble, but in hot water, it dissolves. **Gulrajani (1988)** got that the similar result. It is assumed that long protein molecules of sericin are broken down into smaller fractions that can be dispersed or dissolved in hot water.

3.1.2 Degree of deacetylation

On the basis of potentiometric titration of chitosan solution, the graph with the variation of pH versus the added volume of base has two inflexion points is obtained in the **Figure 3.1**. The first and second inflection points are found after adding 7 ml (V1) and 18 ml (V2) NaOH solution respectively. The first inflection point corresponds to neutralization of HCl, and the second inflection point to neutralization of the ammonium ions from chitosan. The difference of the volumes of these two points (V2-V1)

corresponds to the acid consumed by the amine groups. And finally, degree of deacetylation was found 90% according to the equation number (1).

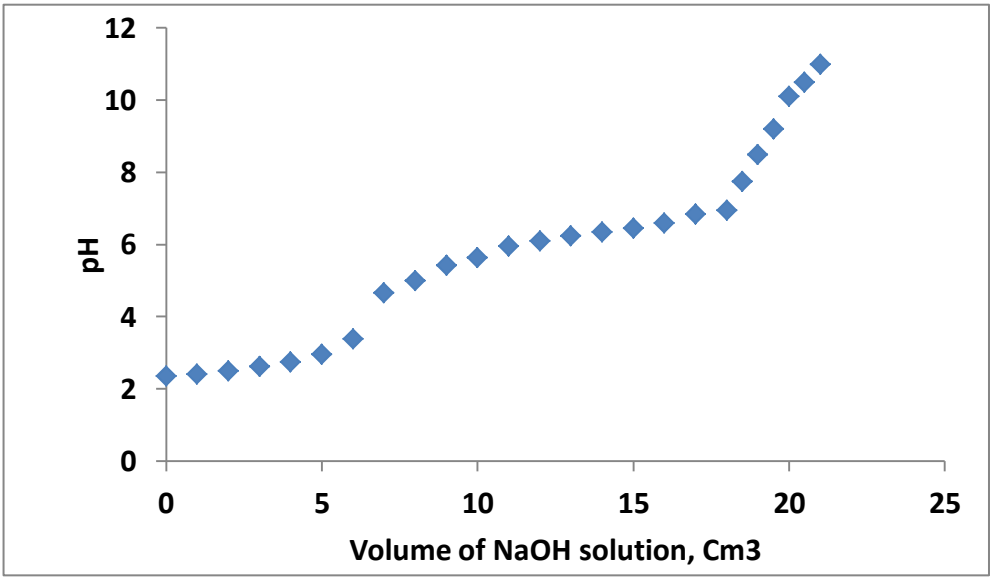


Figure 3.1: pH versus volume of NaOH solution

3.1.3 UV absorption

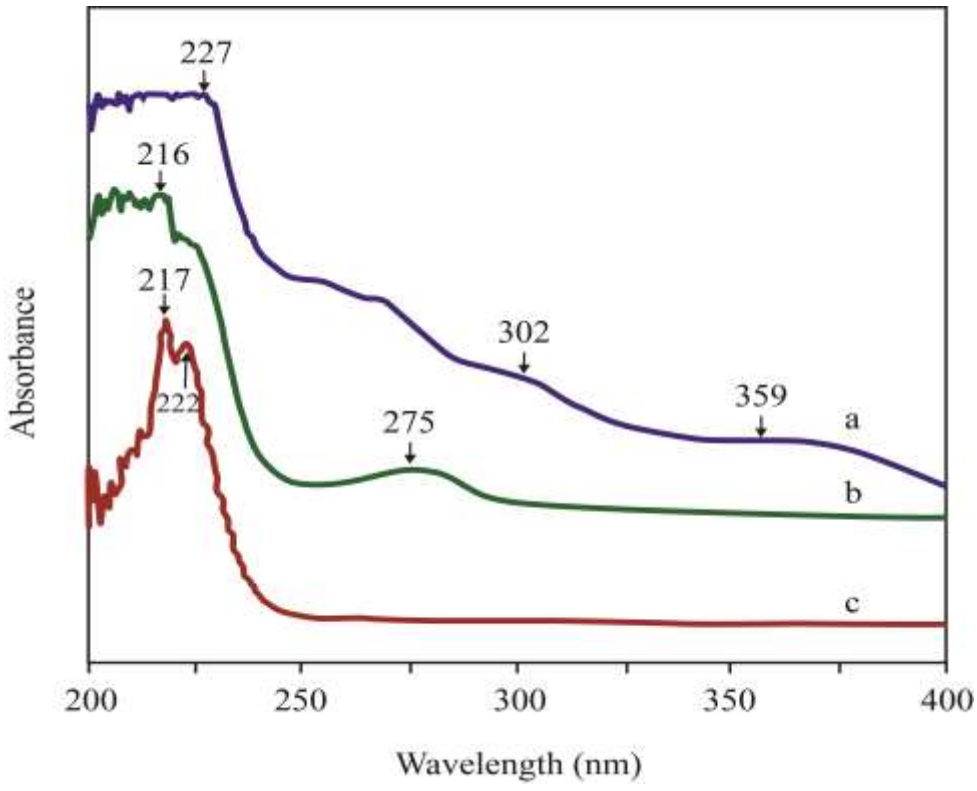


Figure 3.2: UV Spectra of (a) *Aloe vera*, (b) Silk sericin, and (c) Chitosan solution

Aloe vera extracts solution showed UV absorption peaks at 227, 302 and 359 nm in **Figure 3.2 (a). Ray et al. (2013a)** were found UV spectra of *Aloe vera* absorption peaks

at 270 nm (UV-C region), 290 nm (UV-B region) and 350 nm (UV-A region)**]. Ozsoy et al. (2002)** identified the phenolic group of *Aloe vera* at the absorption band between 320 and 380 nm. Long-term exposure to UV-B radiation (315–280 nm) may have a variety of negative effects on the human skin, immune system, and can also cause skin cancer. Overexposure to UV radiation increases the risk of malignant melanoma by causing DNA damage. UV radiation is prominent factor of 92 % melanoma disease **(Davies et al., 2002).**

The absorption peak of silk sericin solution shows at 216 and 275 nm of wavelength shown **Figure 3.2 (b)**. Silk sericin is largely composed of protein, which accounts for approximately 90% of its structure and contains 18 distinct amino acids. Sericin contains approximately 32% serine, along with other amino acid like aspartic acid, threonine, glycine and so on which are attributed to the physico-chemical and functional properties of sericin. Near about 70% hydrophilic amino acids are existed in 18 kinds amino acids which is responsible for good solubility and water absorbability of sericin. On the contarary, only 6.6% aromatic amino acids absorb harmful UV irradiation among the 18 kinds of amino acids **(Wu et al., 2007)**. In the UV zone, proteins usually have two absorbance peaks, one between 215 and 240 nm and the other between 260 and 290 nm. The peptide bonds of amino acid absorb 215- 240 nm region UV light. Tryptophan, tyrosine, and phenylalanine are aromatic amino acids that absorb UV radiation in the 260-290 nm range **(Gupta et al., 2014).** The silk sericin solution showed absorbance peak at 216 nm and 275 nm as shown in **Figure 4** which indicates that sericin absorb UV rays in that region.

UV absorption of chitosan solution was found at 217 and 222 nm wavelength in **Figure 3.2 (c)**. Assume that absorption around this region is due to the presence of amide linkages in chitosan. Chitosan exhibit two chromophoric groups like *N*-acetylglucosamine and glucosamine which absorb UV range light (**Tyagi et al., 1996**)**.** Generally, the solar UV radiation is classified as UV-A (320 to 400 nm), UV-B (290 to 320 nm) and UV-C (200 to 290 nm). As stated above, chitosan absorbs UV-C region radiation. Usually ozone layer absorbs UV-C region rays. Increased environment pollution especially CO_2 emission has caused a depletion of ozone layer. As a result, UV-C region rays come to earth easily which are absorbed by chitosan.

Above discussion established that *Aloe vera* solution absorbed three region of UV radiation. On the other hand, silk sericin and chitosan solution only absorbed the UV-C region radiation. Therefore, it can be said that *Aloe vera* is very much effective to resist all region of UV radiation.

3.1.4 Fourier-transform infrared spectrophotometer (FTIR) Analysis

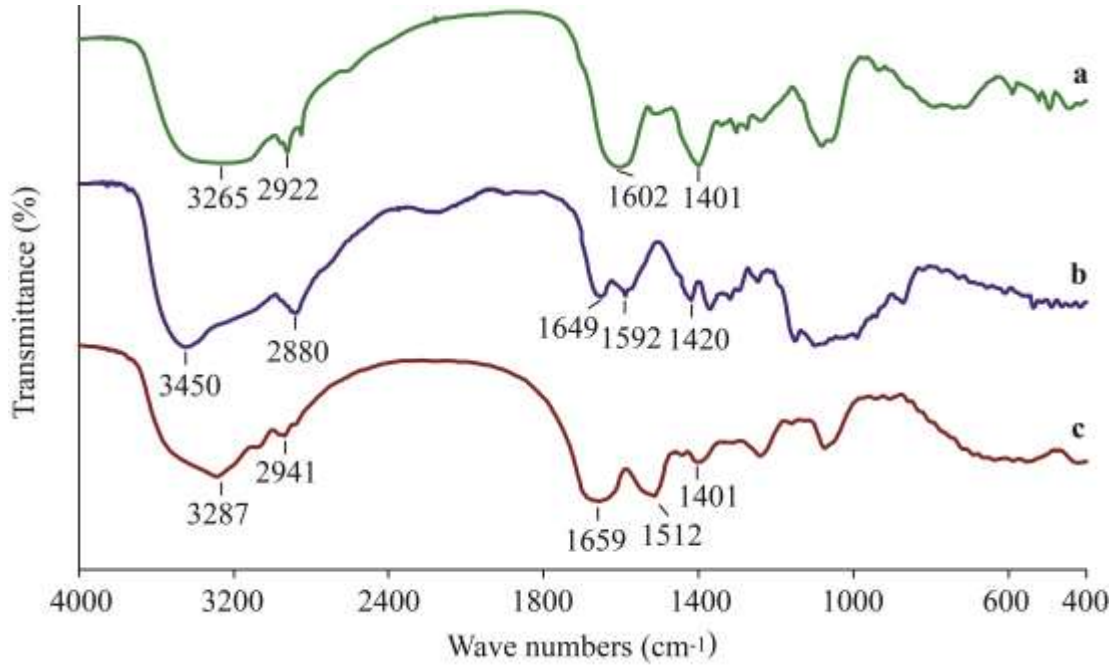


Figure 3.3: FTIR spectra of (a) *Aloe vera*, (b) Chitosan, and (c) Silk sericin

Figure 3.3 (a) showed the FTIR spectrum of *Aloe vera*. The peaks of 2922 cm^{-1} and 1401 cm^{-1} corresponds to - CH_2 and COO^- groups respectively. The prominent broad peak about 3265 cm-1 is due to phenolic -OH stretching, which is related to aloe-specific phenolic such as flavonoids and anthraquinons. The occurrence of carbonyl compounds was shown by another mild strength peak at 1602 cm-1, which was given the C=O stretching vibration. Similar spectrum of *Aloe vera* were found by **Ray & Aswatha** (**2013b**).

The broad FTIR spectrum of chitosan were around 3450 cm^{-1} which indicates O-H and N-H stretching vibration in **Figure 3.3 (b).** The sharp moderate peak is located at 2880 cm^{-1} and 1420 cm^{-1} represent stretching and bending vibrations respectively of C-H (CH_3 and CH_2). The most important peaks of chitosan are observed at 1649 cm^{-1} and 1592 cm^{-1} represents the C=O stretching vibration (Amide l) of acetyl groups and N-H bending vibration(Amide ll) of amino groups respectively. FTIR analysis of chitosan by **Gbenebor et al. (2017)** has also gotten similar outcome.

The peak at around 3500–3000 cm^{-1} (3287 cm^{-1}) is indicated the N-H stretching vibration of sericin in the **Figure 3.3 (c)**. The weave number at 1659 cm^{-1} revealed the stretching vibration of the C=O. The existence of N-H bending in sericin is supported by the weave numbers 1512 cm^{-1}. The peak at 1401 cm^{-1} represent the CH_3 bending and CH_2 deformation. Similar FTIR peaks of sericin powder were discovered by **Gulrajani et al. (2008), Sarovart et al. (2003)** and **Song & Wei (2006).**

The phenolic group was found in *Aloe vera*. Conversely, the amino group was found in chitosan and sericin. So it can be said that these functional groups are responsible for antibacterial, antioxidant and UV resistant property.

3.1.5 XRD Analysis

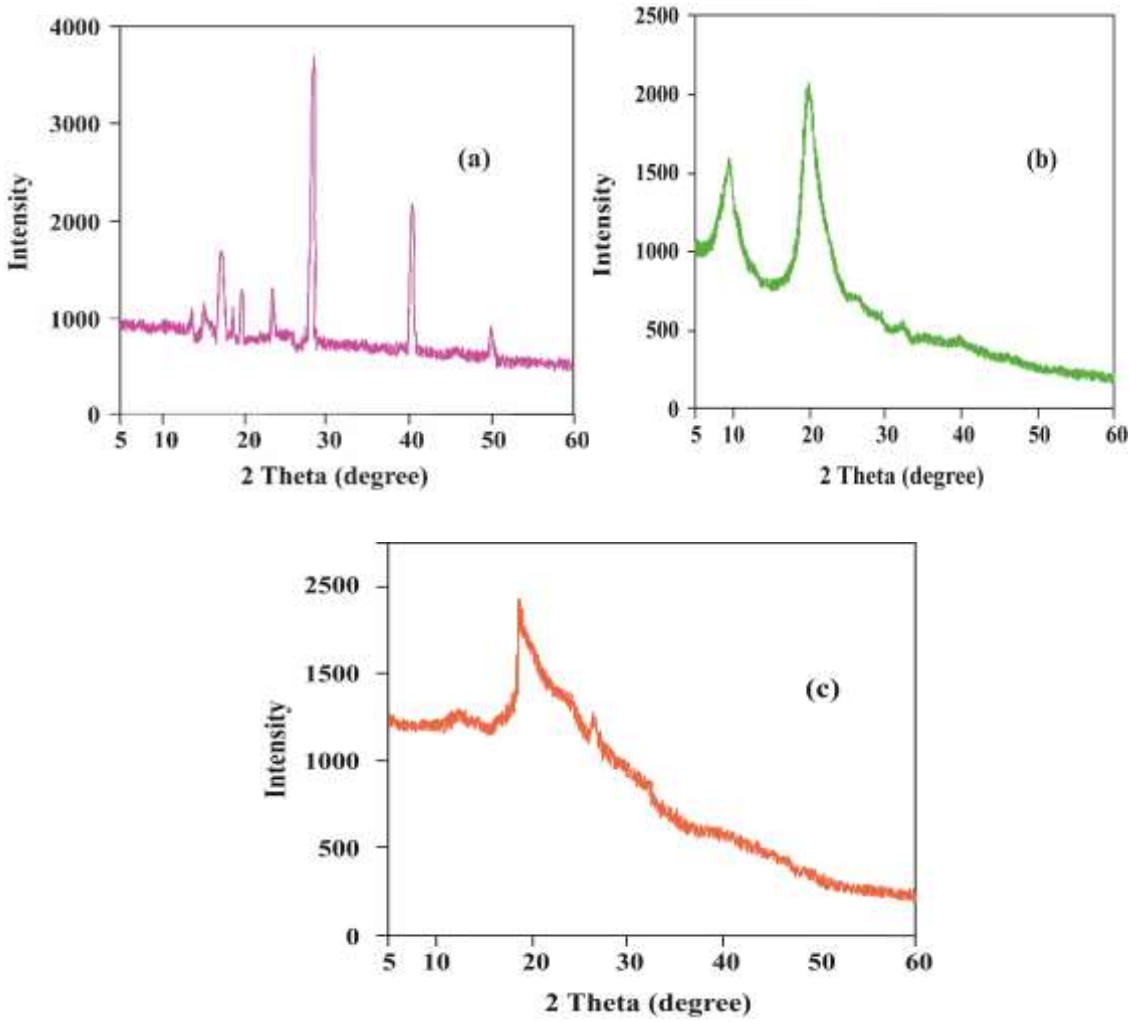


Figure 3.4: XRD Pattern of (a) *Aloe vera*, (b) Chitosan, and (c) Silk sericin

Table 3.1: Measurement of crystallite diameter and'd' spacing of the sample from XRD pattern

Observations	2 theta value (°)	Peak intensity	FWHM in (°)	Crystal Dia. (D) in nm	d spacing in nm
Aloe vera	28.33	3382	0.162	50.36	0.43
Chitosan	20.07	2142	2.861	3.38	0.31
Silk sericin	18.86	1936	3.703	2.17	0.47

Full width of the peak at half maximum (FWHM)

The **Figure 3.4** illustrated the XRD pattern of *Aloe vera*, chitosan and sericin. XRD analysis can be used to characterize the d spacing, full width at half maximum (FWHM) and crystallite diameters of the samples. The major diffraction peak of *Aloe vera* was displayed at 28.33 degree and its peak intensity 3382 counts. The highest peak intensity was illustrated for *Aloe vera* sample, shown in **Table 3.1** and **Figure 3.4 (a).** High peak intensity indicates the highest crytallinity**.** On the other hand, the full width at half maximum (FWHM) value of *Aloe vera* was lowest. Full width and half maximum value (FWHM) was determined with the help of origin pro software *as* the full width at half maximum (FWHM) value is inversely proportional to the crystal diameter according Deby-Scherrer formula **(equation no. 4**). When full width at half maximum value decreases crystal diameter of specimen increases. The crystal diameter of *Aloe vera* was maximum (50.36 nm) because the full length at half maximum value of *Aloe vera* is minimum.

The **Figure 3.4b** showed that the 2-theta value of the chitosan revealed two characteristics peaks at 9.8 and 20.07 degree corresponding to crystal planes of (020) and (110) (**Yen et al., 2009)**. Full width and half maximum value of chitosan were 2.861 and crytrallite diameter was 2.861 nm.

Natural silk is made up of two different types of proteins: crystalline fibroin and amorphous sericin (**Fabiani et al., 1996)**. As seen in Table1, the XRD pattern of silk sericin shows a large diffraction peak at 2θ =18.86 and intensity around 1936 counts. Sericin has a crystalinity diameter of 2.17 nm. Amorphous existence is shown by a low intense peak and a small crystalinity diameter. The highest diffraction peak 2θ=19.2 of sericin was discovered by **Silva et al. (2016), Jo & Um (2015).** This peak specifies the

conversion of the random coil structure into the β-sheet structure due to intermolecular hydrogen bonding between the hydroxyl groups of the amino acids present in sericin.

The crystallite diameter of *Aloe vera* was highest and sericin showed the lowest crystallinity diameter (2.17nm). The inter plane spacing'd' is determined according to equation no. **3.** Inter plane spacing of silk sericin was highest (0.47 nm) and chitosan was lowest (0.31) as shown in the **Table 3.1**.

3.1.6 SEM Analysis

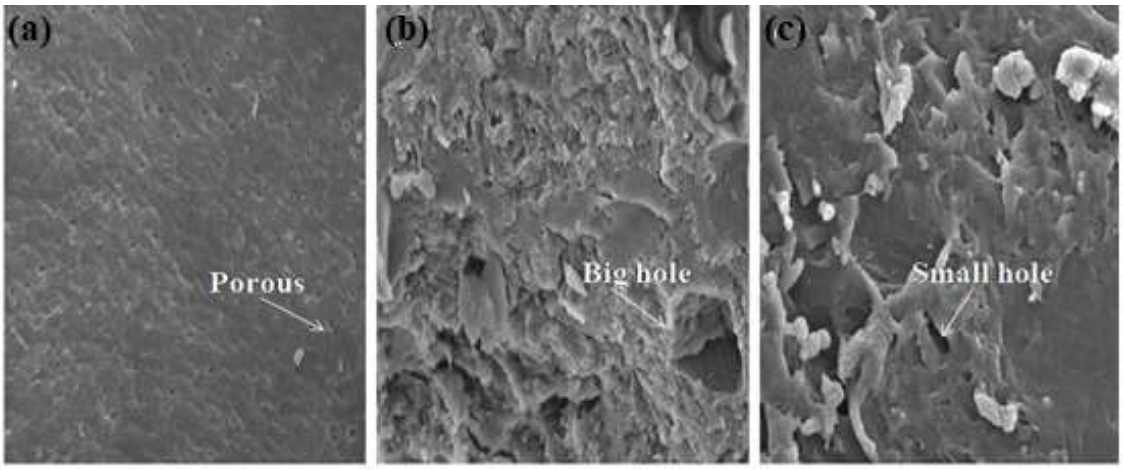


Figure 3.5: SEM image of (a) Chitosan (b) *Aloe vera* (c) Silk sericin

The surface morphology of the *Aloe vera*, chitosan and sericin powder was examined under a scanning electron microscope at 5000 magnifications. Chitosan showed smooth and non-aggregated arrangement of its particles. Additionally, surface morphology of chitosan possesses tiny porous structure as shown in **Figure 3.5 (a).** *Aloe vera* sample exhibits rough and high aggregated appearance as shown in **Figure 3.5 (b)** than that of silk sericin. On the other hand, silk sericin exhibits more sponge like agglomerated form as shown in **Figure 3.5 (c)**. More cracked structure is visible in *Aloe vera* extract powder compared to that silk sericin powder.

Gulrajani et al. (2009) stated that sericin recovered from high temperature and high pressure is mainly in the agglomerated form, which may be attributed to the hydrophilic nature of sericin**.** When sericin is exposed to the air, it absorbs moisture easily and clumps together. This finding supports the present investigation.

3.1.7 Energy-dispersive X-ray spectroscopy (EDS) study

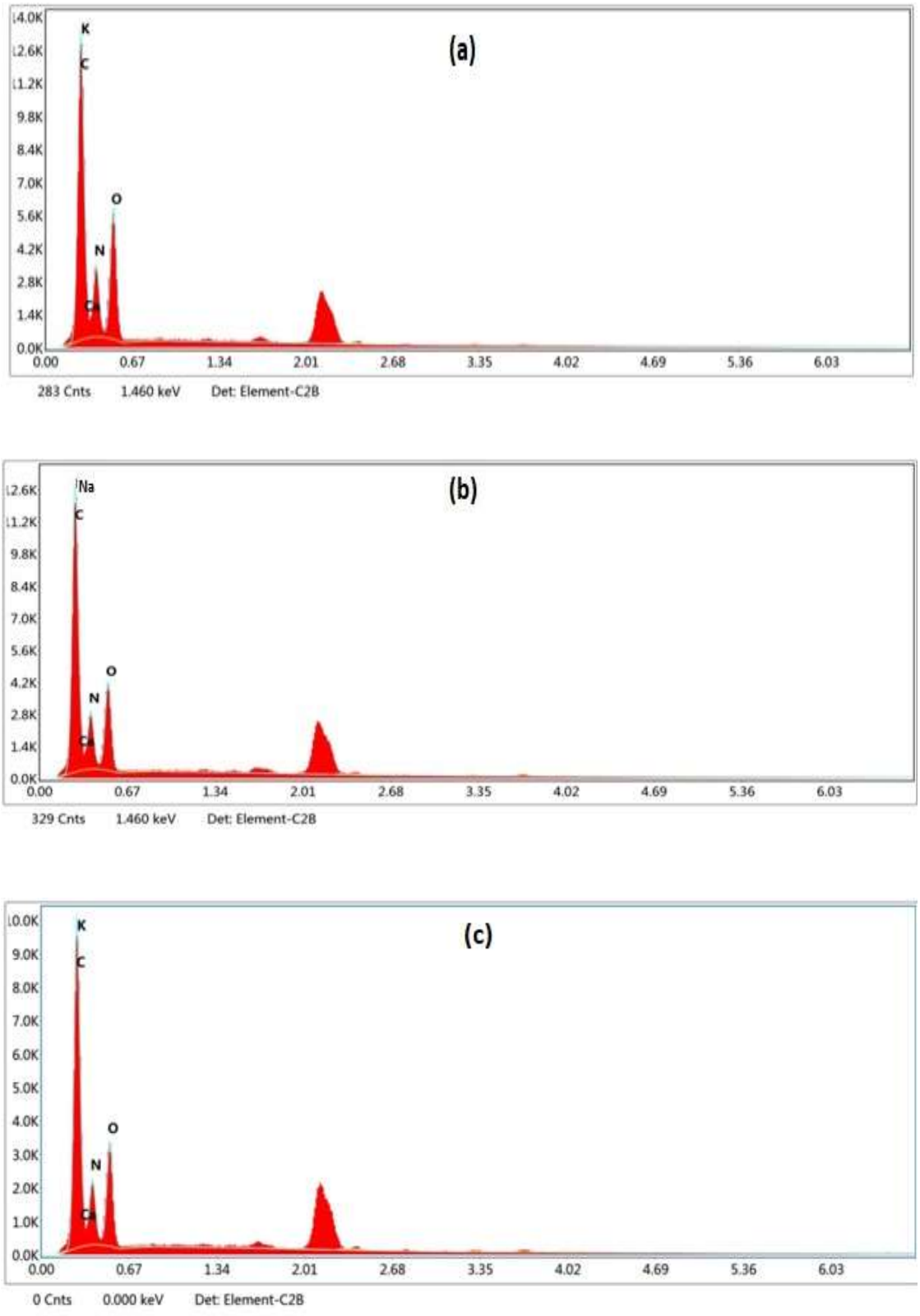


Figure 3.6: EDS pattern of (a) *Aloe vera*, (b) Chitosan, and (c) Sericin

Table 3.2: EDS data of *Aloe vera,* Chitosan and Sericin

Elements	Wt.% of *Aloe vera*	Atomic % of *Aloe vera*	Wt.% of Chitosan	Atomic % of chitosan	Wt.% of sericin	Atomic % of sericin
C K	42.58	48.90	40.72	46.24	39.09	45.22
N K	25.70	25.31	28.19	27.44	25.06	24.85
O K	28.69	24.74	30.50	25.98	33.52	29.11
Na K	0.00	0.00	0.54	0.32	0.00	00
K K	0.86	0.30	0.00	0.00	0.99	0.35
Ca k	2.17	0.75	0.05	0.017	1.34	0.46
Total	100	100	100	100	100	100

Wt. % gives the concentration of the element in terms of the mass fraction of that element in the sample. Atomic (or molar) ratios of the elements are related to the wt.% through the atomic masses of the elements in the sample.

Figure 3.6 displays the spectra of *Aloe vera*, chitosan, and sericin using energy-dispersive X-ray spectroscopy (EDS). The percentage of the elements is presented in **Table 3.2**. Atomic % would be the % as a function of the number of atoms whereas Wt. % would be % as a function of weight. Wt. % gives the concentration of the element in terms of the mass fraction of that element in the sample. The main elements of the three natural antimicrobial agents were C, O and N. The *Aloe vera* powder was possessed with high contents of carbon (42.58%). The amount of N element was 25.70, 28.19 and 25.06 for *Aloe vera*, chitosan and sericin powder respectively. In case of 'N' element, *Aloe vera* and sericin powder presented similar weight and atomic percentage. On the other hand, chitosan holds maximum N element. Sericin contents highest Wt. % regarding O element. Minor elements like Na, K and Ca were observed in *Aloe vera*, chitosan and sericin. Chitosan only exhibited 0.54% Na elements among the samples. K (0.86%) and K (0.99%) were found in *Aloe vera* and sericin powder, respectively. Again *Aloe vera* and sericin powder exhibited Ca (2.177%) and Ca (1.34%) correspondingly.

3.1.8 TGA Analysis

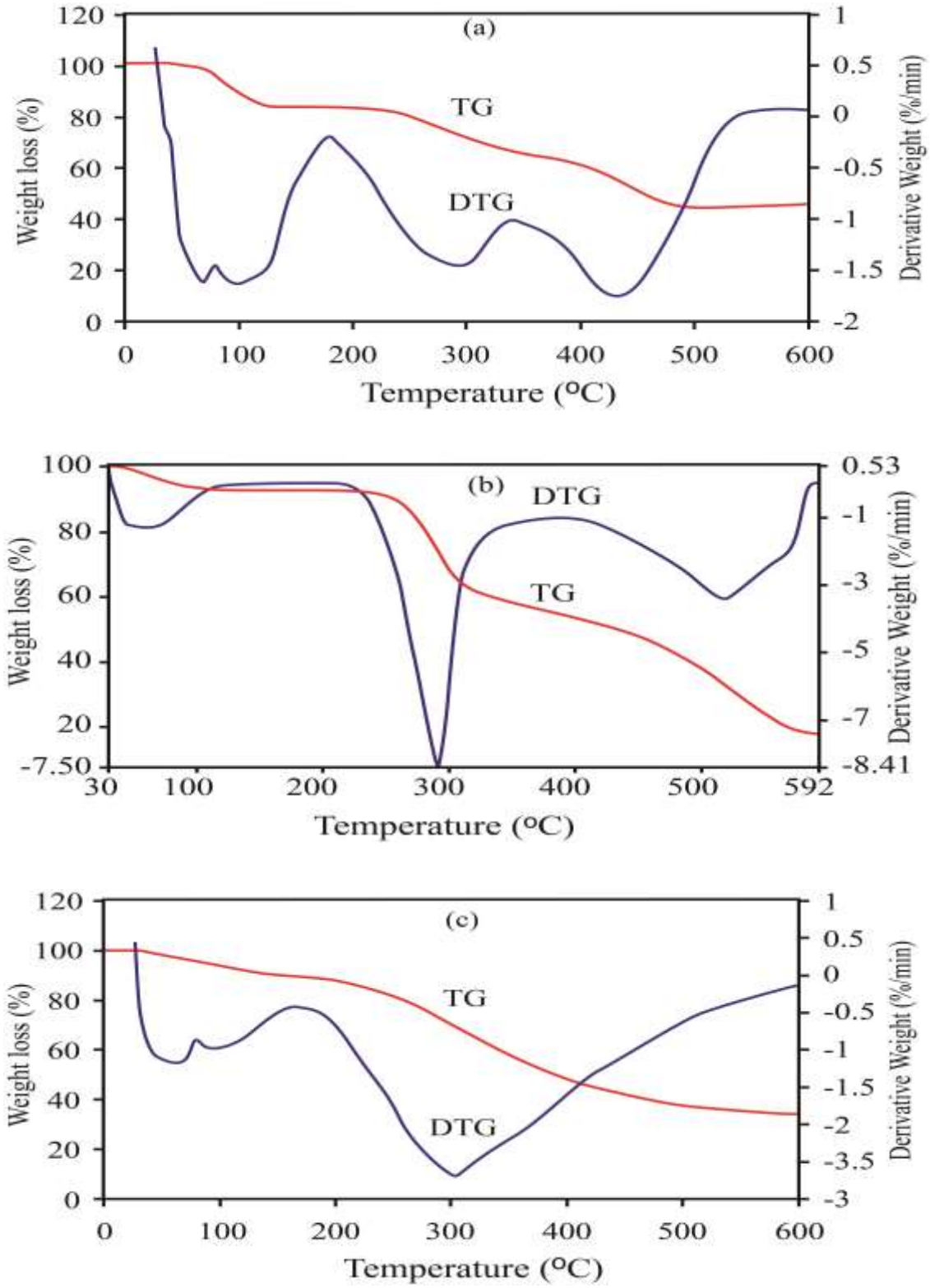


Figure 3.7: TG and DTG curve of (a) *Aloe vera*, (b) Chitosan, and (c) Silk sericin

Table 3.3: Data obtained from TGA and DTG Thermograph

Samples	DTG peak maximum temp. (℃)	Weight loss % at 300℃	Char yield (%) at 600℃
Aloe vera	430	28	45
Chitosan	290	38	15
Sericin	300	30	34

According to Figure 3.7, the mass loss of *Aloe vera*, chitosan, and sericin at 100°C was 11, 7.5, and 6%, respectively, due to moisture loss, low molecular weight solvent loss, and gas loss. The weight loss at 200°C was 17% 9% and 14% for *Aloe vera*, chitosan and sericin respectively. The 28%, 38% and 30% weight loss was occurred at 300°C due to decomposition of the specimen. The major weight loss 39%, 51% and 52% was observed at 400°C for *Aloe vera*, chitosan and sericin specimens. 17%, 19% and 11% weight was lost between 400°C to 500°C for *Aloe vera*, chitosan and sericin specimens respectively. Furthermore a little amount of weight of *Aloe vera* has been increased between 500°C to 600°C. Eventually, 45%, 15% and 34% ash was found for *Aloe vera*, chitosan and sericin specimens respectively as shown in **Table 3.3**. **Chollakup et al. (2015)** also got similar ash content in sericin at 600°C temp. Originally maximum decomposition temperature is determined by DTG thermograph. Maximum decomposition temperature of *Aloe vera* was observed at 430°C temperatures. Besides maximum decomposition temperature of chitosan and sericin were found at 290°C and 300°C. Maximum decomposition temperature of silk sericin was observed at 320° by **Tsukada (1978).** Many factors determine the thermal stability of the materials like molecular weight, nature of chemical structure, Crystallinity and maximum decomposition temperature of polymers.

Thus it can be said that the thermal stability of *Aloe vera* is highest and sericin is second in terms of maximum decomposition temperature. In addition, XRD analysis of crystallinity also supports these findings.

3.1.9 DSC Analysis

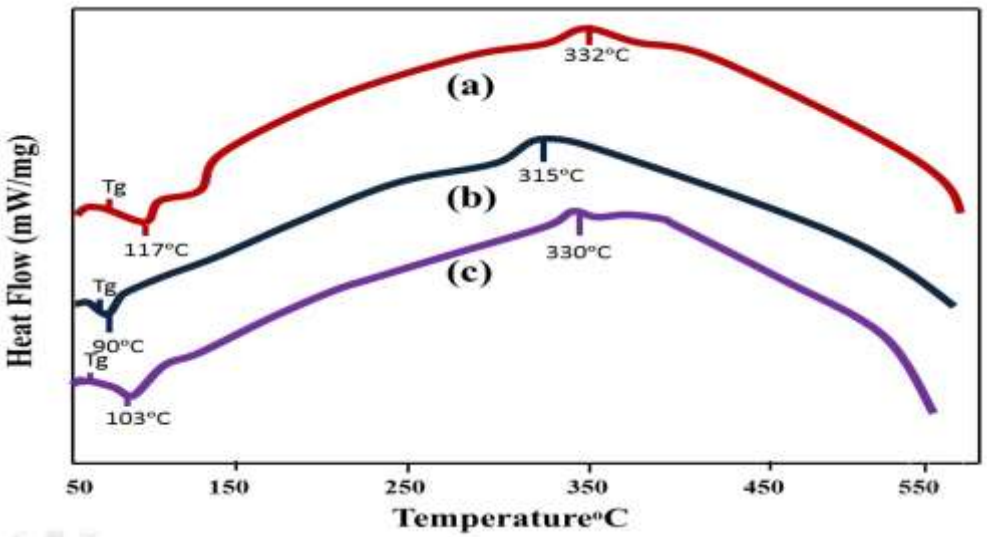


Figure 3.8: DSC curve of (a) *Aloe vera*, (b) chitosan, and (c) Silk sericin

The glass transition temperature (Tg) and thermal stability of biopolymers have been measured using differential scanning calorimetry. Biopolymers usually contain moisture and bound water that isn't fully separated throughout drying. This bound water affects many properties of polymers, such as rheological, transport properties and glass transition temperature. In presence of different amount of moisture in material gives an endothermic peak at different position. Dry and wet samples of the same material will give different Tg as water is present in different forms in macromolecules of biopolymer (**Schubnell & Schawe, 2001).** Normally, the glass transition temperature is identified at the initial change in the slope of the heat capacity of the DSC curve.

Figure 3.8(a) of the DSC thermograph of *Aloe vera* reveals one endothermic peak at 117°C, which is attributable to the removal of absorbed water and one exothermic peak at 332°C is associated with the degradation of the material. In the **Figure 3.8(a)**, Tg value of *Aloe vera* was found nearly at 80°C. **Chakraborty et al. (2019)** found a sharp endothermic peak obtained at 120°C and an exothermic peak was at 337.43°C is associated with the breakage of saccharide ring and the bond rupture of linked groups of *Aloe vera* extract. The glass transition temperature (Tg) is found to be 49°C. **Nindo et al (2010)** found the Tg of *Aloe vera* was nearly 65°C temperature.

Chitosan showed endothermic peak at 90°C and glass transition temperature was approximately at 81°C. The characteristics exothermic peak revealed at 315°C in the **Figure 3.8 (b).** The endothermic peak, also known as the dehydration temperature (TD),

is the temperature at which water is lost due to the hydrophilic groups of chitosan **(Cheung et al., 2002; Kittur et al., 2002**). Chitosan is Polysaccharides based biopolymer and has good affinity to water. It is because chitosan which can be easily hydrated (**Cardenas & Miranda, 2004**). Degradation of acetyl and deacetylated units, glycoside bond cleavage of chitosan was identified by exothermic peak (**Sreenivasan, 1996; Deng et al, 2007**). **Dey et al. (2016)** found the endothermic peak almost at 80°C and an exothermic peak at 304°C from the chitosan DSC thermograph.

The silk sericin illustrated the one endothermic peak at 103°C. The characteristics exothermic peak was shown at 330°C and Tg value was found at 70°C in **Figure 3.8 (c)**. **Dutta et al. (2012)** found two endothermic peak of silk sericin at 65.5°C and the 363.2°C temperature. Other researchers discovered two endothermic peaks of silk sericin in the temperature ranges of 210-220°C and 310-320°C, respectively, leading to thermally mediated molecular motion in an amorphous area and thermal decomposition of silk sericin (**Tao et al, 2016; Oh et al, 2011)**.

3.1.10 Antimicrobial Activity evaluated by qualitative method

The zone of inhibition (ZOI) of *Aloe vera*, chitosan, and silk sericin were evaluated for 10g/l concentration with *E. coli* gram-negative and *S. aureus* gram-positive bacteria**.** **Figures 3.9, 3.10, and 3.11** display the antibacterial activity of *Aloe vera* chitosan and silk sericin against *E. coli* gram-negative and *S. aureus* gram-positive bacteria, respectively. The zone of inhibition of *Aloe vera*, chitosan, and silk sericin was 2.5, 3 and 1 mm respectively for *E. coli* gram-negative bacteria as shown in **Table 3.4**. Again the zone of inhibition of *Aloe vera*, chitosan, and silk sericin was 4, 5, and 1.5 mm for *S. aureus* gram-positive bacteria. The zone of inhibition of chitosan was greater than that of *Aloe vera* and silk sericin solution. *Aloe vera*, on the other hand, had stronger antimicrobial activity than silk sericin solution. In addition, zone of inhibition of chitosan was more neat and clean than the *Aloe vera* and sericin.

Table 3.4: Zone of inhibition (ZOI) of 10 g/l *Aloe vera*, Chitosan and sericin solution against *E. coli* and *S. aureus* bacteria

Type of Bacteria	ZOI for 10 g/l *Aloe vera* Sol^n	ZOI for 10 g/l Chitosan Sol^n	ZOI for 10 g/l Silk sericin Sol^n
E. coli	2.5 mm	3 mm	1 mm
S. aureus	4 mm	5 mm	1.5 mm

The zone of inhibition of *Aloe vera* chitosan and silk sericin was greater for *S. aureus* gram-positive bacteria than *E. coli* gram-negative bacteria as shown in the **Table 3.4**. The reasons are gram-negative bacteria are harder to gram-positive bacteria because of the extra outer membrane on gram-negative bacteria. In addition, gram-negative bacteria contain 10% peptidoglycan of cell wall and high lipid content due to outer membrane. On the other hand, Gram-positive bacteria contain 90% peptidoglycan of cell wall and low lipid content (**Krasner, 2009)**.

Aloe vera holds different ingredient like polysaccharide acemannans, anthraquinones and tannins which have antibacterial properties. These components are thought to have the ability to inactivate enzymes, denature proteins, and damage membranes, limiting microbe development by interfering with cell function or reproduction (**De Rodrıguez et al., 2005**).

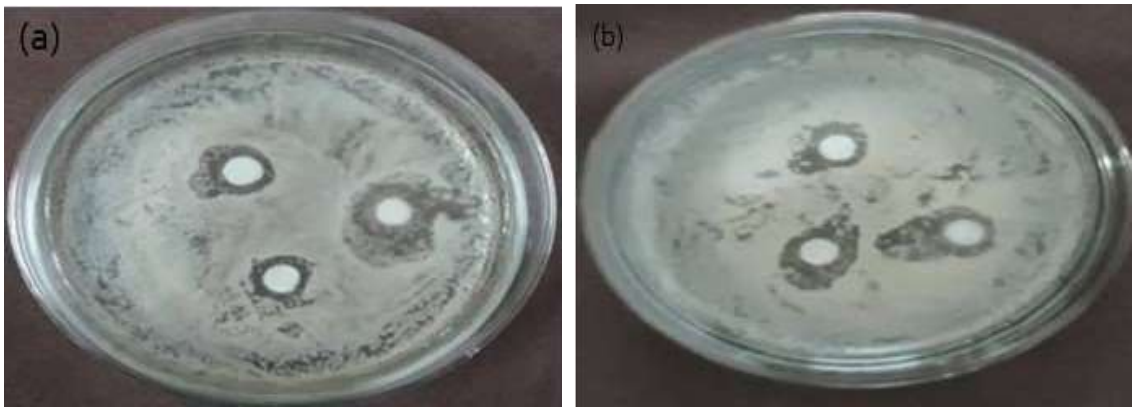


Figure 3.9: Zone of inhibition of 10 g/l *Aloe vera* solution against (a) *E. coli* gram-negative and (b) *S. aureus* gram-positive bacteria

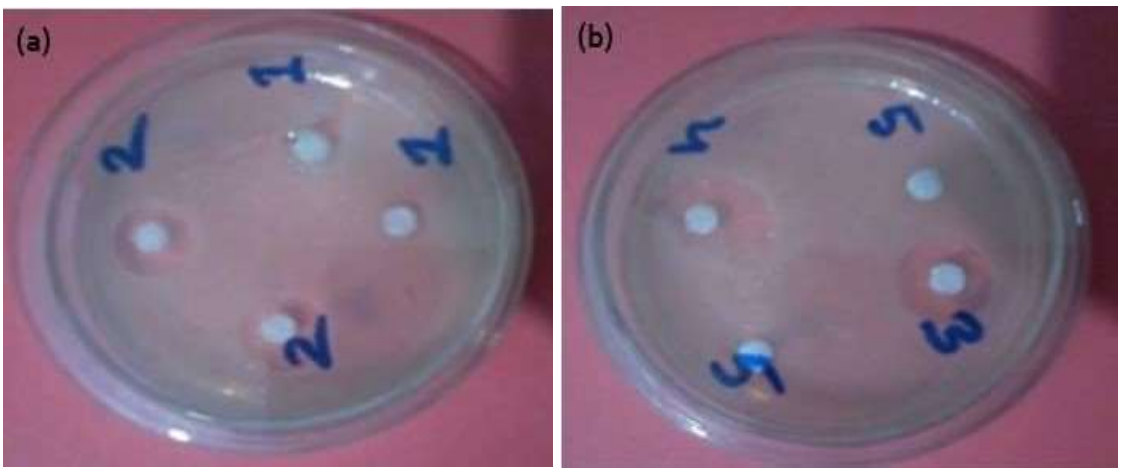


Figure 3.10: Zone of inhibition 10 g/l chitosan solution against (a) *E. coli* gram-negative and (b) *S. aureus* gram-positive bacteria

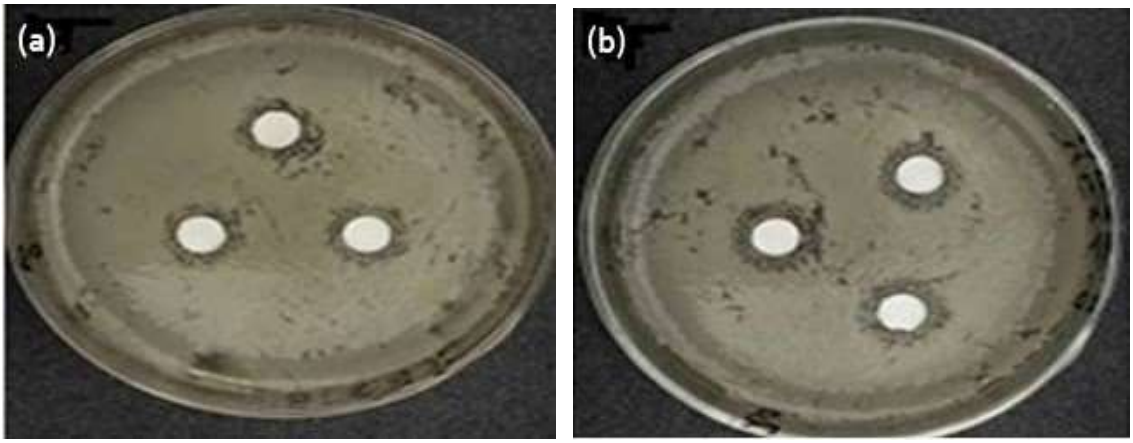


Figure 3.11: Zone of inhibition of 10 g/l sericin solution against (a) *E. coli* gram-negative, and (b) *S. aureus* gram-positive bacteria

Many hypotheses have been explained the mechanism of antibacterial activity of chitosan. Three reactive functional groups are exhibited in chitosan (amino/acetamide group at the C2 position of each deacetylated unit and both primary and secondary hydroxyl groups at the C6 and C3 positions).

The antimicrobial activity of chitosan is due to the association of the positively-charged amino group ($-NH_3^+$) of chitosan is bind with the negatively-charged carboxylate group ($-COO^-$) located on the cell surfaces of bacteria. As a result, cell surface alteration and alters cell permeability are happened by above electrochemical binding. Consequently, leading to disruption of the cell membrane of bacteria followed by leakage of intracellular substances, such as electrolytes, proteins, amino acids, glucose, UV absorbing materials and lactate dehydrogenase. In this way chitosan show their bacteriostatic and bactericidal effect (**Lim & Hudson., 2004; Helander et al., 2001**).

Senakoon et al. (2009) studied on mode of antimicrobial action of eri silk sericin through scanning electron microscopy against *S. aureus* and *E. coli* bacteria. They observed that silk sericin inhibit the cell division and cell growth in bacteria media, which is expected as the cause of the early declining phase, together with the induction of membrane dysfunction. Moreover, the observation of scanning electron microscopy also illustrated that silk sericin causing the cell deformation, cells with reduced sizes, and cell shrinkage of bacteria. In this way metabolism of bacteria is bring to a close.

3.1.11 Antimicrobial Activity evaluated by optical density

The concentration of bacterial cells in the sample is directly proportional to the sample's absorbance. The absorbance values indicate the optical density (OD) the bacterial cells. The optical density was measured of bacterial control solution and control solution plus chitosan, *Aloe vera* and silk sericin solution at different interval.

Table 3.5: Determination of optical density (O. D) value and bacterial reduction% (B. R) at different time interval for *S. aureus* gram-positive bacteria

Inoculation at 37℃	Optical density (O.D) value						B. R %
	0 hrs.	2 hrs	4 hrs.	6 hrs.	8 hrs.	10 hrs.	10 hrs.
N. Broth+ *S. aureus* (control)	0.02	0.557	0.873	1.078	1.298	1.675	-
N. Broth + *S. aureus*+ A. vera	0.02	0.357	0.462	0.612	0.740	0.802	52
N. Broth + *S. aureus*+ Chitosan	0.02	0.243	0.414	0.523	0.691	0.745	56
N. Broth+ *S. aureus*+ Sericin	0.02	0.467	0.652	0.813	0.924	1.121	33

*Nutrient broth (N. broth)

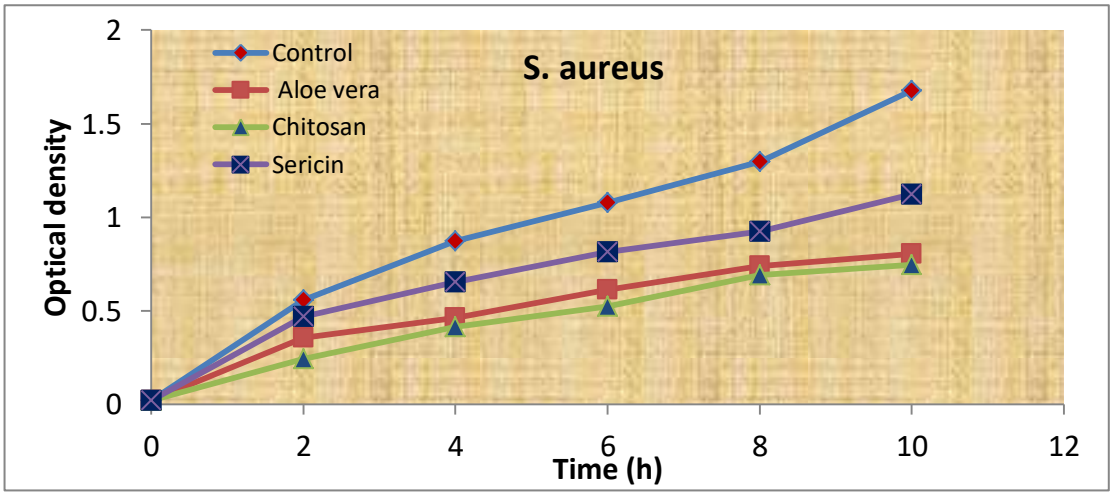


Figure 3.12: The growth curves of *S. aureus* bacterial solution in the presence of 1% *Aloe vera*, 1% chitosan and 1% sericin solution

There was no major difference in bacterial growth between *Aloe vera* and chitosan, according to the findings (**Figure 3.12 & 3.13**). On the other hand, bacterial growth in the presence of sericin was more compared to *Aloe vera* and chitosan (**Figure 3.12 & 3.13**). The peak or maximum growth was observed for *S. aureus* (control) and the absorbance was1.675, while the lowest growth was found in chitosan containing *S.*

aureus medium absorbance was 0.745, and chitosan containing *E. coli* medium absorbance was 0.872 at the end of the 10-hour incubation. The results of the bacteriostatic growth curves were indicating that chitosan has a prominent anti-microbial effects on *E. coli* and *S. aureus*. Lower optical density means higher antimicrobial activity.

Table 3.6: Determination of optical density (O. D) value and bacterial reduction% (B. R) at different time interval for *E. coli* gram-negative bacteria

Inoculation at 37℃	**Optical density (O.D) value**						**B. R %**
	0 hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	10 hrs.
N. Broth+ *E. coli* (control)	0.02	0.557	0.873	1.078	1.298	1.675	-
N. Broth + *E. coli* + A. vera	0.02	0.397	0.492	0.672	0.785	0.875	48
N. Broth + *E. coli* + Chitosan	0.02	0.295	0.482	0.585	0.734	0.787	53
N. Broth+ *E. coli* + Sericin	0.02	0.507	0.692	0.872	0.997	1.146	32

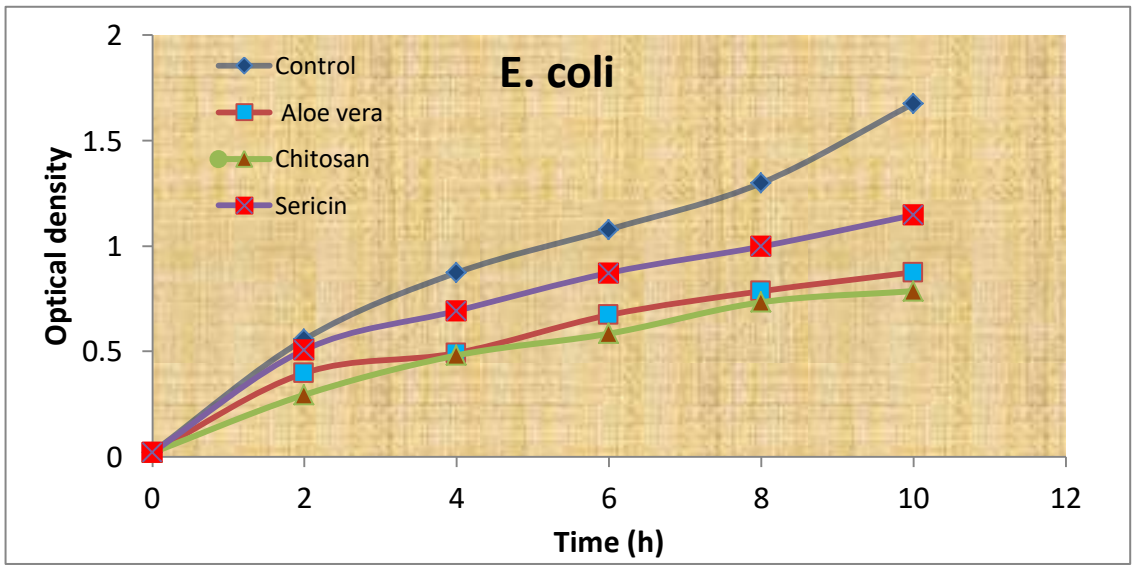


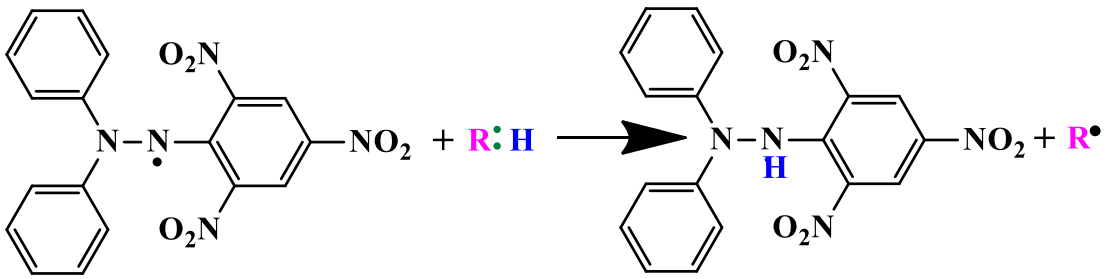
Figure 3.13: The growth curves of *E. coli* bacterial solution in the presence of 1% *Aloe vera*. 1%, chitosan, and 1% sericin solution

The bacterial reduction percentage was calculated according to equation **7**. The bacterial reduction percentage was 52, 56, and 33 for *Aloe vera*, chitosan and sericin respectively against *S. aureus* gram-positive bacteria as shown in the **Table 3.5**. Again the bacterial reduction percentage was 48, 53, and 32 for *Aloe vera*, chitosan and sericin respectively against *E. coli* gram-negative bacteria as shown in the **Table 3.6**. The results have been revealed that bacterial reduction percentage was more for *S. aureus* than *E. coli*. It is assumed that gram-negative bacteria are stiffer to gram-positive bacteria because of the

extra outer membrane on gram-negative bacteria. In addition, gram-negative bacteria contain 10% peptidoglycan of cell wall and high lipid content due to outer membrane. On the other hand, gram-positive bacteria contain 90% peptidoglycan of cell wall and low lipid content (**Krasner, 2009)**.

3.1.12 Antioxidant activity

The stable free radical2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) is used to test antioxidant activity. The odd electron in the DPPH radical is responsible for the highest absorbance at 517 nm as well as the visible deep purple hue. It forms the hydrogen bond by reacting with suitable reducing agents. The DPPH is decolorized and the absorbance is reduced as free radicals accept an electron donated by an antioxidant compound **(Sultana et al., 2009; Cuendet et al., 1997; Burits & Bucar 2000; Amarowicz et al., 2004**). **Figure 3.14** illustrates the action process of antioxidants against DPPH free radicals (**Liang & Kitts, 2014)**.



DPPH (purple color) **DPPH-H (color less)**

Figure 3.14: The action mechanism of the antioxidant agent on 2, 2 di-phenyl 1-picryl hydrazyl (DPPH) free radicals (R: H represents antioxidant) (Liang & Kitts, 2014)

Table 3.7 shows the free radical scavenging behavior of *Aloe vera* chitosan and sericin solution. The percentage of radical scavenging activity improved as the concentration of *Aloe vera* chitosan and sericin solution was increased. As *Aloe vera* chitosan and sericin concentrations rise, UV absorption decreases, suggesting that free radical scavenging activity rises. In another way, as the concentration of the specimen solvent rises, absorption reduces as the specimen donates protons to the free radical continuously. In comparison to other solutions, *Aloe vera* extract solution has the best scavenging percentage. **Ray et al. (2013a)** found that phenolics in aloe gel had the capacity to scavenge DPPH free radicals, and that phenolics are the main contributor to antioxidant

activity in aloe gel. Phenol compounds may either capture or scavenge free radicals through a sequence of reactions with antioxidant enzymes (**Reynolds & Dweck, 1999**). *Aloe vera* extract's antioxidant function is enhanced by the use of integral phenolic OH (**Ray et al., 2013c**).

The free radical scavenging operation of chitosan and sericin is almost identical, as seen in **Table 3.7**. The free radical scavenging activity of 8 mg/ml sericin and chitosan was 47 and 49 respectively.

Table 3.7: Radical scavenging activity (RSA) of specimen

Concentration of specimen	RSA % of *Aloe vera*	RSA % of Chitosan	RSA % of Sericin
2 mg/ml	65	40	35
4 mg/ml	76	44	39
6 mg/ml	85	47	43
8 mg/ml	90	49	47
10 mg/ml	93	53	52

Fan et al. (**2009**) extracted silk sericin from bombyx mori silk cocoon by heating process at 95°C for 120 min. They found that DPPH scavenging rate of 8 mg/ml of silk sericin solution was nearly 75% which was higher than present study result. It is assumed that extraction time is the main responsible factor for DPPH scavenging activity. As the present study silk sericin extracted from bombyx mori silk cocoon by autoclaving process at 120°C for only 20 min. Silk sericin acts as antioxidant agent as the residual free $–NH_2$ groups of sericin can react with that the free radicals (DPPH solution) to form stable molecules and the $–NH_2$ groups can form ammonium groups (NH_4^+) by capturing an hydronium ion from the solution.

10 mg/ml of chitotan solution showed 53% free radical scavenging activity as shown in **Table 3.4**. **Kanatt et al. (2008)** found, scavenging ability of 10 mg/ml chitosan on 1, 1-diphenyl-2-picrylhydrazyl radicals was 46.4-52.3%. It is assumed that the residual free $–NH_2$ group of chitosan is responsible for antioxidant activity. The DPPH radicals can react with residual free –NH2 group of chitosan to form stable molecules and the $–NH_2$ groups can form ammonium groups (NH_3^+) by capturing an hydronium ion from the solution (**Yen et al., 2008).**

The capacity of a substance to scavenge DPPH radicals is a measure of its antioxidant activity. Therefore, it is not surprising to conclude that *Aloe vera* chitosan and sericin solution could have contained electron donors that reacted with free radicals to transform them to more stable products and avoid the radical chain reaction.

These results indicated that *Aloe vera* chitosan and sericin solution was a natural antioxidant with potent anti-oxidative activity. Reactive oxygen species have been linked to a number of oxidative stress-related disorders. These natural antioxidants can be useful in combating oxidative stress-related diseases such as cardiovascular disease, rheumatoid arthritis, ulcerative colitis, and neurological degenerative diseases. These natural antioxidant agents are hoped to be used as a food supplement, cosmetic, biomedical application, pharmaceutical, and medical industry.

3.1.13 UV Protection Factor

Table 3.8 shows that UV protection factor of *Aloe vera* extraction film was the best compared to other materials. Conversely, the position of the UV protection factor of silk sericin producing film is second. UV protection factor rises as the concentration rises for all samples. The UPF rating of 10 mg/ml concentrated *Aloe vera*, chitosan, and silk sericin film were 10.4, 4.46, and 7.4 respectively.

Table 3.8: UV protection factor (UPF) value

Observation	UPF rating
5 mg/ml *Aloe vera*	8.23
10 mg/ml *Aloe vera*	10.4
5 mg/ml Chitosan	3.64
10 mg/ml Chitosan	4.46
5 mg/ml Sericin	6.3
10 mg/ml Sericin	7.4

Aloe vera had a two-fold higher UPF ranking than chitosan. Among the sample, chitosan showed lowest UPF values. The equation number **(5)** revealed that the UPF rating is inversely proportional to the transmittance value of the specimens. So it can be said that the transmittance value of chitosan producing film was highest. N-acetyl glucosamine and glucosamine are two chromophoric groups contained in chitosan. These are made from chitin that has been partly deacetylated. UV resistance is aided by these chromophoric types (**Tyagi et al., 1996**).

3.2 Characterization of modified fabric

3.2.1 FTIR analysis

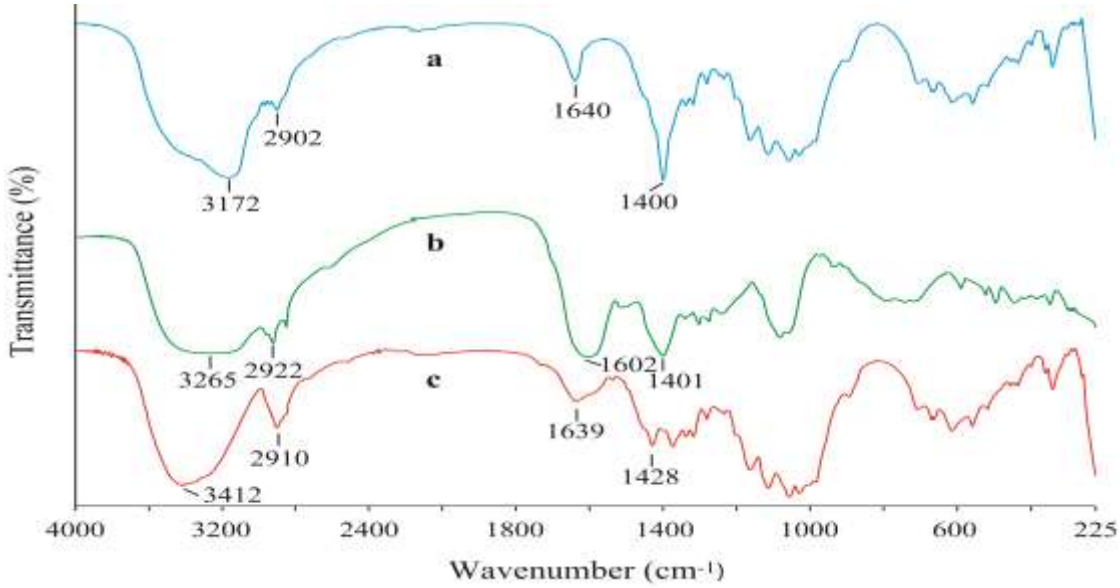


Figure 3.15: FTIR Spectra of (a) Cotton, (b) *Aloe vera* and (c) *Aloe vera* modified fabric

Figure 3.15a shows a wide peak at 3172 cm-1 in the FTIR spectra of scoured bleached cotton, which was attributed to OH stretching vibrations of cellulose hydroxyl groups in the 3100-3600 cm-1 range. The spectra of the scoured bleached cotton fabric also showed typical distinctive peaks at 1400 cm-1 and 2900 cm-1, respectively, which were assigned to C-H bending and stretching bands. Moreover, the H–O–H stretching vibration of absorbed water in carbohydrate is responsible for the peak at 1640 cm-1 **(Chung et al., 2004).**

The distinctive peak of cotton fabric at 3172 cm-1 (**Figure 3.15a**) was relocated to 3412 cm-1 **(Figure 3.15c**) after *Aloe vera* treatment, indicating intermolecular hydrogen bonding between the untreated cotton bleached fabrics and *Aloe vera*. The peak of untreated cotton fabric at 1400 cm-1 is transferred to 1428 cm-1 after treatment, indicating that *Aloe vera* and untreated cotton samples are attached. Furthermore, untreated cotton wave numbers 2902 cm-1 and 1640 cm-1 (**Fig. 3.15a**) are converted to 2910 cm-1 and 1639 cm-1 (**Figure 3.15c).**

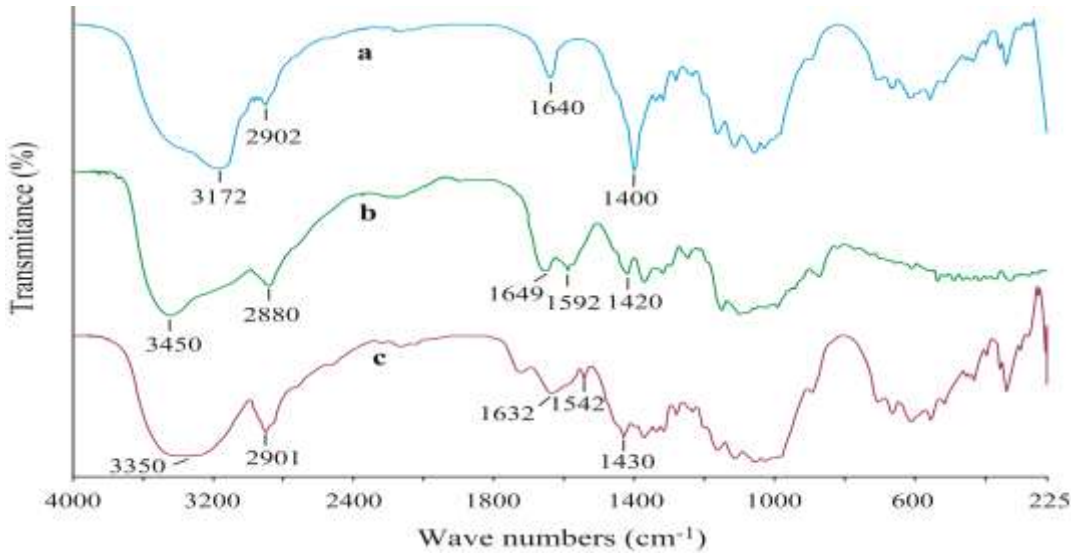


Figure 3.16: FTIR Spectra of (a) Cotton, (b) Chitosan and (c) Chitosan modified fabric

The **Figure 3.16 (a, b, c)** represents the untreated cotton fabric, chitosan and chitosan modified fabrics respectively. After chitosan treatment, the treated sample showed, at 3350 cm^{-1} (**Figure 3.16c)** that indicated the intermolecular hydrogen bonding between the cotton and chitosan. The most important peaks of chitosan are observed at 1649 cm^{-1} and 1592 cm^{-1} represents the C=O stretching vibration (Amide l) of acetyl groups and N-H bending vibration (Amide ll) of amino groups respectively. The peak in the chitosan-treated sample was 1542 cm-1, which was completely absent in the untreated cotton fabric. Cotton wave number 1640 cm-1 (Figure 3.18a) has been changed to 1632 cm-1. Above findings demonstrated that chitosan had been successfully integrated into the cotton fabric.

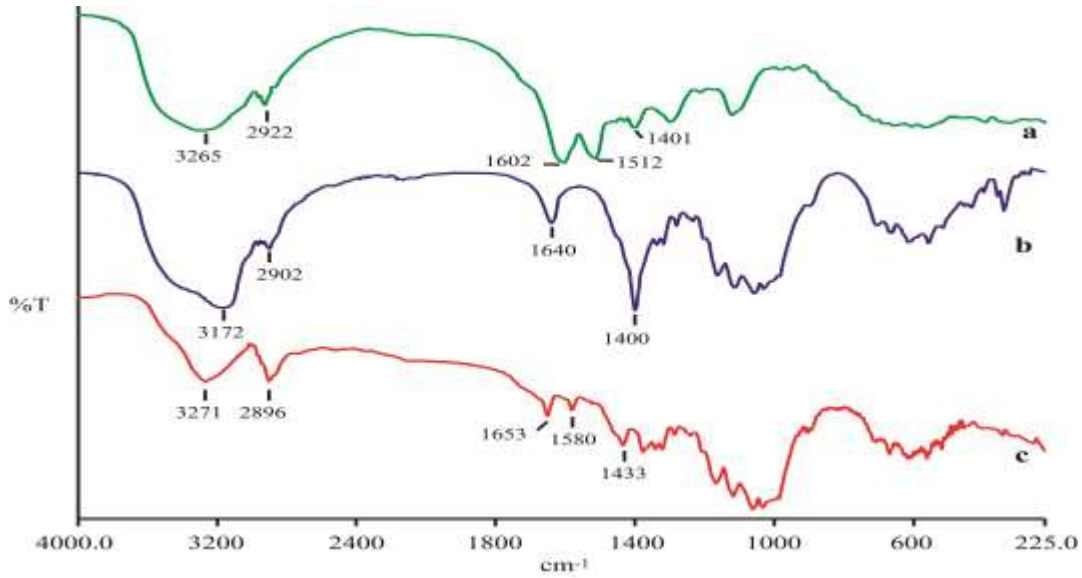


Figure 3.17: FTIR Spectra of (a) Sericin, (b) Cotton and (c) Sericin modified fabric

The new characteristic peak was found at 1580 cm^{-1} in sericin modified fabric (**Figure 3.17c**) which was absent in untreated cotton fabric (**Figure 3.17b**). After application of sericin, the peak at 3172 cm^{-1} and 2902 cm^{-1} of untreated cotton fabric was shifted to 3271 and 2896 cm^{-1} which were indicated the intermolecular hydrogen bonding between untreated cotton fabric and sericin. Sericin modified fabric (**Figure 3.17c**) also exhibited modified peaks at 1653 and 1433 cm^{-1}. Above results indicated that sericin had been integrated effectively into the cotton fabric.

3.2.2 XRD Analysis

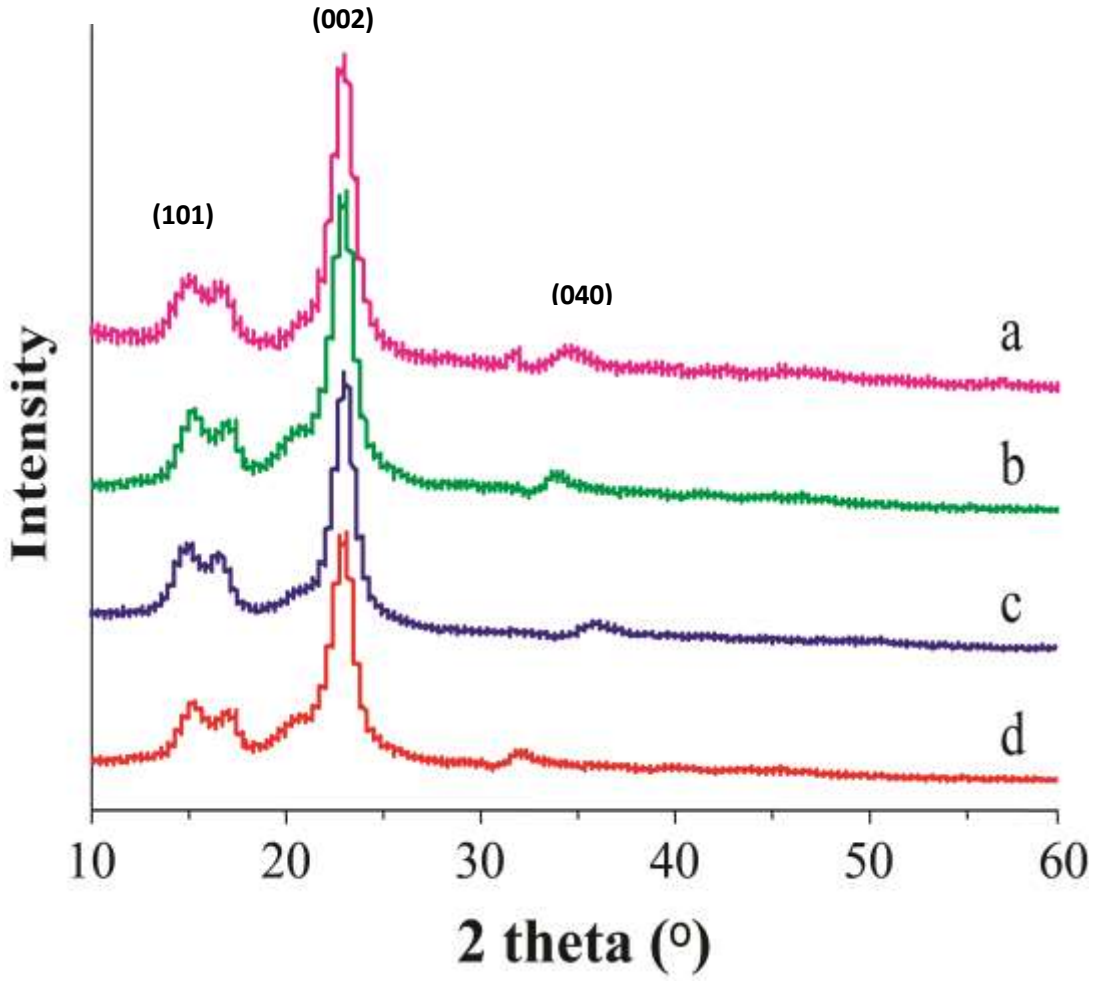


Figure 3.18: X-Ray diffraction pattern of: (a) Cotton fabric, (b) *Aloe vera*-treated fabric and (c) Chitosan-treated fabric (d) Sericin treated fabric

The 2-theta value of the untreated sample (cotton) showed three consecutive peaks at 15.78, 22.94, and 34.8 corresponding to crystal planes of (101), (002), and (040), respectively, as shown in **Figure 3.18** and **Table 3.9**. This outcome is consistent with that of **Altınışık et al. (2013).**

Table 3.9: Measurement of and crystallite index (CI) and crystallite diameter (C.D) of the sample from XRD pattern

Observations	2 theta value (º)	Peak intensity at crystallite region (count)	Peak intensity for amorphous region at 18º	C. I (%)	FWHM in Radian	C. D in nm
Cotton fabric	22.94	2847	541	81	0.0330	4.28
Aloe vera trated	22.95	4695	838	82	0.0342	4.14
Chitosan treated	22.78	5283	936	83	0.0333	4.25
Sericin treated	22.72	5044	840	84	0.0336	4. 20

Equation no. 5 was used to compute the crystallite index. The crystallite index of untreated sample, *Aloe vera*, chitosan and sericin treated sample was 81, 82, 83 and 84% respectively.

These findings showed that there was little distinction among the samples. As illustrated in **Table 3.9**, the full width at half maximum (FWHM) value of *Aloe vera*, chitosan, and sericin-treated samples increased slightly when compared to untreated samples. According to the Deby-Scherrer formula, the full width at half maximum (FWHM) value is inversely proportional to the crystal diameter **(equation no. 4).** With the assistance of origin pro software, the full width at half maximum value (FWHM) was calculated. The crystal diameter of the specimen reduces as the full width at half maximum value rises. The crystal diameter of untreated sample, *Aloe vera*, chitosan and sericin treated sample was 4.28, 4.14, 4.25 and 4.20 respectively. The untreated sample had a slightly larger crystal diameter than the *Aloe vera*, chitosan, and sericin-treated samples because the untreated sample's full width at half maximum value was the smallest. The crystallite diameter of *Aloe vera* extract modified cotton fabric was marginally less than the untreated sample, according to **Xu and Chen (2011).** Moreover, when compared to pure cotton, the crystallite diameter of nano chitosan treated cotton fabric decreased. (**Wang et al., 2014**).

Cotton, *Aloe vera*, chitosan and sericin treated cotton have nearly identical X-ray diffraction characteristic peaks, indicating that the cotton fibre cellulose did not undergo any significant modification. After *Aloe vera*, chitosan and sericin treatment, the intensity of X-ray diffraction peaks at $2\theta = 22.94°$ for cotton cellulose decreased slightly.

3.2.3 SEM analysis

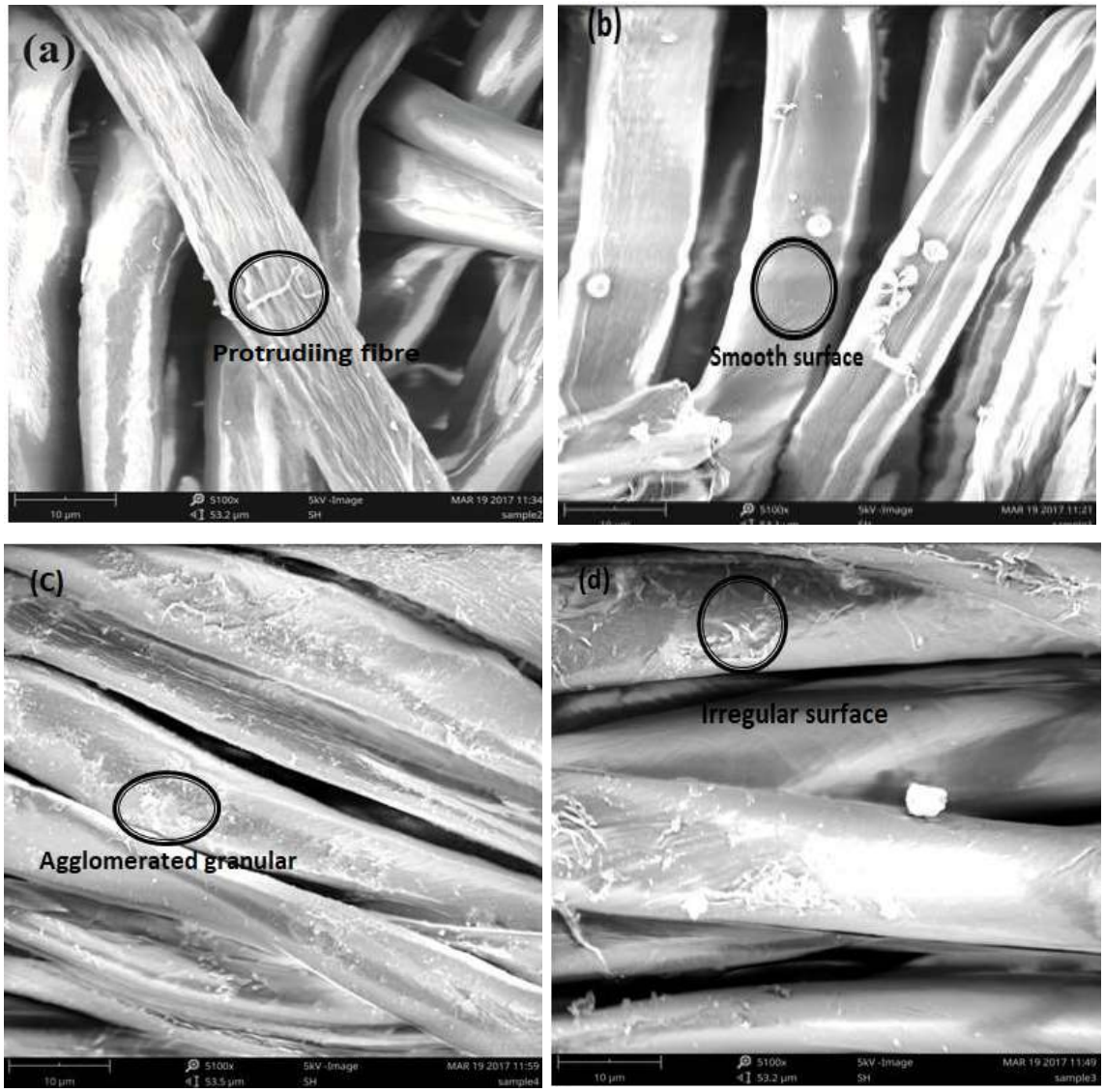


Figure 3.19: SEM photograph of: (a) Untreated fabric, (b) Chitosan-treated fabric, (c) *Aloe vera*-treated fabric, and (d) Sericin-treated fabric.

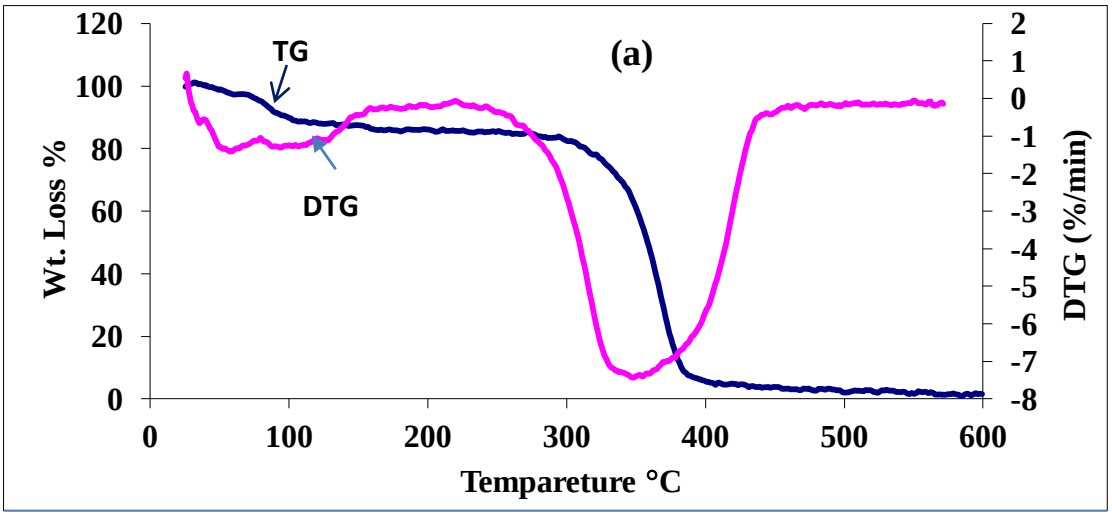
A scanning electron microscope (SEM) was used to examine the surface morphology of the treated and untreated fabrics. **Figure 3.19(a)** shows a significant amount of protruding fibers on the surface of an untreated cotton fabric sample which responsible for roughness. But all of the treated fabrics had a protruding fibers-free surface as shown in **Figure 3.19 (b, c, d)** (**Sathianarayanan et al., 2010; Lim & Hudson, 2004)**. Despite the fact that antibacterial agent treatment filled the pores of untreated fabrics, a slightly irregular surface was found on all antibacterial agent treated samples as agglomerated granular was easily visible on the treated cloth in **Figure 3.19(b, c, d)**, suggesting that antimicrobial agents were effectively bound to the untreated fabric

3.2.4 TGA Analysis

The mass loss was detected at 14%, 1%, 8%, and 4% of the untreated fabric, *Aloe vera*, chitosan, and sericin-treated samples, respectively, at 200°C, according to **Figure 3.20** and **Table 3.10**. This mass loss is due to vaporization of moisture and the low molecular weight. More significant mass loss occurs between 200°C and 400°C due to decomposition of the specimen. Maximum decomposition temperature of untreated, *Aloe vera*, chitosan, and sericin-treated samples was 354, 297, 337, 340 correspondingly. The results clearly indicate that the decomposition rate of untreated cotton fabric was maximum and *Aloe vera*-treated sample was minimum. In this regard, it can be said that thermal stability of *Aloe vera* treated fabric was highest. 45, 21, 46 and 31% mass loss was observed at DTG peak maximum temp. (°C) for untreated, *Aloe vera*, chitosan, and sericin-treated samples, respectively. Thereafter, little weight lost was observed between 400°C to 600°C. Untreated, *Aloe vera*, chitosan, and sericin-treated samples yielded 2, 45, 10, 34 percent of char yield, respectively. These finding indicates that, the untreated sample contained the least amount of ash.

Table 3.10: Data obtained from TGA and DTG Thermograph

Samples	Wt. loss % at 200°C	DTG peak maximum temp. (°C)	Weight loss %	har yield (%) at 600 (°C)
Untreated	14	354	45	2
Aloe vera treated	1	297	21	45
Chitosan treated	8	337	46	10
Sericin treated	4	340	31	34



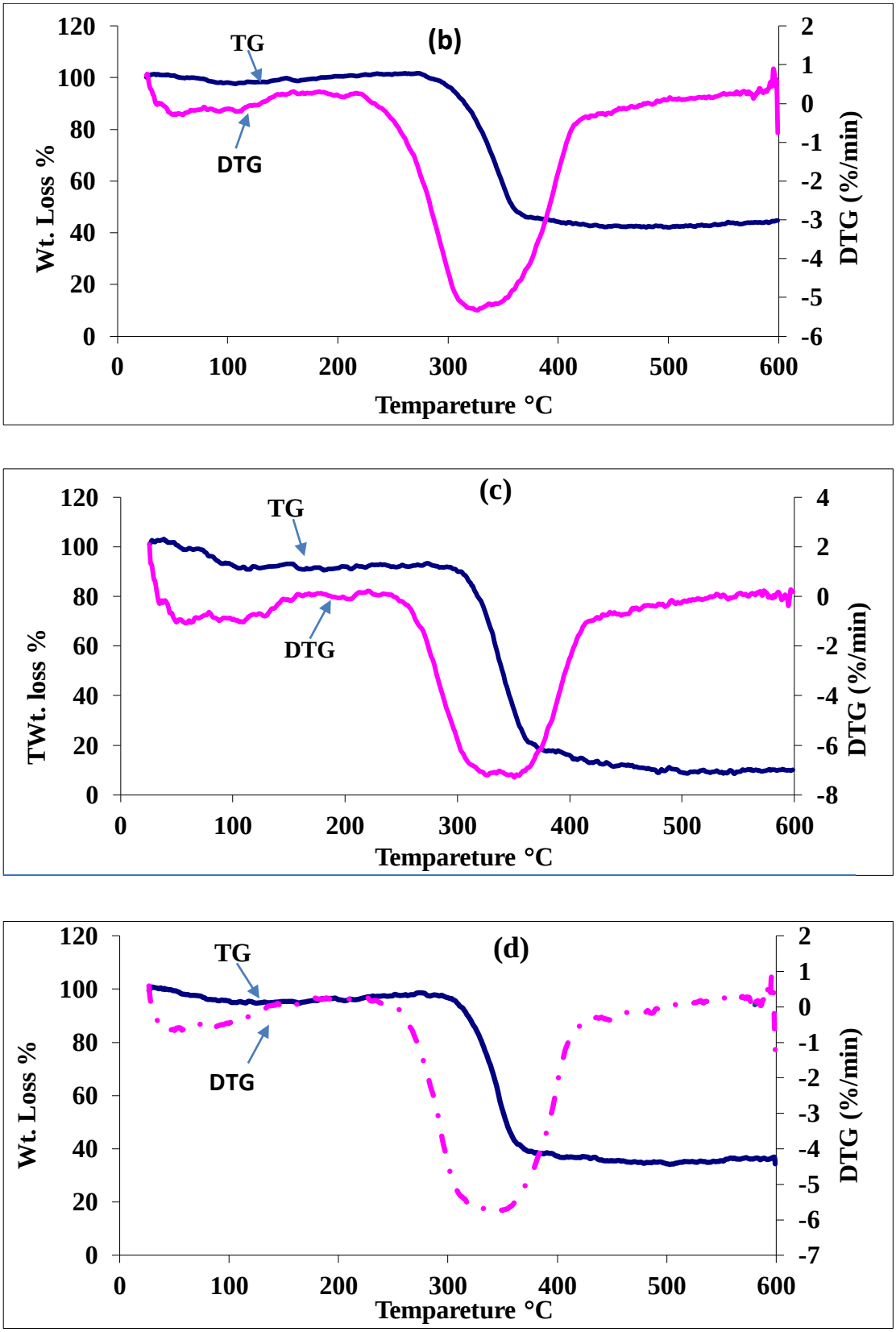


Figure 3.20: TGA & DTG graph of (a) Untreated cotton, (b) *Aloe vera* treated, (c) Chitosan treated and (d) Sericin treated fabric

3.2.5 DSC analysis

The DSC curve **Figure 3.21 (a)** was given a broad exothermic peak at 370°C which pointed out the maximum decomposition temperature of cellulose. The exothermic peak of *Aloe vera*, chitosan, and sericin treated fabrics is moved to a lower temperature of 345°C, 340°C, and 306°C, respectively. This may be due to break down the intermolecular hydrogen bonding of the *Aloe vera*, chitosan and sericin treated fabric.

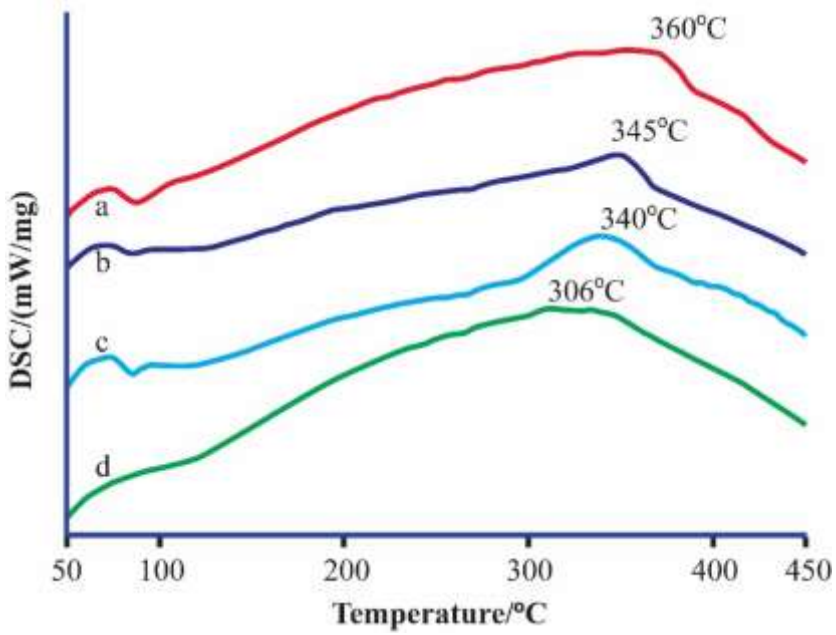


Figure 3.21: Differential scanning calorimetry diagram of: (a) Untreated fabric, (b) *Aloe vera* treated fabric, (c) Chitosan treated fabric, and (d) Sericin treated fabric

Another research found that chitosan-treated cotton decomposes at a lower temperature than untreated cotton fiber (**53. Yang et al, 2014**). It is assuming that the antimicrobial agent not only cross linked with amorphous region but also accessed into the crystalline phase of cotton cellulose and causing disruption of some intermolecular hydrogen bonding of the crystalline regions of cotton.

3.2.6 Antimicrobial activity of treated fabric by qualitative method

It can be seen from **Table 3.11 and Figure 3.22** that the circular zone of inhibition increases with increasing concentrations of *Aloe vera*, chitosan, and sericin. 10 g/l *Aloe vera*, chitosan and sericin showed zone of inhibition in 1.5, 2.3 and 0.7 mm respectively. Chitosan treated fabric showed maximum antibacterial activity. As higher zone of inhibition indicates the higher antibacterial activity.

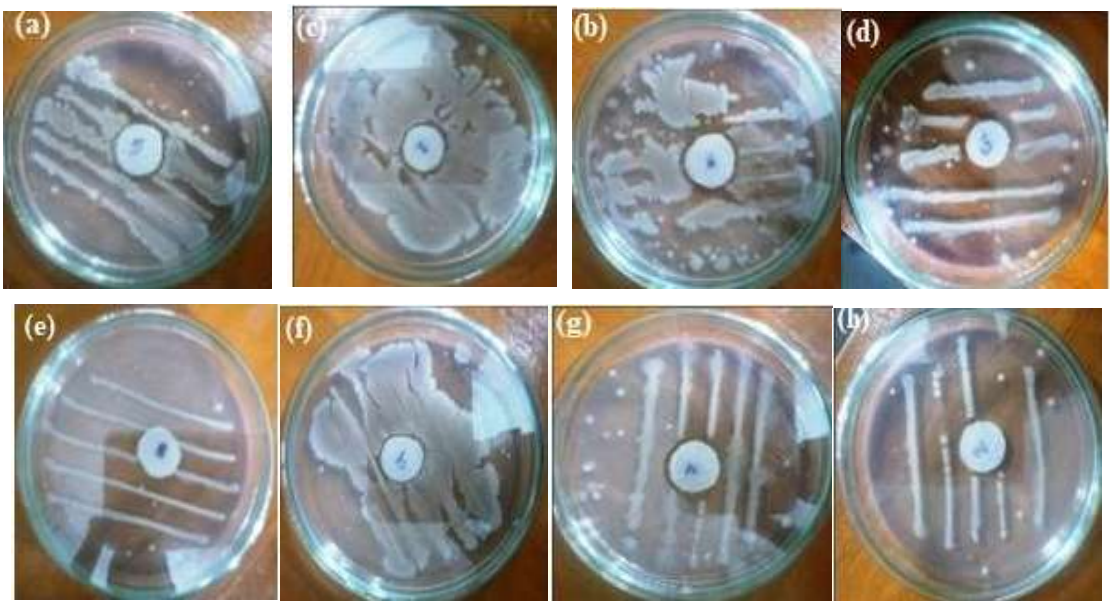


Figure 3.22: Photographs showing zone of inhibition of treated sample (a). 5 g/l *Aloe vera*, (b). 10 g/l *Aloe vera*, (c). 5 g/l Chitosan, (d). 10 g/l Chitosan, (e). 5 g/l sericin, (f). 10 g/l sericin, (g). 5 g/l chitosan and 5 g/l *Aloe vera*, (h). 5 g/l chitosan and 5 g/l sericin

Table 3.11: Zone of inhibition of *Aloe vera*, chitosan, and sericin treated fabrics against *S. aureus* gram-positive bacteria

Observation	Zone of inhibition in mm
5g/l *Aloe vera*	1
10 g/l *Aloe vera*	1.5
5 g/l Chitosan	1.2
10 g/l Chitosan	2.3
5g/l Sericin	0.2
10 g/l Sericin	0.7
5 g/l Chitosan + 5 g/l *Aloe vera*	1.9
5 g/l Chitosan + 5 g/l Sericin	1.4

Conversely, Sericin-treated cotton has the lowest antibacterial efficacy. The combined treatment of 5 g/l chitosan and 5 g/l *Aloe vera* showed a 1.9 mm zone of inhibition against *S. aureus* bacteria, which was higher than the 10 g/l *Aloe vera* treated fabric. In the other side, fabric treated with 5 g/l chitosan and 5 g/l sericin displays a 1.4 mm zone of inhibition against *S. aureus* bacteria, which was better than 10 g/l sericin treated fabric.

3.2.7 Antimicrobial activity of treated fabric by quantitative method

Table 3.12: Bacterial reduction (B. R) rate of bleached cotton fabric treated by *Aloe vera,* chitosan, and sericin against *S. aureus* and *E. coli* bacteria

Observation	B. R (%) for *S. aureus*	B. R (%) for *E. coli*	B. R (%) after five wash for *S. aureus*	B. R (%) after five wash for *E. coli*	B. R (%) after ten wash for *S. aureus*	B. R (%) after ten wash for *E.coli*
5 g/l Chitosan	66	58	54	47	42	34
10 g/l Chitosan	86	77	75	66	61	53
5g/l *Aloe vera*	58	53	50	44	38	32
10 g/l *Aloe vera*	76	69	65	57	53	44
5g/l Sericin	40	34	32	27	25	20
10 g/l Sericin	50	43	40	36	32	27
Combind-1	81	74	70	65	58	55
Combind-2	55	49	44	41	34	33
*Combind-1: 5 g/l Chitosan+ 5 g/l *Aloe vera*; *Combined-2: 5 g/l Chitosan + 5 g/l Sericin						

Table 3.12 indicates that the bacterial reduction rate improved as the concentrations of *Aloe vera*, chitosan, and sericin increased. Bacterial reduction rates for 5 g/l and 10 g/l *Aloe vera*-treated fabric were 58 and 76 percent, respectively for *S. aureus* gram-positive bacteria. Bacterial reduction values for chitosan-treated fabric of 5 g/l and 10 g/l were 66 and 86 percent, correspondingly. Again bacterial reduction values for sericin-treated cloth of 5 g/l and 10 g/l were 40 and 50 percent, respectively. The combined antimicrobial agent (5 g/l chitosan and 5 g/l *Aloe vera*) treated fabric reduced bacteria by 81 percent. The combined antimicrobial agent (5 g/l chitosan and 5 g/l *sericin*) treated fabric reduced bacteria by 55 percent. However, at a concentration of 10g/1, the bacteria reduction rate of each individual *Aloe vera* and sericin treated fabric was lower than that of combined treated fabric.

In case of *E.coli* gram negative bacteria, bacterial reduction rate was always lower than *S. aureus* gram-positive bacteria. Because gram-negative bacteria are thought to be harder than gram-positive bacteria due to the additional outer membrane on gram-negative bacteria.

Another research showed that the mixture of *Aloe vera* and neem had the best antibacterial efficacy as compared to *Aloe vera* or Neem alone **(Khurshid et al., 2015)**. The explanation for this is that when a single antimicrobial agent is applied to a textile surface, fewer functional groups are formed. on the other hand, two antimicrobial agents create more functional groups on the textile surface and have a synergistic effect. As a result, the increased amount of new functional groups found in antimicrobial treated textiles attacks the bacteria's cell wall and greatly inhibits microbial development. Therefore, it can be said that combined antimicrobial agents have greater biocidal efficacy than single antimicrobial agent **(Ammayappan & Moses, 2009).**

The durability of antibacterial activity after repeated washing was also assessed. The findings explicitly show that subsequent washing reduces bacterial reduction. After 10 washes, the bacterial reduction in case of *S. aureus* was reduced from 86 percent to 61 percent for 10g/l chitosan-treated fabric and from 76 percent to 53 percent for 10 g/l *Aloe vera*-treated fabric.

The bacterial reduction rate was reduced from 77 percent to 53 percent for 10g/l chitosan-treated fabric and from 69 percent to 44 percent for 10 g/l *Aloe vera*-treated fabric after ten washes for *E. coli* gram- negative bacteria. Again bacterial reduction rate was reduced from 43 to 27 for 10 g/l sericin treated fabric as seen in **Table 3.12**. All of the treated fabric samples maintained 50 to 70 percent antimicrobial activity after 10 washing cycles, according to the findings.

After 10 washing cycles, **Khushid et al. (2015)** discovered that *Aloe vera* and Neem treated cloth contained 50 to 60% antimicrobial activity. **Sathianarayanan et al. (2010**) also discovered that after 10 washing cycles, 60 to 70 percent of the antimicrobial activity of tulsi and pomegranate treated cotton fabric was retained. Overall 50 to 70% antibacterial activity retains the natural antimicrobial agent treated cotton fabrics after 10 washing cycle. Therefore, it is proved that the present work did not show poor wash durability.

3.2.8 Antioxidant activity of treated fabric

Figure 3.23 demonstrate the free radical scavenging behavior of *Aloe vera*, chitosan and sericin treated fabrics. The percentage of radical scavenging activity improved with increasing concentrations of *Aloe vera*, chitosan and sericin, according to the findings. As the concentration of *Aloe vera* chitosan and sericin increases, the UV absorption of DPPH decreases, suggesting increased free radical scavenging activity. Other way absorption decreases gradually with increasing concentration of specimen treated fabric as specimen donate protons to this free radical. In contrast to chitosan and sericin treated fabrics, *Aloe vera* treated fabric displayed the highest scavenging percentage. Chitosan and sericin-treated fabric had almost identical free radical scavenging behavior.

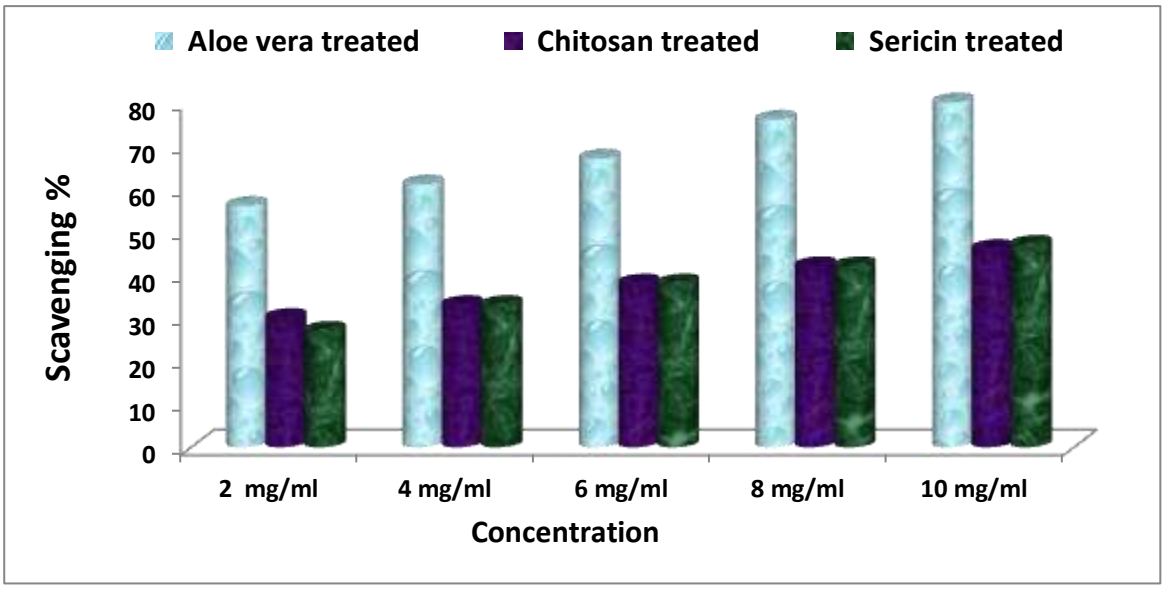


Figure 3.23: Radical Scavenging activity (RSA) of specimen treated fabric

Ray et al. (2013a) found that phenolics in aloe gel have the ability to scavenge DPPH free radicals, and that phenolics are the major contributor of antioxidant activity in aloe gel. Free radicals can be trapped directly by phenol compounds or scavenged through a series of reactions with antioxidant enzymes (**Reynolds & Dweck, 1999**). The abundance of the integral phenolic OH in *Aloe vera* extract contributes to its antioxidant activity (**Ray et al., 2013c**).

3.2.9 UV resistivity of modified fabric

UV rays are usually blocked by the ozone layer. However, owing to climate change and depletion of the ozone layer, UV rays are now quickly coming the planet. Therefore, Sunburn, early skin aging, asthma, and skin cancer are all caused by UV rays.

UV resistivity of modified fabric was determined by UV Protection Factor (UPF). UPF rating of *Aloe vera*, chitosan and sericin showed in **Figure 3.26.** UPF rating increases by increasing the concentration of *Aloe vera*, chitosan and sericin. The fabric treated with *Aloe vera* had the highest UV safety factor. *Aloe vera*'s highest efficiency ensures it can absorb more UV rays and protect the human body. Since polyphenols and flavonoids in *Aloe vera* help to block UV rays **(Strid & Porra, 1992; Landry et al., 1995).**

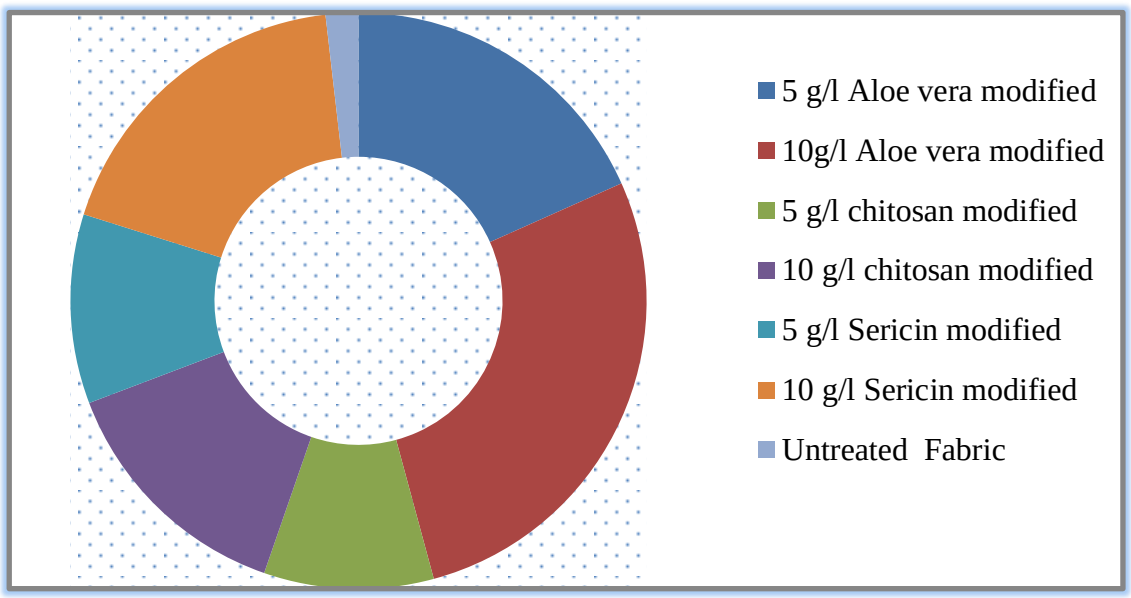


Figure 3.24: UV protection factor (UPF) of treated and untreated fabrics

Fabric treated with 5 g/l *Aloe vera* had a tenfold increase in absorbency compared to untreated fabric. Among the samples, the chitosan-treated fabric performed the worst. **Kotb et al. (2014)** discovered that chitosan can form a transparent film on cotton cloth, allowing UV rays to pass through easily**.**

3.2.10 Weight add-on (%), thickness and GSM

Table 3.13: Weight add-on (%), thickness, GSM of *Aloe vera*, chitosan, and sericin treated fabric

Concentration	Add on (%) of A. vera	Thickness (cm) of A. vera	GSM of A. vera	Add on (%) of Chitosan	Thickness (cm) of Chitosan	GSM of Chitosan	Add on (%) sericin	Thickness (cm) of sericin	GSM of sericin
00	-	0.021	122	-	0.021	122	-	0.021	122
2 g/l	2.0	0.022	124	3.2	0.025	125	2.8	0.024	126
4 g/l	2.2	0.023	126	3.6	0.027	127	3.4	0.026	129
6 g/l	2.6	0.024	129	4.2	0.029	129	3.9	0.028	132
8 g/l	2.9	0.025	130	4.7	0.031	132	4.4	0.030	134
10 g/l	3.1	0.026	131	5.4	0.035	135	5.0	0.033	136

Table 3.13 shows that as the concentration of antimicrobial agents rose, the weight add-on proportion increased, indicating that antimicrobial agents adhered to the cloth during finishing. Consequence, the treated fabric's GSM was raised. The add-on% of chitosan-treated fabric was greater than that of *Aloe vera* and sericin treated fabric, in the same concentration. It is assumed that this is due to the high degree of affinity of chitosan to cotton fabric. As a result, the GSM and thickness of chitosan-treated fabric improved.

3.2.11 Whiteness Index

The whiteness index of the fabric was decreased gradually by treatment with increased of *Aloe vera*, chitosan and sericin concentrations as shown in **Fig. 3.25**. The use of *Aloe vera*, chitosan, sericin, and critic acid at high temperatures resulted in yellowing of finished fabrics. Maximum yellowness meant lowest amount whiteness was found for sricin treated fabric owing to that sericin contains a mass of aromatic groups and tends to cause yellowness at high temperature.

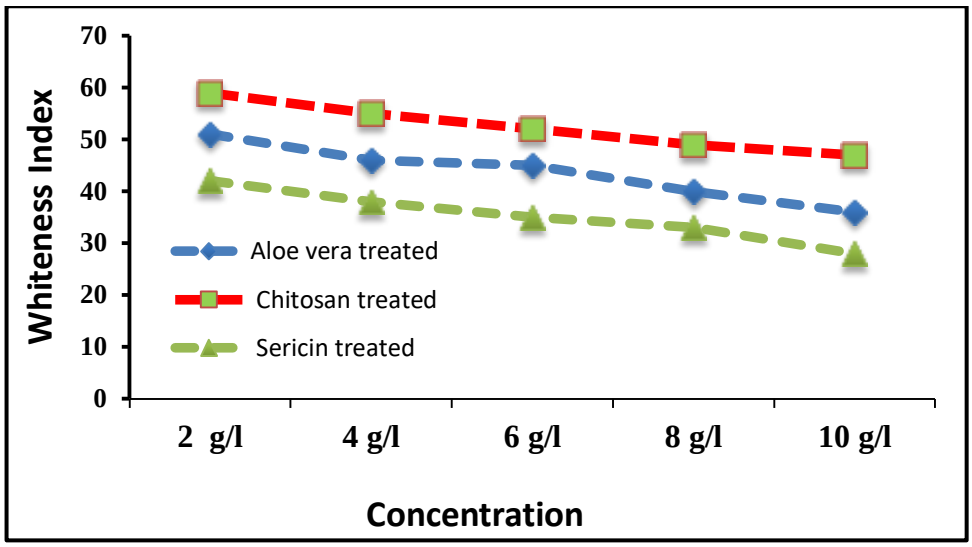


Figure 3.25: Whiteness Index (WI) of *Aloe vera*, Chitosan and Sericin treated fabric

On the other hand, the color of sericin also exhibits yellowness. In addition to maximum whiteness was retained the chitosan treated sample. Because visual appearance of chitosan powder was off white. Whiteness decreased about 21%, 9% and 35% with 2g/l *Aloe vera*, chitosan and sericin treatment respectively with compared to untreated fabric. As a result, chitosan-only treatment is better for maintaining cloth whiteness compared with *Aloe vera* and sericin treatment.

3.2.12 Absorbency test

The capillary flow of water through the cotton causes fabric wicking **(Sharabaty et al., 2008).** Water absorbency height of untreated and critic acid treated fabrics was 4.8 cm and 3.1 cm, respectively (see the appendix-4)

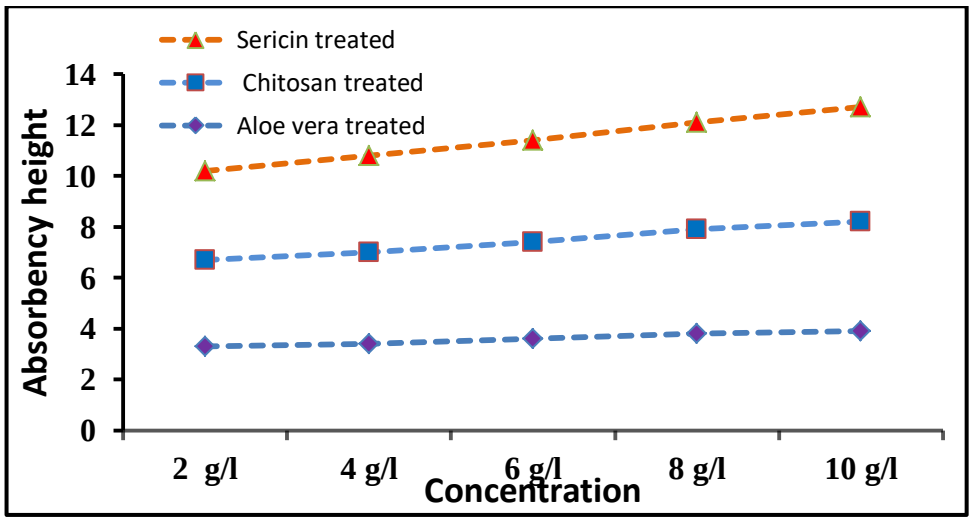


Figure 3.26: Water absorbance height of *Aloe vera*, chitosan and sericin treated fabric

According to the findings, water absorbency of critic acid treated fabrics is reduced by nearly 35% compared to untreated fabric. On the other hand, water absorbency was improved after treatment of *Aloe vera*, chitosan and sericin compared to critic acid treated fabric which could be attributed to the hydrophilic groups containing in the *Aloe vera*, chitosan and sericin. Absorbency of sericin treated fabric was maximum and Aloe treated fabric was minimum as shown in **figure 3.26.**

3.2.13 Tensile strength

Figure 3.27 displays the tensile strengths of untreated and treated samples in the warp direction. The tensile strength of treated samples was dramatically decreased after treatment with *Aloe vera*, chitosan, and sericin. Tensile strength was declined by 22%, 30% and 28% for 2 g/l *Aloe vera*, chitosan and sericin treated samples respectively compared to that of untreated samples. Strength losses by 22–33%, 30–40% and 28-39% were found for 2 to 10 g/l *Aloe vera*, chitosan and sericin treated samples respectively compared to that of untreated samples. Hydrolysis of cotton cellulose macromolecules during treatment in the presence of critic acid as a cross linking agent was most likely the cause of the strength loss. Because cotton is alkali resistant but not acid resistant.

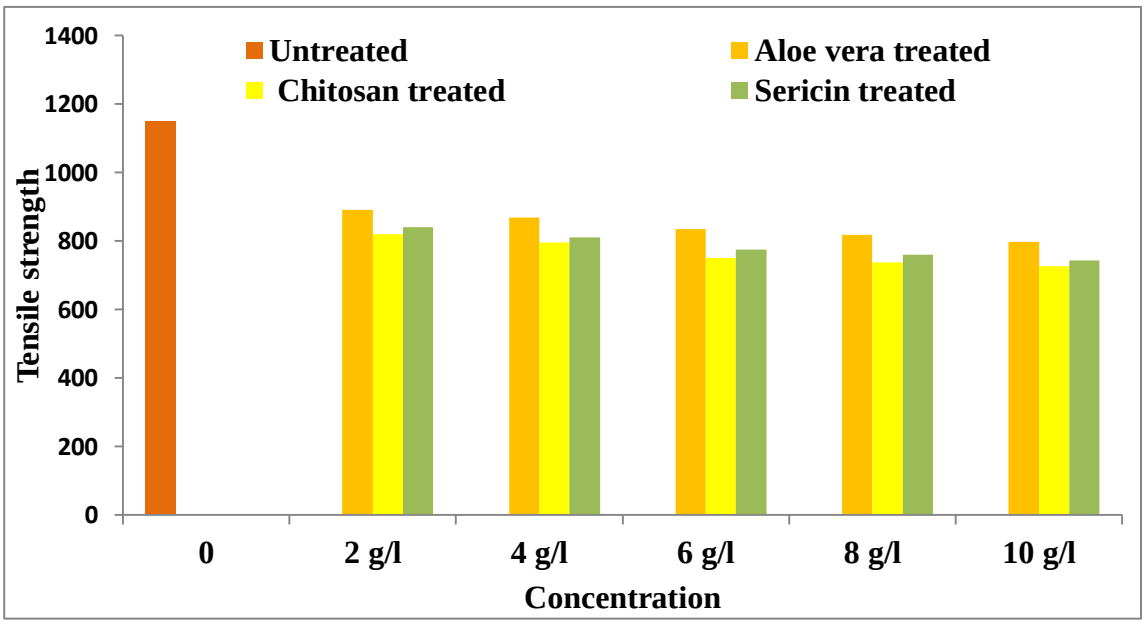


Figure 3.27: Tensile strength of *Aloe vera*, chitosan and sericin treated fabrics

The tensile strength of the treated fabric did not decrease dramatically as the concentration of finishing agent was increased. It can be said that, cross linking agent is

the most responsible factor for strength loss rather than concentration varied. The strength loss of chitosan and sericin treated fabric was greater than that of *Aloe vera* treated fabric. The tensile strength of *Aloe vera* (5 g/l) and chitosan (5 g/l) combined treated fabric was 740 N which was higher than 10 g/l chitosan treated sample and lower than 10 g/l *Aloe vera* treated fabric. Conversely, 5 g/l chitosan and 5 g/l sericin combined treated fabric was 730 N which was higher than 10 g/l chitosan treated sample and lower than 10 g/l sericin treated fabric.

3.2.14 Bending length and flexural rigidity measurement

The **Figure 3.28** shows that the *Aloe vera*, chitosan and sericin treatment affects the fabric stiffness mostly. Fabric bending length gradually increases for 2g/l to 10g/l of *Aloe vera*, chitosan and sericin treated sample. Bending length increase means increase of the fabric stiffness. The bending length of *Aloe vera*, chitosan and sericin treated fabric of 10 g/l concentration was 3.2, 3.6, and 3.7 respectively. These results indicate that bending length (cm) of sericin treated fabric was maximum.

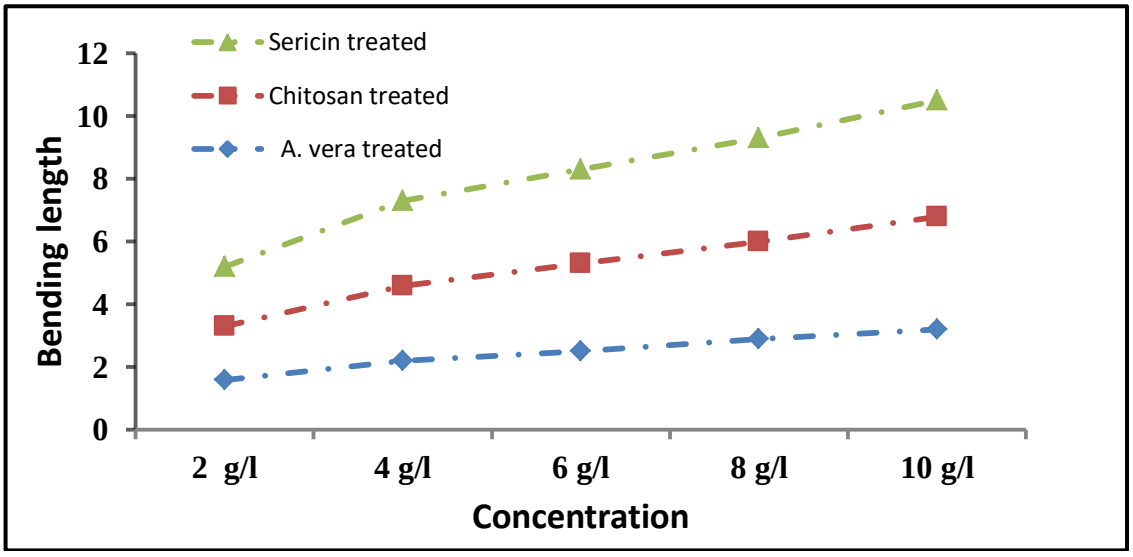


Figure 3.28: Bending length of *Aloe vera*, chitosan and sericin treated fabric

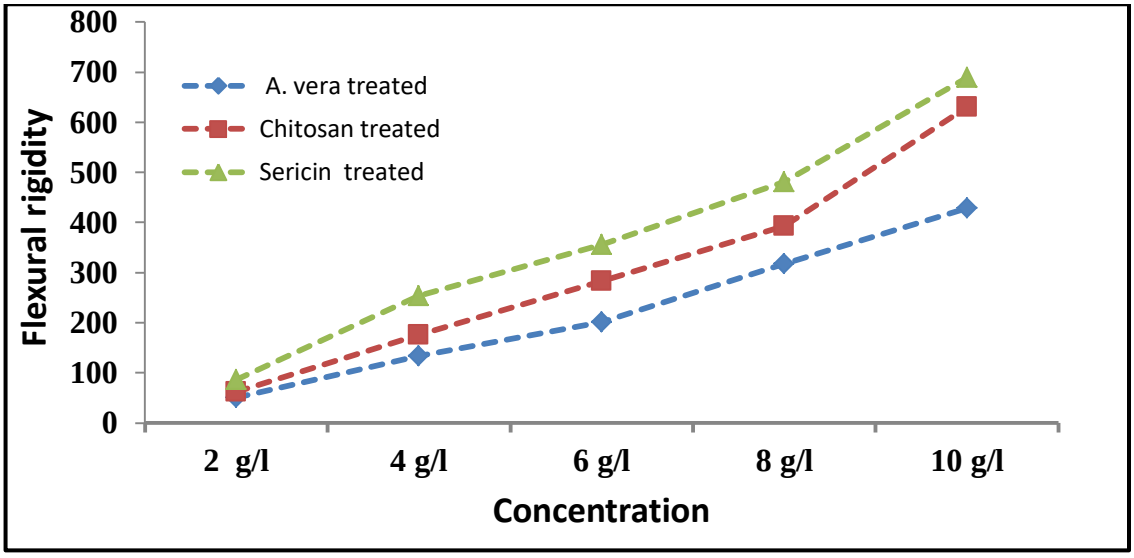


Figure 3.29: Flexural rigidity of *Aloe vera*, chitosan and sericin treated fabric

Fabric flexural rigidity gradually increases for 2g/l to 10g/l of *Aloe vera*, chitosan and sericin treated sample. The flexural rigidity of *Aloe vera*, chitosan and sericin treated fabric of 10 g/l concentration was 429, 629, and 688 respectively. These results indicate that flexural rigidity of sericin treated fabric was maximum.

3.2.15 Abrasion resistance and soil degradation

Thread breakage was not observed in chitosan, *Aloe vera*, or sericin-treated samples at 10,000 rubs per cycle (**Table 3.14**). Thus, antimicrobial finish did not influence the abrasion resistance of cotton fabric. Maybe, this is because the antimicrobial finish has no harmful effects on the chemical composition of the cellulose. **Bonin's** findings were also identical to these outcomes (**60. 2008**).

Table 3.14: Abrasion resistance and Soil degradation test result (sample buried for 30 days) of treated and untreated samples fabric

Observation	No. of rub cycles	Abrasion resistance	After soil degradation Weight loss (%)
Untreated fabric	10,000	No thread breakage	71
10 g/l Chitosan treated	10,000	No thread breakage	38
10 g/l *Aloe vera*-	10,000	No thread breakage	50
10g/l Sericin-treated	10,000	No thread breakage	58

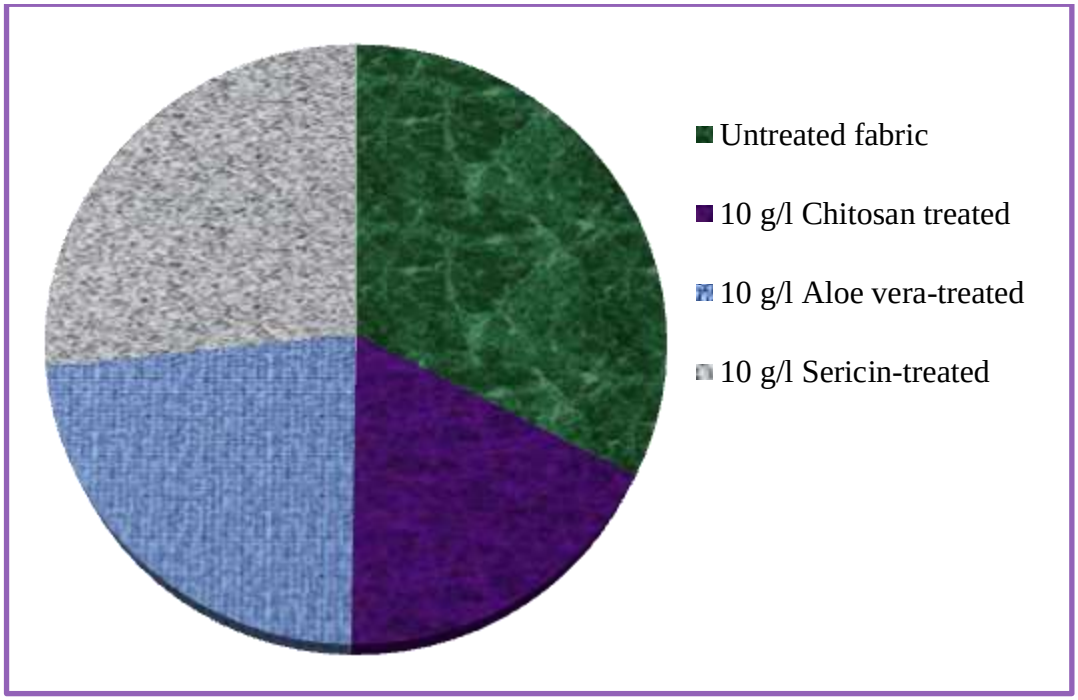


Figure 3.30: Soil degradation test result

The untreated sample experienced the biggest weight loss as a result of soil deterioration as shown in **Figure 3.30**. Since, the untreated cotton was easily invaded by soil microorganisms. **Table 3.14 displays that**, the 10 g/l chitosan-treated sample showed the least degradation (38 percent), demonstrating chitosan's high antimicrobial activity. The soil degradation of *Aloe vera* treated sample was next least, with 50% weight loss. On the other hand, weight loss was 58%, for sericin treated samples. Thus, chitosan showed the greatest antimicrobial activity. The findings of soil degradation show that treated samples are still biodegradable and thus environmentally sustainable, despite their modest resistance to soil degradation.

3.2.16 Crease recovery angle (CRA)

In contrast to the untreated cotton fabric, the *Aloe vera*, chitosan, and sericin treated cotton fabric demonstrated increased crease recovery angle as shown in **Figure 3.31.** For 2 g/l *Aloe vera*, chitosan, and sericin treated cotton, the crease recovery angle improved by 12 percent, 15 percent, and 14 percent. The crease recovery angle of cellulose fabric was poor, since cellulose molecules chains slipped when samples were wrinkled. Poly carboxylic acid (critic acid) was used as cross linking agent during finishing.

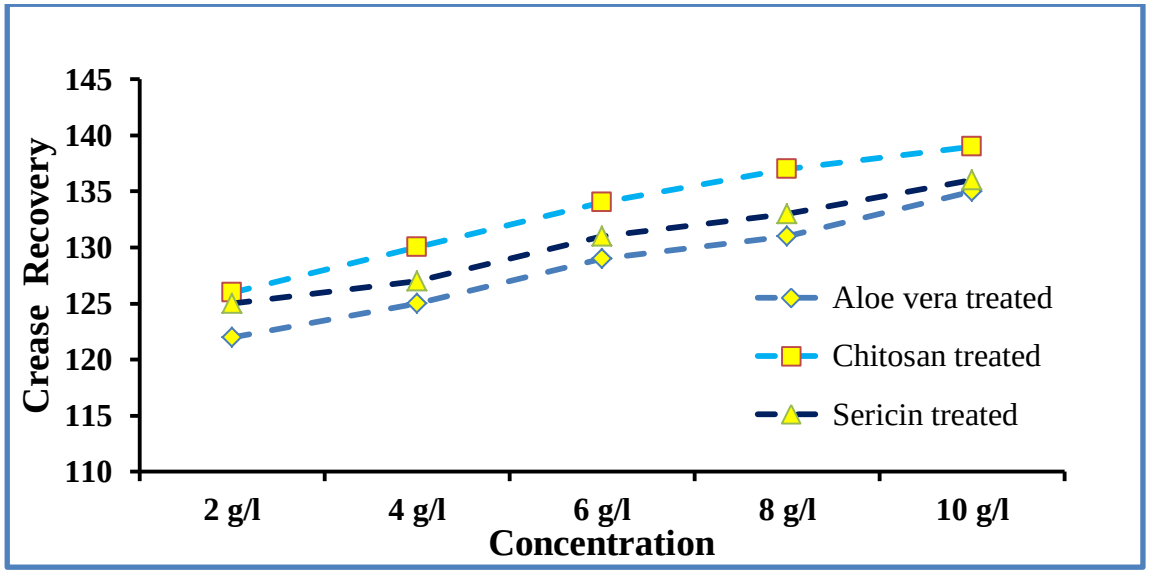


Figure 3.31: Crease recovery of *Aloe vera*, chitosan and sericin treated fabric

This cross linking agent restricted the relative slippage among the cellulose molecule through esterification reaction. Consequently, the crease recovery angles of the treated fabric were increased. On the other hand, with increasing of solution concentration, crease recovery angle was not increased significantly. Among the treated fabric, crease recovery angle of chitosan treated sample was maximum as the thickness of chitosan treated fabric is greater than *Aloe vera* and sericin treated cotton fabric.

3.2.17 Thermal Conductivity

Thermal conductivity is a measure of heat flow through a material. The rate of thermal conductivity decreased with concentration of antimicrobial agents. The thickness of treated fabric increased with concentration of antimicrobial agent, causing the rate of heat transfer to decrease. Due to the greater thickness of chitosan-treated fabric, the rate of thermal conductivity is less than that of *Aloe vera* and sericin-treated fabric as shown in the **Table 3.15.** Antimicrobial agents also change the surface morphology of treated fabric, which also creates barriers to thermal conductivity.

The SEM results showed that the surface of treated fabric was smoother than that of untreated fabric, as the *Aloe vera*, chitosan and sericin filled the pores of the untreated fabric. As a result, thermal conductivity is reduced.

Table 3.15: Thermal conductivity (T. C) and thermal resistivity (T. R) of *Aloe vera*, chitosan and sericin treated fabric

Concentration	T. C of A. vera (W/mk) $\times 10^{-3}$	T. R of A. vera (m^2k/W) $\times 10^{-2}$	T. C of chitosan (W/mk) $\times 10^{-3}$	T. R of chitosan (m^2k/W) $\times 10^{-2}$	T. C of sericin (W/mk) $\times 10^{-3}$	T. R of sericin (m^2k/W) $\times 10^{-2}$
2 g/l	5.428	4.053	3.990	6.265	4.361	5.503
4 g/l	4.551	5.053	3.799	7.107	4.121	6.309
6 g/l	4.384	5.474	3.624	7.450	3.867	7.240
8 g/l	3.914	6.387	3.336	9.292	3.453	8.688
10 g/l	3.526	7.373	3.167	11.051	3.231	10.213
Thermal conductivity of untreated fabric was 6.138 W/mk $\times 10^{-3}$						

Thermal resistance is an assessment of the material's ability to prevent heat passing through it. Thermal resistance is greatly influenced by fabric thickness. Since the thermal insulation of a garment increases as its thickness increases. Thermal resistance is a function of the thickness and thermal conductivity of a fabric. However, the results reveal that thermal conductivity increases the thermal resistance decreases and vice versa. 10 g/l chitosan-treated fabric showed the greatest thermal resistance, due to high amount of antimicrobial agent absorbed. Greater absorption of antimicrobial agent was increased the thickness the fabric and increases the GSM. Thermal resistance of chitosan-treated fabric was higher than that of *Aloe vera*, and sericin-treated fabric at all levels of concentration**.**

Aloe vera-treated fabric is more comfortable to wear in warmer climates, due to its lesser thermal resistance and greater thermal conductivity. Chitosan-treated fabric feels warmer to wear and will be suitable for slightly-cold weather, due to its lower thermal conductivity.

3.2.18 Water vapor permeability

Water vapor permeability refers to the ability of water vapor to pass into clothing. Permeability of water vapor is desirable; otherwise humidity is trapped between skin and cloth. When humidity is trapped on the skin, heat accumulates in the body. Heat and humidity impaction causes discomfort. Moisture vapor transfer refers to a fabric's ability

to transfer perspiration and thereby enhance comfort. When moisture vapor does not pass freely through the fabric, this leads to sweat accumulation. As a result, the heat is unable to dissipate, causing displeasure condition. Lower fabric mass per square meter and thickness values, which allow for easy water vapor passage through the fabrics, are thus preferred. The rate of water vapor permeability of the treated fabric was increased with concentration of *Aloe vera*, chitosan, and sericin treatment.

Table 3.16: Water vapor permeability of *Aloe vera*, chitosan and sericin treated fabric

Concentration	Water vapor permeability of *Aloe vera* treated (gm./m^2/day)	Water vapor permeability of chitosan treated (gm./m^2/day)	Water vapor permeability of sericin treated (gm./m^2/day)
2 g/l	883	885	887
4 g/l	886	888	891
6 g/l	889	893	894
8 g/l	891	896	897
10 g/l	894	898	899
*Water vapor permeability of untreated fabric was 910 gm./m2/day *Water vapor permeability of critic acid treated fabric was 875 gm./m2/day			

Among the treated samples, chitosan and sericin-treated samples showed little bit better water vapor permeability compared to that the *Aloe vera* treated fabric as shown in **Table 3.16**. The key cause for this distinction is that cloth treated with chitosan or sericin absorbs water vapor easily and then evaporates quickly.

3.2.19 Air permeability

The rate of air flow through the cloth is measured by air permeability. The air permeability of the fabrics is affected by the finishing treatment. The thickness of the cloth was improved by increasing the concentration of antimicrobial agents added to the fabric from 2g/1 to 10 g/l. For this reason, the air permeability was reduced slightly. *Aloe vera*, chitosan, and sericin treated fabrics had lower air permeability than untreated fabrics, according to the test results reported in **Table 3.17.** *Aloe vera*, chitosan and sericin treatments filled the pores of the fabrics and hence led to a decrease in the air permeability.

Table 3.17: Air permeability of *Aloe vera*, chitosan and sericin treated fabric

Concentration	Air permeability of *Aloe vera* treated fabric ($cm^3/cm^2/sec$)	Air permeability of chitosan treated fabric ($cm^3/cm^2/sec$)	Air permeability of sericin treated fabric ($cm^3/cm^2/sec$)
2 g/l	126	120	123
4 g/l	124	117	122
6 g/l	121	115	120
8 g/l	118	111	117
10 g/l	115	108	113
Air permeability of untreated fabric was 130 $cm^3/cm^2/sec$			

The rising concentration of antimicrobial agent in solution is thought to be a contributing factor to the reduced air permeability of cotton fabrics. In comparison to *Aloe vera* and sericin-treated cloth, the coating layer thickness of chitosan-treated fabric was greater. As a result, chitosan-treated fabric has a lower air permeability than *Aloe vera*-and sericin-treated fabrics as shown in **Table 3.17**. Discomfort occurs as the volume of air permeability reduces.

3.2.20 Statistical Analysis

Table 3.18: Correlation coefficient (C. C) of fabric thickness (F. T) verses thermal conductivity (T. C), Air permeability (A. P) and Water vapour permeability (W.V.P)

Observation	C. C (r) for *Aloe vera* treated sample	C. C (r) for chitosan treated	C. C (r) for sericin treated sample
F. T verses T.C	-1	-0.98	-0.99
F. T verses A. P	-0.97	-0.99	-0.98
F. T verses W.V.P	1.0	0.97	0.96
F. A verses W.V.P	0.98	0.98	0.99

(**For details see the appendix: 8-11**); **#Fabric absorbency (F. A)**

For *Aloe vera*, chitosan, and sericin treated fabrics, the correlation coefficient values of fabric thickness verses thermal conductivity and fabric thickness verses air permeability

show a complete negative correlation in **Table 3.18**. These findings indicate that thermal conductivity and air permeability values decrease when the fabric thickness increases On the other hand, perfect positive correlation was found fabric thickness verses water vapor. Finally, **Table 3.18** shows a positive correlation between fabric absorbency and water vapor permeability, for *Aloe vera*, chitosan and sericin treated fabrics. Fabric absorbency increases, water vapor permeability also increases.

Table 3.19: Chi-Square (χ^2) hypothesis test observation in terms of Air permeability value of treated fabric (Degree of freedom: (3-1) = 2; Level of significance; 5%)

Observation	Calculated value of *Aloe vera* treated	Calculated value of chitosan treated	Calculated value of sericin treated	Tabulated value	Comments
6 g/l	2.06	2.32	5.20	5.99	N. H test is accepted
8 g/l	3.34	3.91	8.34		N. H test is accepted for A. vera and chitoan but rejected for sericin treated fabric
10 g/l	5.20	6.68	11.184		N. H test is accepted for A. vera but rejected for chitosan and sericin treated fabric

 # (For details see the appendix-12); # Null hypothesis (N. H)

For hypothesis test, 6, 8, and 10 g/l *Aloe vera*, chitosan and sericin treated fabrics were taken. Since lower concentration finishing agents did not affect the thermal comfort of treated fabric. Hypothesis test result revealed that null hypothesis test is accepted for 6 g/l *Aloe vera*, chitosan and sericin treated fabric as seen in **Table 19**. This results indicates that there was no significant difference between treated fabric and untreated fabrics in terms of air permeability only for 6 g/l concentration. Besides, 8 g/l *Aloe vera*

and chitosan treated fabrics were accepted but 8 g/l sericin treated fabric was rejcted. Finally, null hypothesis test of 10 g/l *Aloe vera* treated fabric was accepted but 10 g/l chitosan and sericin treated fabrics were rejected.

Table 3.20: Chi-Square (χ^2) hypothesis test observation for thermal conductivity value of treated fabric (Degree of freedom: (3-1) = 2; Level of significance; 5%)

Observation	Calculated value of *Aloe vera* treated	Calculated value of chitosan treated	Calculated value of sericin treated	Tabulated value	Comments
6 g/l	1.228	2.523	3.088	5.99	N. H test is accepted
8 g/l	2.55	3.523	3.838		N. H test is accepted
10 g/l	3.32	3.880	4.314		N. H test is accepted

 # (For details see the appendix-13)

Table 3.20 revealed that null hypothesis test was accepted for 6, 8, and 10 g/l *Aloe vera*, chitosan and sericin treated fabrics in terms of thermal conductivity**.** This findings indicate that there was no significant difference between treated fabric and untreated fabrics in terms of thermal conductivity.

Table 3.21: Chi-Square (χ^2) hypothesis test observation for water vapor permeability value of treated fabric (Degree of freedom: (3-1) = 2; Level of significance; 5%)

Observation	Calculated value of *Aloe vera* treated	Calculated value of chitosan treated	Calculated value of sericin treated	Tabulated value	Comments
6 g/l	1.456	0.95	0.85	5.99	N. H test is accepted
8 g/l	1.192	0.64	0.56		N. H test is accepted
10 g/l	0.84	0.48	0.40		N. H test is accepted

 # (For details see the appendix-14)

In terms of water vapour permeability, null hypothesis tests were accepted for 6, 8, and 10g/l *Aloe vera*, chitosan, and sericin treated fabrics (**Table 3.21**). According to these data, there was no significant change in water vapour permeability between treated and untreated fabrics.

It is clearly seen from the **Table 3.19, 3.20, 3.21** that all the calculated values are lower than the tabulated values at a 5% level of significance except 8 g/l chitosan and 10 g/l chitosan and sericin treated fabrics for air permeability. The results indicate that there is no significant difference between treated fabric's and untreated fabric's thermal comfort properties. The three terms: thermal conductivity: air permeability; and water vapour permeability; define thermal comfort. Therefore, the comfort of bleached cotton woven fabric was not influenced by *Aloe vera*, chitosan and sericin treatments.

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Chapter Four
CONCLUSION

Natural compounds' biocompatibility and biodegradability encouraged researchers to use them in the pursuit of a safe world, and hence for the development of environmentally sustainable textiles. The current research describes a more environmentally friendly method of developing bioactive textiles by combining *Aloe vera*, chitosan, and silk sericin. The pad-dry-cure method was used to successfully attach *Aloe vera*, chitosan, and sericin to cotton woven fabric. In the presence of chitosan and sericin, the water absorbency of treated fabric was shown to be better than that of fabrics in the presence of *Aloe vera*. The most responsible factors for thermal comfort in wearing textiles are air permeability, water vapor permeability, and thermal conductivity. From the experimental results, the following conclusions may be drawn.

a) *Aloe vera*, chitosan and sericin showed UV ray absorption capacity. Among the specimen *Aloe vera* solution absorbed three region of UV radiation. On the other hand, *Aloe vera*, chitosan, and sericin treated fabric also showed UV radiation resistance property which may be protected the human body from skin related diseases like sunburn, premature skin ageing, allergies and skin cancer.

b) Important functional groups of *Aloe vera*, chitosan and sericin were identified from different characteristics peaks of FTIR. Additionally, the attachment of *Aloe vera*, chitosan and sericin on cotton woven fabric was confirmed by FTIR spectroscopy.

c) Crystallite diameters of the *Aloe vera*, chitosan and sericin have determined from XRD pattern and data. Besides, X-ray diffraction characteristic peaks of cotton, *Aloe vera*, chitosan and sericin treated cotton are almost similar, which proved that the cotton fibre cellulose did not produce any remarkable changes after *Aloe vera*, chitosan and sericin incorporation.

d) Quantity of carbon, nitrogen and hydrogen of *Aloe vera*, chitosan and sericin were found from EDS data.

e) Weight loss percentage of *Aloe vera*, chitosan and sericin was evaluated from TGA curve and decomposition temperature was identified by DTG graph. Glass transition temperature and exothermic peak temperature were determined by DSC graph.

f) *Aloe vera*, chitosan, and sericin exhibited antimicrobial activity. Among samples chitosan showed excellent antimicrobial activity. Moreover, Antimicrobial activity of treated fabric has been proven by established tests, which shows the possibility of application of the product in personal care and wound dressings.

g) *Aloe vera*, chitosan and sericin illustrate antioxidant activity. *Aloe vera* displayed admirable antioxidant activity. The free radical scavenging activity of *Aloe vera* chitosan and sericin treated fabric was revealed effectively. It is expected that antioxidant finished clothing to minimize or stabilize the free radicals of the outer side of the body. In this way, natural antioxidant inhibits to develop chronic diseases in our body.

h) Finally agglomerated granular are clearly visible on the treated fabric in SEM image, which offers the evidence of *Aloe vera*, chitosan and sericin were successfully attached on the untreated fabric.

i) Statistical analysis confirmed that there is no significant difference between treated fabric's and untreated fabric’s thermal comfort properties. Generally, thrmal comfort is decreased after finishing treatment. Moreover water vapor permeability was increased in modifified fabric which indicates improve thermal comfort.

j) Finally, it is concluded that *Aloe vera*, chitosan, and sericin modified fabrics are mostly biodegradable which ensured the ecofriendly property and can be used as protective textile materials.

4.1 Future trend

Antimicrobial textiles for biological protection are needed for many applications, including medical textiles, sportswear, hygiene products and military uniforms. Sustainable antimicrobial technology is a new direction for future advancement with the understanding that antimicrobial textiles should be safe for humans and the atmosphere, cost efficient, need less energy to manufacture, and be sustainable and recyclable. Antibacterial technologies should be employed in medical textiles like surgical gowns, drapes in such a way that to meet the requirement of sterilization. Overall, the development of perfect biocidal textiles is still a challenge to textile scientist.

Appendices

Appendix-1
Table: Radical scavenging activity (RSA) of specimen treated fabric

Concentration	RSA % of *Aloe vera* treated	RSA % of Chitosan treated	RSA % of Sericin treated
2 mg/ml	56	30	27
4 mg/ml	61	33	33
6 mg/ml	67	38	38
8 mg/ml	80	42	42
10 mg/ml	85	46	47

Appendix-2
Table: Ultra violet protection factor (UPF) of treated and untreated fabrics

Observation →	Untreated	5 g/l A. vera	10 g/l A. vera	5 g/l chitosan	10 g/l chitosan	5 g/l Sericin	10 g/l Sericin
UPF Rating	0.25	2.50	3.75	1.30	1.90	1.45	2.50

Appendix-3
Table: Whiteness index (WI) of treated and untreated fabric

Concentration	WI of *Aloe vera* treated	WI of Chitosan treated fabric	WI of Sericin treated fabric
2 g/l	51	59	42
4 g/l	46	55	38
6 g/l	45	52	35
8 g/l	40	49	33
10 g/l	36	47	28
#WI of untreated fabric 65			

Appendix-4
Table: Water absorbency heaight of treated and untreated fabrics

Concentration	Absorbency height (cm) of *Aloe vera* treated fabric	Absorbency height (cm) of chitosan treated fabric	Absorbency height (cm) of sericin treated fabric
2 g/l	3.3	3.4	3.5
4 g/l	3.4	3.6	3.8
6 g/l	3.6	3.8	4.0
8 g/l	3.8	4.1	4.2
10 g/l	3.9	4.3	4.5
# Absorbency height of untreated fabric was 4.8 cm # Absorbency height of critic acid treated fabric was 3.1 cm			

Apendix-5
Table: Tensile strength of Aloe vera, chitosan and sericin treated fabrics

Concentration	Tensile strength (N) of *Aloe vera* treated fabric	Tensile strength (N) of chitosan treated fabric	Tensile strength (N) of sericin treated fabric
2 g/l	890	820	840
4 g/l	868	795	810
6 g/l	835	750	775
8 g/l	818	737	760
10 g/l	797	726	742
Tensile strength of untreated fabric was 1148 N			

Appendix- 6
Table: Bending length and Flexural rigidity of treated nd untreated fabric

.Concentration	B. L. (cm) of A. vera treated	F. R of A. vera treated (mg-cm)	B. L. (cm) of chitosan treated	F. R of chitosan treated (mg-cm)	B. L. (cm) of sericin treated	F. R of sericin (mg-cm)
2 g/l	1.6	50.79	1.7	61.41	1.9	86.42
4 g/l	2.2	134.16	2.4	175.56	2.7	253.91
6 g/l	2.5	201.56	2.8	283.18	3.0	356.40
8 g/l	2.9	317.05	3.1	393.24	3.3	481.55
10 g/l	3.2	429.26	3.6	629.85	3.7	688.88
Bending length and flexural rigidity of untreated fabric are 1.3 cm and 26.80 mg-cm						

Appendix- 7
Table: Crease recovery of treated nd untreated fabric

Concentration	Crease recovery angle (º) of *Aloe vera* treated	Crease recovery angle (º) of chitosan treated	Crease recovery angle (º) of sericin treated
2 g/l	122	126	125
4 g/l	125	130	127
6 g/l	129	134	131
8 g/l	131	137	133
10 g/l	135	139	136
# Crease recovery angle of untreated sample is 107º			

Appendix- 8
Table: Correlation coefficient between thermal conductivity (T.C) and thickness (T) of Aloe vera, chitosan and sericin treated fabric

Concentration	T.C of *Aloe vera* treated (W/mk)×10^{-3}	T. (cm) of *Aloe vera* treated	T.C of chitosan treated fabric (W/mk)×10^{-3}	T.(cm) of Chitosan treated	T.C of sericin treated fabric (W/mk)×10^{-3}	T.(cm) of Sericin treated
2 g/l	5.428	0.022	4.361	0.024	3.990	0.025
4 g/l	4.551	0.023	4.121	0.026	3.799	0.027
6 g/l	4.384	0.024	3.867	0.028	3.624	0.029
8 g/l	3.914	0.025	3.453	0.030	3.336	0.031
10 g/l	3.526	0.026	3.231	0.033	3.167	0.035
Correlation	**-0.97**		**-0.99**		**-0.98**	

Appendix- 9
Table: Correlation coefficient between air permeability (A.P) and thickness (T) of Aloe vera, chitosan and sericin treated fabric

Concentration	**A.P** of *Aloe vera* treated $cm^3/cm^2/sec$	T. (cm) of *Aloe vera* treated	A.P of chitosan treated fabric $cm^3/cm^2/sec$	T.(cm) of Chitosan treated	A.P of sericin treated fabric $cm^3/cm^2/sec$	T.(cm) of Sericin treated
2 g/l	126	0.022	123	0.024	120	0.025
4 g/l	124	0.023	122	0.026	117	0.027
6 g/l	121	0.024	120	0.028	115	0.029
8 g/l	118	0.025	117	0.030	111	0.031
10 g/l	115	0.026	113	0.033	108	0.035
Correlation	**-1**		**-0.98**		**-0.99**	

Appendix- 10

Table: Correlation coefficient between water vapor permeability (WVP) and thickness (T) of Aloe vera, chitosan and sericin treated fabric

Concentration	**WVP** of *Aloe vera* treated ($g/m^2/day$)	T. (cm) of *Aloe vera* treated	**WVP** of chitosan treted ($g/m^2/day$)	T.(cm) of Chitosan treated	**WVP** of sericin treated ($g/m^2/day$)	T.(cm) of Sericin treated
2 g/l	883	0.022	885	0.024	887	0.025
4 g/l	886	0.023	888	0.026	891	0.027
6 g/l	889	0.024	893	0.028	894	0.029
8 g/l	891	0.025	896	0.030	897	0.031
10 g/l	894	0.026	898	0.033	899	0.035
Correlation	**1**		**0.97**		**0.96**	

Appendix- 11

Table: Correlation coefficient between water vapor permeability (WVP) and waer absorbency height (WAH) of Aloe vera, chitosan and sericin treated fabric

Concentration	**WVP** of *Aloe vera* treated ($g/m^2/day$)	WAH (cm) of *Aloe vera* treated	**WVP** of chitosan treted ($g/m^2/day$)	WAH (cm) of Chitosan treated	**WVP** of sericin treated ($g/m^2/day$)	WAH(cm) of Sericin treated
2 g/l	883	3.3	885	3.4	887	3.5
4 g/l	886	3.4	888	3.6	891	3.8
6 g/l	889	3.6	893	3.8	894	4.0
8 g/l	891	3.8	896	4.1	897	4.2
10 g/l	894	3.9	898	4.3	899	4.5
Correlation	**1**		**0.97**		**0.96**	

Appendix-12

Table: Chi-Square ($\chi 2$) hypothesis test observation in terms of air permeability value of treated fabrics

Air permeability of *Aloe vera* treated fabrics					
Observations	(f_o) of 6 g/l *Aloe vera* treated fabric	(fo) of 8 g/l *Aloe vera* treated fabric	(fo) of 10 g/l *Aloe vera* treated fabric	Expected frequency (f_e)	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	121	118	115	130	2.06 for 6 g/l
2	123	117	116	130	3.34 for 8 g/l
3	119	119	114	130	5.20 for 10 g/l
Air permeability of Chitosan treated fabrics					
Observations	'f_o' of 6 g/l chitosan treated fabrics	'f_o' of 8 g/l chitosan treated fabrics	'f_o' of 10 g/l chitosan treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	120	117	113	130	2.32 for 6 g/l
2	119	118	114	130	3.91 for 8 g/l
3	121	116	112	130	6.68 for 10 g/l
Air permeability of Sericin treated fabrics					
Observations	'f_o' of 6 g/l sericin treated fabrics	'f_o' of 8 g/l sericin treated fabrics	'f_o' of 10 g/l sericin treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	115	111	108	130	5.20 for 6 g/l
2	116	112	109	130	8.34 for 8 g/l
3	114	110	107	130	11.18 for 10 g/l

 # Observed frequency (f_o) means *Aloe vera*, chitosan and sericin treated fabric's observations; # expected frequency (f_e) means untreated fabric's observation

Appendix-13

Table: Chi-Square ($\chi 2$) hypothesis test observation in terms of thermal conductivity value of treated fabrics

Thermal conductivity of *Aloe vera* treated fabrics					
Observations	(f_o) of 6 g/l *Aloe vera* treated fabric	(fo) of 8 g/l *Aloe vera* treated fabric	(fo) of 10 g/l *Aloe vera* treated fabric	Expected frequency (f_e)	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	4.384	3.914	3.526	6.138	1.228 for 6 g/l
2	4.369	3.902	3.506	6.138	2.55 for 8 g/l
3	4.98	3.925	3.544	6.138	3.32 for 10 g/l
Thermal conductivity of Chitosan treated fabrics					
Observations	'f_o' of 6 g/l chitosan treated fabrics	'f_o' of 8 g/l chitosan treated fabrics	'f_o' of 10 g/l chitosan treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	3.867	3.453	3.231	6.138	2.523 for 6 g/l
2	3.836	3.432	3.211	6.138	3.523 for 8 g/l
3	3.894	3.474	3.53	6.138	3.880 for 10 g/l
Thermal conductivity of Sericin treated fabrics					
Observations	'f_o' of 6 g/l sericin treated fabrics	'f_o' of 8 g/l sericin treated fabrics	'f_o' of 10 g/l sericin treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	3.624	3.336	3.167	6.138	3.088 for 6 g/l
2	3.608	3.317	3.148	6.138	3.838 for 8 g/l
3	3.641	3.352	3.186	6.138	4.314 for 10 g/l

Appendix-14

Table: Chi-Square ($\chi 2$) hypothesis test observation in terms of water vapour permeability value of treated fabrics

Water vapour permeability of *Aloe vera* treated fabrics					
Observations	(f_o) of 6 g/l *Aloe vera* treated fabric	(fo) of 8 g/l *Aloe vera* treated fabric	(fo) of 10 g/l *Aloe vera* treated fabric	Expected frequency (f_e)	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	890	891	894	910	1.456 for 6 g/l
2	889	890	893	910	1.192 for 8 g/l
3	888	892	895	910	0.84 for 10 g/l
Water vapour permeability of Chitosan treated fabrics					
Observations	'f_o' of 6 g/l chitosan treated fabrics	'f_o' of 8 g/l chitosan treated fabrics	'f_o' of 10 g/l chitosan treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	893	896	896	910	0.95 for 6 g/l
2	892	895	895	910	0.64 for 8 g/l
3	894	897	897	910	0.48 for 10 g/l
Water vapour permeability of Sericin treated fabrics					
Observations	'f_o' of 6 g/l sericin treated fabrics	'f_o' of 8 g/l sericin treated fabrics	'f_o' of 10 g/l sericin treated fabrics	Expected frequency ($f_{e)}$	$\frac{\sum(f_o - f_e)^2}{f_e}$
1	894	897	899	910	0.85 for 6 g/l
2	893	896	898	910	0.56 for 8 g/l
3	895	898	900	910	0.40 for 10 g/l

List of Publications

1. Md. Ibrahim H. Mondal, and **Joykrisna Saha (2019).** Antimicrobial Activity, UV Resistant and Thermal Comfort Properties of Chitosan and *Aloe vera*-Modified Cotton Woven Fabric. **Journal of Polymers and the Environment, 27(2), 405–420.**

2. **Joykrisna Saha**, Md. Ibrahim H. Mondal*, Md. Rezaul Karim Sheikh and Md. Ahsan Habib, **(2019)**. Extraction, Structural and Functional Properties of Silk Sericin Biopolymer from Bombyx mori Silk Cocoon Waste**. Journal of Textile Science & Engineering**, **9 (1), 1-5**.

3. Md. Ibrahim H. Mondal, **Joykrisna Saha** and Md. Ashadur Rahman **(2021).** Functional Applications of *Aloe vera* on Textiles: A Review**, Journal of Polymers and the Environment, 29, 993-1009.**

4. **Joykrisna Saha** and Md. Ibrahim H. Mondal (2021). Antimicrobial Textiles from Natural Resources-Types, Properties and Processing, In *Antimicrobial Textiles from Natural Resources,* **(pp. 1-43), Woodhead Publishing**.

5. **Joykrisna Saha**, and Md. Ahsan Habib and Md. Ibrahim H. Mondal **(2021).** Investigation of Antibacterial, Antioxidant, UV Resistance Properties of Chitosan, *Aloe vera,* and Silk sericin. **Journal of textile science & Fashion Technology**, **8(3), 1-5.**

Papers Presented in National/ International Conferences

1. "Antimicrobial Activity and Other Properties of Bleached Cotton Woven Fabric Developed by Using Harbal *Aloe vera* Extract and Chitosan" **Joykrisna Saha** and Md. Ibrahim H. Mondal, 1st International Conference on Engineering Materials and Metallurgical Engineering, ICEMME-2016, PP&PDC, BCSIR, Dhaka, Bangladesh 22-24, December, 2016 (**Poster presentation-26**)

2. "Study on Antimicrobial Activity and Thermal Comfort Properties of Chitosan and *Aloe vera* Modified Cotton Woven Fabric" **Joykrisna Saha**, Md. Ibrahim H. Mondal, Conference on Material Science And Nano-electrochemistry, CMSN 2017, Dept. of Chemistry, Rajshahi University, Bangladesh, 8-9 April, 2017 (**Oral Presentation-19)**

3. "Cotton Fabric Modified With Silk Sericin To Improve Textile Performance For Medical Application" **Joykrisna Saha**, Rezaul Karim Sheikh and Md. Ibrahim H. mondal, 39th Annual Conference of Bangladesh Chemical Society, 17-19, October, 2018 **(Poster Presentation-183**)

4. "Structural and Functional Properties of Silk Sericin From *Bombyx Mori* Silk Cocoon Waste Biopolymer" **Joykrisna Saha** and Md. Ibrahim H. Mondal, 1st National conference on Sustainable Textile and Apparel Engineering, Mawlana Bashani Science and Technology University, 23Feb. 2019, (**Oral Presentation ID: OP-03)**

5. "Investigation of Antibacterial, Antioxidant and UV Resistance Properties of Chitosan, *Aloe vera* and Silk Sericin" **Joykrisna Saha** and Md. Ibrahim H. Mondal, IC^4ME^2-2019, Faculty of Engineering, Rajshahi University, **(Poster Presentation-205).**

6. "Influence of Degree of Deacetylation on Physical, Structural and Functional Properties of Chitosan" **Joykrisna Saha** and Md. Ibrahim H. Mondal, Bangladesh Chemical Society Conference, **9 -10** November, 2019, (**Oral presentation: PN-OP-06).**

7. "*Aloe vera* Gel in Wound Healing, Medical Textiles and Personal Care Products" Md. Ibrahim H. Mondal, **Joykrisna Saha** and Md. Ashadur Rahman, Bangladesh Chemical Society Conference, 9-10 November, 2019, (**Oral presentation: NP-OP-10)**.

8. Cotton fabric modified with silk sericin and chitosan to improve textile performance for medical applications, **Joykrisna Saha**, Md. Ibrahim H. Mondal and Md. Rezaul Karim Sheikh, **BCSIR Congress**, 12-14, December, 2019, **Oral presentation:1B (5).**

9. Modification of cotton fabric with *Aloe vera* and silk sericin to develop antibacterial and antioxidant performance for personal care products, **Joykrisna Saha**, Md. Ibrahim H. Mondal, International Conference on Science and Technology for Celebrating the Birth Centenary of Bangabandhu (**ICSTB-2021**), 11-13, March, Oral **presentation** (OP-A22