SYNOPSIS

“Development of Nano-composite Textiles for Bio-medical Application”

The thesis entitled **“Development of nano-composite textiles for bio-medical application.”** comprises of six chapters briefly summarized here.

1.0 INTRODUCTION:

Chemically synthesized silver nano has been given preference amongst antibacterial components used for producing non-woven based nano composites. These composites are widely used as bio medical textile material for products like bandages, gloves, gowns, aprons etc. Silver nano particles (AgNPs) have reported premier effectiveness in this area and its long term stability in the nano scale was attributed to reducing and capping chemical reagents like; Sodium Borohydride. But looking at the application condition, where textile silver nano composite is going to make a direct contact with human body use of natural eco-friendly reducing and capping agents, like; extracts of tulsi or neem or aloe-vera etc. are analysed and preferred for the purpose being. Milkweed (Calotropis) leaves are well proven from its wide span use made in rural areas for treating burns, fungal attack and wound healing mainly. They have inherently inbuilt high level of antibacterial potential, still remained unexplored on statistical ground. Thereby a novel silver nano composite was engineered in the present study by using medicinally proven antibacterial constituent milkweed extract in association with silver nitrate ($AgNO_3$) for medical application. Antibacterial potential of milkweed leaves extract without AgNPs in non-woven composites was also worked out parallel along with their various physical, comfort related, morphological and elemental assessment.

2.0 LITERATURE REVIEW:

2.1 Biomedical Textiles and Its Applications

Biomedical is the term related to the technology and engineering design employed for medical equipments used by living beings. Whereas term “Biomedical Textile”, represents textile products and structures developed for medical and biological applications. Such products have been found very useful in first aid;

clinical as well as hygienic purpose [1-5]. Hygiene behaviour of the material used for the medicinal purpose is of prime importance. Many researches have been done on hygienic activities, especially antibacterial activities of the medicinal fabrics used in the products coming in direct contact with the human skin like; gloves, mask, caps, etc. [6, 7]. Bifurcation of Bio-medical textiles as per application areas can be briefly summarized as follow (Graphical representation shown in figure 1).

a) Protective and healthcare textiles: Surgeons wear, Operating drapes and Staff uniforms, etc.

b) External devices: Wound dressings, Bandages, Pressure garments, Prosthetic socks, etc.

c) Implantable materials: Sutures, Vascular grafts, Artificial ligaments, etc.

d) Hygiene products: Incontinence pads, Nappies, Tampons, Sanitary towels, etc.

e) Extracorporeal devices: Artificial liver, Artificial kidney, Artificial lung, etc.

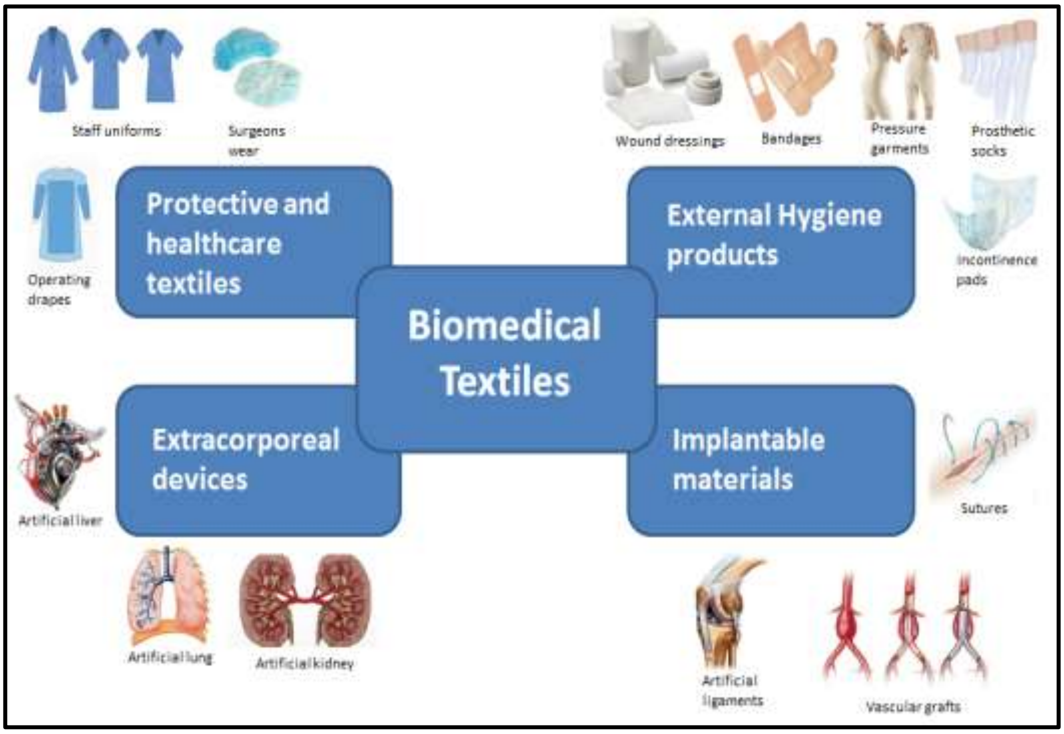

Figure 2.1: Application areas of biomedical textiles

Structure of a biomedical textile is driven by the need/s of its end use application. The main criterions include:

1) Function: The textile needs to fulfil the purpose for which it has been designed, e.g.; swabs require use of an absorbent textile, a biodegradable textile for sutures and hospital bed sheets should be made not only durable but comfortable to the patient as well.

2) Biocompatibility: This refers to the reaction of the textile material with blood and tissues in the body; especially when they make direct contact. Implantable devices should have more potential for reaction than external devices and therefore, usually subjected to tighter regulations. For example an artificial ligament is set permanently in the human body and thereby it should be able to react with blood cells and the surrounding tissue. Conversely an external bandage is deployed temporarily and contacts the outer skin tissue only.

3) Cost: This factor depends mainly on resultant of; the raw materials cost, manufacturing process cost and product shelf life demanded. Just for an example; surgeon’s gowns and swabs usually discarded after single use, should have a low production cost while vascular grafts and artificial skin last long in human body, should allowed to undergo a relatively higher production cost.

4) Product approval: Medical products/tools/accessories need to undergo medico legal approval before putting into use. Each country has its own regulations and standards to be followed for medical textiles. However the European Union has introduced Community Legislation to govern medical devices.

2.2 Synthesis of nano-particles

Nanoparticle synthesis refers to ways and means used for creating nanoparticles. Nanoparticles can be derived from larger particles, or synthesized by different techniques, for example, nucleate and develop particles from fine molecular distributions in liquid or vapour phase. Synthesis can also comprise functionalization by conjugation to bioactive molecules like silver, copper, zinc etc. Such biologically active metals, known as bio metals are known to human beings since long back in the form of “grandma recipe”. Salts, complexes, colloid particles, “silver” water were commonly used house hold remedies for antimicrobial, bactericidal in many dieses [8-10].

2.2.1 Chemical and Physical synthesis of nano-particles

Modern scientists offer an innovative complex antibacterial and antiviral nano systems on the basis of metal oxides or intermetallic oxide compounds, such as TiO_2, SnO_2, ZnO_2 and SiO_2 etc. These compounds are functionalized by organic or organometallic molecular structures capable to connect ions of the transition bio metals, such as Ag+ and Cu++. Such systems can be used for manufacturing of medicinal and non-medicinal means, dermatological compounds and creams, for bactericidal modification of surfaces and coatings in living quarters, industrial and specialized premises [6, 8, 10, 11]. Chemically synthesized antibacterial agent; silver nano ($AgNO_3$) colloidal has consistently found preference for imparting desirable hygienic activities over natural reagents due to its proactive performance [12, 13].

2.2.2 Green synthesis of nano-particles

Although chemical and physical methods are effective, they come with various inadequacies. The chemical and physical methods require high energy resources and generate toxic by-products and hazardous wastes. Recent researches have indicated presence of toxic chemicals with the chemically synthesized nanoparticles making them relatively unfavourable for biomedical applications. Issues associated with energy balance and toxic by-products, have been overcome by switching over to complete green techniques or biological synthesis of nano-particles. Many biological resources like plants, microorganisms, alga, and yeasts are used for the purpose being [6, 10, 14-17]. Biosynthesis is beneficial over other techniques because of its low cost, rapidity, solitary synthesis, high yield, and biocompatibility. Moreover, the size can be controlled simply by regulating the concentrations, pH, and temperature [9, 10]. In the present scenario the abundant availability, low cost and a rich source of bio-reducing agents; plants and plant products have found priority over the other options in biosynthesis of nanoparticles.

2.3 Non-woven in bio-medical application

Non-woven based bio-medical textiles have become the material of choice for many tissue engineering and regenerative medicine applications. This is mainly due to their superior surface area, high void volume, excellent permeability, and low production cost. Wide application ranges starting from orthopaedic restoration and wound management to cosmetic surgery have been covered by them [5, 18]. Most notably, the ability to encourage cellular in-growth through specific spacing, layer thickness, and material integrity allows for customized performance and controlled absorption profiles [3, 4, 18].

Current non-woven application areas include:

- Surgical mask
- Surgical gowns
- Surgical drapes
- Surgical gloves
- Bed sheets in hospitals
- Scaffolds for tissue engineering
- Scaffolds for stem cell applications
- Dental and cosmetic applications
- Absorbable additives for cements and hydrogels
- High surface area material for trauma applications
- Sensors, Markers, Washers, Spacers, Cuffs etc.

3.0 RESEARCH GAP AND OBJECTIVE OF THE STUDY:

The milkweed (Calotropis) is one of the traditional therapist's plants because of its potential antibacterial and toxicological activity [19, 20]. It possesses cardenolides, triterpenes, flavonoids, sterols, saponins, diterpenes, resins, tannins, alkaloids and steroids [19, 21]. These phytochemicals presents in the plants extracts can act as reducing as well as capping agents for the formation of nano particles and also contribute in antibacterial activities [9, 22, 23]. It is widely studied in the field of medicine for its numerous pharmacological properties especially rich phytochemical contents and testified successfully for various drugs

[22]. However, these specialities have been yet not utilized in bio-medical textiles. Thereby present research work was carried out to fill this research gap.

This work aimed to develop non-woven based silver nano-composite textile materials for the bio-medical applications enriched with antibacterial component of milkweed leaves extract used during green synthesis as reducing and capping agent.

Accordingly primary purpose of this work was to synthesize the silver nano particles through the green synthesis technique by using the milkweed leaves extract as reducing and capping agent. Selection of silver in the study was mainly attributed to its well-documented antimicrobial status capable of killing bacteria, fungi and certain viruses [24]. The effect of varying concentration of silver and milkweed leaves extract on antibacterial activities of the resulting nano-composite textile materials were investigated. An antibacterial activity efficacy of self-sufficient antibacterial milkweed extract/nonwoven composite was also worked out at its optimum concentration level in the absence of silver nano particles. Engineered composite was verified for its non-toxicity to human body on handle or direct body contact.

4.0 Experimental:

The entire experiment was conducted on the laboratory set up. Required non-woven materials were procured from the reputed medical textile industries. Fresh milkweed leaves were collected and extract of desired concentrations were prepared at the laboratory end.

An entire research work is divided into four main sections & schematically given in figure 4.1. Their objectives are summarized briefly in table 4.1.

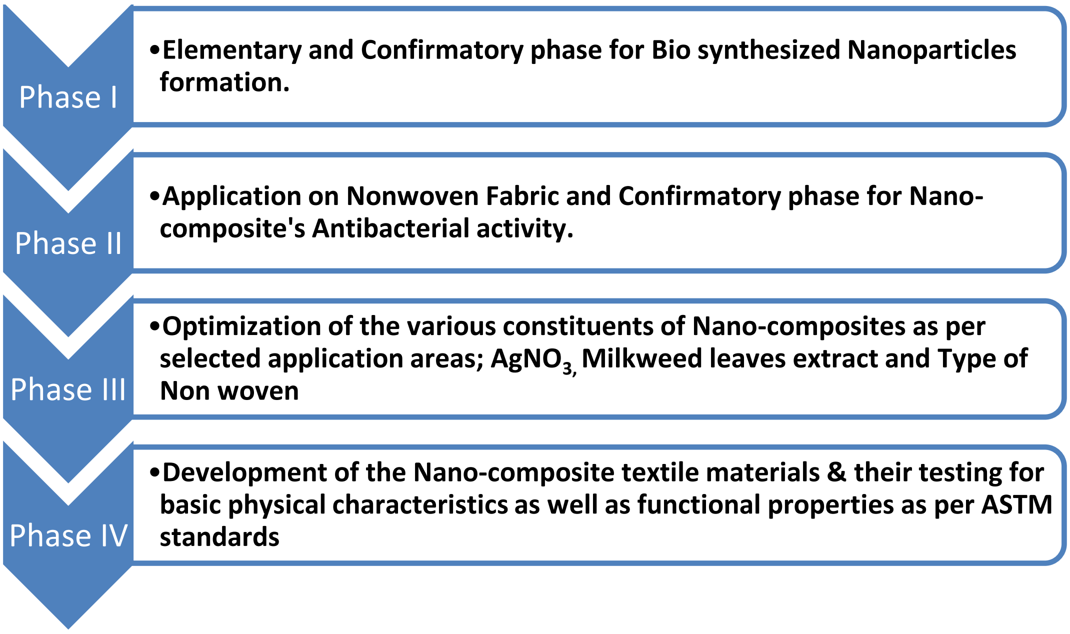

Figure 4.1: Plan of Experimental Work

Table 4.1: Objectives of Research design in different phases of Experiment

Stages	Objective/s
Phase I	**Elementary and Confirmatory phase for Bio synthesized Nanoparticles formation** This phase dealt with the preparation of the silver nanoparticles by chemical and proposed green synthesis techniques. The experiments involved in this phase were carried out for the foundry evaluation of the synthesized nanoparticles with new Milkweed as reducing and capping agent thereby nomenclated as “Elementary and Confirmatory phase for Nanoparticles formation”. This phase of experiments were divided into two sub groups; Part-I and Part-II. **Part-I** It concise about the preparation of the silver nanoparticles by customary chemical synthesis technique.

This part of experiments has been designed with fundamental empathetic of the chemical synthesis method of the silver nitrate salt ($AgNO_3$). In this Elementary study chemical synthesis analysis of the silver nitrate salt was done by three techniques, viz; i)Forward synthesis method (Top down approach), ii) Reverse synthesis method (Bottom up approach) and iii) Reduction method (By tri-sodium citrate).

Part-II It précised about the preparation of the silver nanoparticles by green synthesis technique. This set of experiments was designed for the confirmation of the nanoparticles formation via green synthesis route from the silver nitrate salt, thereby designated as "Confirmatory part for nanoparticles formation". In this part, green synthesis of the silver nitrate salt was done with Milkweed leaves extract but without using any hazardous chemicals by Forward synthesis method (Top down approach) and Reverse synthesis method (Bottom up approach).

The prepared silver nanoparticles in both the series of pilot trials were subjected for preliminarily Nanoparticle analysis; size, stability etc. using standard evaluation techniques for comparative analysis apart from confirmation for nano particle formation.

Phase II **Application on Nonwoven fabric and Confirmatory phase for Nano-composite's Antibacterial activity**

This phase dealt with utilisation of green synthesized silver nanoparticles in the preparation of the Polyester-Viscose nonwoven based nano-composite textile materials. The prepared nano-composite textile materials were then evaluated for their efficacy in antibacterial activities. The prepared nano-composite textile material were also analysed in terms of changes in their structural, physical and mechanical properties on reinforcing nanoparticles.

Phase III **Optimization of the various constituents of Nano-composites as per selected application areas; $AgNO_3$, Milkweed leaves extract and Type of Nonwoven**

This phase has dealt with optimization of the silver nitrate ($AgNO_3$) salt and Milkweed leaves extract in the synthesis of desirable AgNPs. Pilot trials were conducted by varying concentration of both the constituents; concentration of the $AgNO_3$ salt was studied based on molarity (Mole, M) of the silver nitrate i.e. 0 to 1000 mM, whereas the concentration of the milkweed leaves extract was 05%, 10%, 15%, and 20% respectively. Stability of the colloidal, particle size analysis and antibacterial activities were analysed in these series of pilot trials. Based on results best colloidal solution was selected. Pilot trials were also conducted for defining efficacy for antibacterial activities of natural capping agent used, viz; Milkweed treated composites in the absence of well recognized $AgNO_3$, antibacterial component.

Phase IV **Development of the Nano-composite textile materials & their testing for basic physical characteristics as well as functional properties as per ASTM standards**

This phase deals with development of various nano-composite textile materials by application of the optimized colloidal solution. Different nano-composite textile material will be prepared from non-woven textile material like, PV (Polyester-Viscose) Non-woven, PV with PE (Polyethylene) coated non-woven, PV with PU (Polyurethane) coated non-woven etc. The developed nano-composite textile material will be useful in antibacterial/hygienic non-woven textile materials for biomedical applications.

A brief mention about the experiments conducted, results obtained and their analysis have been given.

4.1 Preparation of silver nano particle ($AgNO_3$) colloidal

Silver nano particle colloidal was synthesized at laboratory scale by treating $AgNO_3$ salt with milkweed leaves extract. Effect of varying concentration of both the participating constituents on stability of the colloidal, particle size and antibacterial activities was analysed by conducting series of pilot trials. For selecting best colloidal solution, concentration of the $AgNO_3$ salt was studied based on molarity(Mole, M) of the silver e.g. i) Major scale (1000 mM, 500 mM, 200 mM, and 100 mM), ii) Minor scale (100 mM, 75 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 05 mM and 01 mM). Whereas the concentration of the milkweed leaves extract was 05%, 10%, 15%, and 20% respectively. An optimized colloidal solution in terms of stability of the colloidal, particle size and antibacterial activities was used in the subsequent phases of the work to prepare hygienic non-woven textile materials for biomedical applications on laboratory scale.

4.2 Preparation of nano-composite textile material

After preparation of the silver nano particle colloidal different samples of the non-woven textile materials were developed. Like, 1) Untreated non-woven fabric, 2) Non-woven fabric treated with silver nano-particle colloidal solution, and 3) Non-woven fabric treated only with milkweed leaves extract. Here both the treated samples were made by pad-dry-cure method. Initially the PV (Polyester-Viscose, 70-30) non-woven fabric of 40 GSM (Grams per square metre), which is widely used for different bio-medical applications was used for the preparation of silver nano composite. Subsequently the selected silver nano colloidal was used in different non-woven fabrics which are used in various bio-medical applications.

4.3 Testing and evaluation of nano-composite textile material

After preparation of all the samples, they were evaluated and analysed by different evaluation methods, viz;

1) Physical assessment of the untreated and treated textile materials like, GSM (Gram per square meter) as per ASTM D 3776, Thickness (ASTM D1777), Bending modulus (ASTM D1388), and Crease recovery angle (AATCC 66-2003).

2) Comfort associated properties like, Air permeability (ASTM D737), and Overall moisture management capability (OMMC) (AATCC-195), UV-Transmission (AS/NZ 4399:19961, AATCC TM 183-2000, EN 13758: 1:2001, GB/T18830).

3) Morphological and Elemental assessment of the nano-composite textile material by ESEM (Environmental Scanning Electron Microscopy), EDAX (Energy Dispersive X-ray Analysis), FT-IR (Fourier-transform infrared spectroscopy), Particle size Analysis, Particle size distribution by "ImageJ software" etc. [25].

4) Antibacterial assessment of the nano-composite textile material by AATCC-147 (Qualitative, Parallel streak) and AATCC-100 (Quantitative) method against different bacterial culture viz, Staphylococcus aureus and Escherichia coli [26].

5.0 RESULTS AND DISCUSSION:

Evaluation of the developed nano-composite textile materials at different concentration levels as well as only milkweed extract composite were done with reference to untreated sample and results were compared with standardize values wherever needed to determine their proficiency.

The results of characterisation by ESEM, EDAX, FT-IR, Particle size analysis and statistical distribution revealed the uniform distribution and formation of the silver nano particles on the surface of the green synthesized AgNPs treated PV non-woven composite textile material. ESEM images of composite material shown in figure 5.1. Here the elemental assessment (EDAX) spectrum has confirmed presence of the composition of silver (Ag) in the green synthesized AgNPs composite.

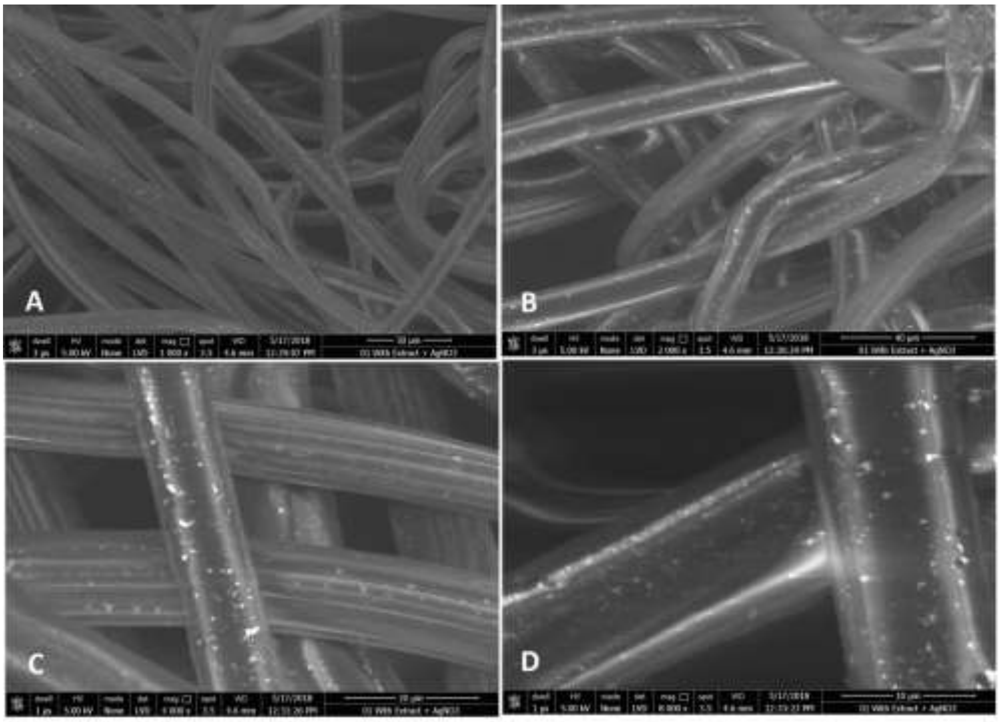

Figure 5.1:
The ESEM images of green synthesized AgNPs non-woven composite.

As shown in figure 5.2 the result of the antibacterial test methods for optimized AgNPs treated PV non-woven fabric was shown maximum antibacterial activity against both the bacterial culture viz, Staphylococcus aureus and Escherichia coli. However, the most fascinating finding during the study was very close antibacterial activities shown by milkweed composite (treated with 10% concentration) to that of similar level AgNPs treated fabric. The average zone of inhibition (mm) is given in table 5.1.

Table 5.1: Average zone of inhibition (mm)

Organism	**Sample 00***	**Sample 01***	**Sample 02***
Staphylococcus aureus (SA)	None	23.75	12
Escherichia Coli (EC)	None	24.50	12

* Sample 00: Untreated PV non-woven fabric
Sample 01: Treated with silver nano-particle colloidal solution (Nano-composite textile material)
Sample 02: Treated only with milkweed leaves extract

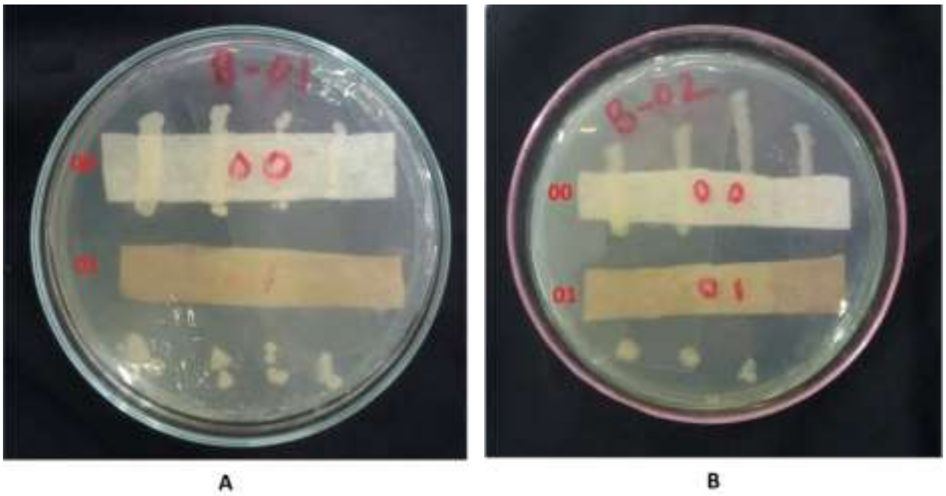

Figure 5.2:
Antibacterial assessment of the untreated nonwoven (00) and PV nano-composite (01) with Staphylococcus Aureus (A) and Escherichia Coli (B) respectively.

The chemical properties of the prepared nano-composite textile material were measured by FTIR analysis. Figure 5.3 represents the FTIR spectral characterization peaks of the PV nano-composite textile material. The result of the FTIR spectral characterization peaks revealed different chemical compositions like, N-H stretching vibration of primary amines, C–H stretching vibration, carboxyl groups (C=O) stretching, cyclic alkene (C=C) stretching vibration, alcoholic (O–H) bending vibration, phenolic (O–H) bending vibration and aromatic amine (C–N) stretching vibration. Different physical properties of the prepared PV nano-composite textile material was also measured and compared with untreated PV non-woven textile material.

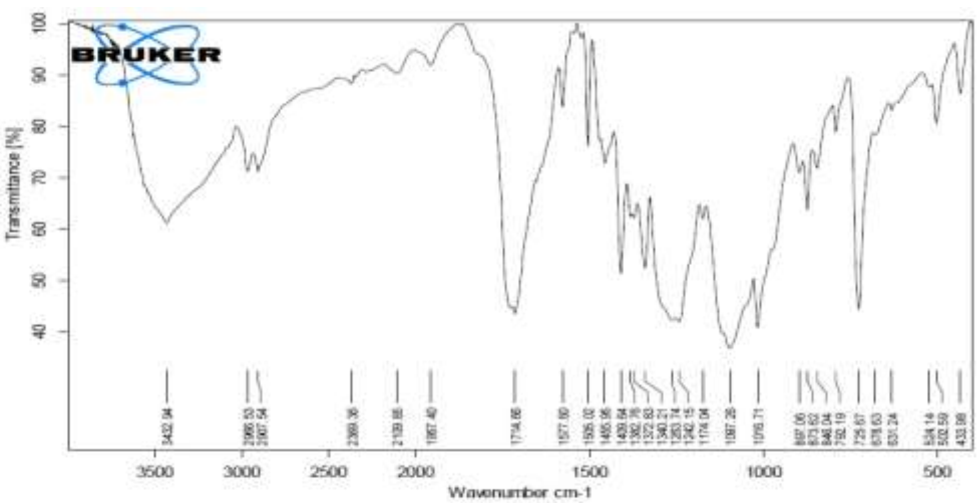

Figure 5.3:
FTIR spectral characterization peaks of the PV nano-composite textile material

Physical properties of PV nano-composite textile material are given in Table 5.2. The GSM and thickness of the prepared nano-composite textile material have shown positive changes after application of the nanoparticles i.e. GSM has shown negligible shuffle of 2.7 %, and similarly a very very small increase in thickness value; 0.72%. The low stress properties like, bending modulus and crease recovery of the prepared nano-composite textile material have shown consistent behaviour. Insignificant decrease was observed in bending modulus value in length wise direction. A small decrease in crease recovery angle (3-3.5%) was observed on the addition of AgNPs in the surface of the PV non-woven textile material. A small descend of 2.0% in the air permeability of the prepared PV nano-composite textile material was observed on the deposition of the nanoparticles. The produced PV nano-composite textile material has executed good moisture management capabilities and graded under the category of moisture management fabric.

Table 5.2: Physical properties of PV nano-composite textile material

Physical Properties				
Properties	**Untreated Nonwoven**		**PV nano-composite textile material**	
GSM (Gram per Sq. meter)	40.904		42.043	
Thickness (mm)	0.415		0.418	
Low stress Properties				
	length wise	width wise	length wise	width wise
Bending Modulus (g/cm^2)	9.43	0.87	6.70	1.28
Crease Recovery angle	116.4^o	111^o	112.3^o	107.7^o
Comfort Related Properties				
Air Permeability ($m^3/m^2/h$)	4753		4658	
OMMC grade	0.3041 (Poor)		0.4132 (Good)	

6.0 CONCLUSION:

In Phase I, the chemical synthesis of the silver nitrate salt was done by differenct techniques. The objective mode colour stability test observation opted for the prepared colloidal solution the best results were realized with the reverse synthesis method (Bottom up approach). Thereby, the reverse synthesis method was used during the entire study. During confirmatory phase forward and reverse synthesis methods were used in the synthesis of the silver nitrate salt with milkweed leaves extract. The green synthesis of the silver nitrate salt was successfully carried out irrespective of the technique used at the selected milkweed concentration. Formation of silver nanoparticles was preliminarily ascertained by visual observation and then SEM analysis. The results of the SEM micrographs have revealed not only formation but uniform distribution of the silver nanoparticles.

During phase II, PV nano-composite textile material was prepared by reinforcing the green synthesized nanoparticles via pad-dry-cure method. The results of the antibacterial test method AATCC-147 (Parallel streak method) revealed that the prepared PV nano-composite textile material has shown sensible antibacterial activity against both the bacterial culture i.e. Staphylococcus aureus and Escherichia coli. The prepared nano-composite textile material (Polyester-Viscose non-woven fabric) has confirmed enhanced antibacterial activities compared to chemically synthesize colloidal treated composite, mainly due to presence of self-sufficient antibacterial milkweed in the structure. The ESEM analysis has revealed excellent deposition of AgNPs on the surface of the prepared nano-composite textile material with used deposition technique. The presence and nanoparticle size distribution was preliminarily confirmed by EDAX and 'ImageJ 1.53a' software respectively. The PV nano-composite textile material has not exhibited any significant variations in their structural and physical properties at nano level add on.

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