Wound healing potential of bioactive film incorporated with essential natural polymers and silver nanoparticles of Tridax procumbens Linn.

NANDINI.A

(17PBC011)

Thesis submitted to Avinashilingam Institute for
Home Science and Higher Education for Women,
Coimbatore-641 043

In partial fulfillment of the requirement for the Degree of
Master of Science in Biochemistry

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Signature of the Guide

ACKNOWLEDGEMENT

It is with the choice of blessings and the divine grace of **LORD ALMIGHTY** that any human endeavour is achieved. In addition to the will of supreme divinity, the willingness of many subject experts and erudite scholars to extend their assistance and help for the completion of a work plays, indeed a vitaly important role.

I take the opportunity of expressing my sincere thanks to **Dr. Padmashri. P.R. KrishnaKumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore, for providing the opportunity and infrastructure to undertake this investigation.

I immensely thank **Dr. Premavathy Vijayan**, Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the entire facilities essential to carry out and complete the study.

I record my sincere thanks to **Dr. S. Kowsalya**, Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for timely help rendered to carry out the work.

I express my special gratitude to **Dr. S. Annapurani**, Former Professor and Head, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, her sustained support throughout the study.

I record my sincere thanks to **Dr. P.R. Padma**, Dean, Faculty of Science and Professor and Head, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the opportunity and for the timely help rendered to carry out the work successfully.

I owe my indebtedness, profound and deepest thanks to my guide **Dr. T. Angayarkanni**, Associate Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her excellent guidance, immense tolerance, meticulous care, good support, creative influence, thoughtfull advice, steady encouragement, motherly love throughout the research and motivation right from the selection of topic to the compilation and completion of the work efficiently and effectively.

I submit my sincere thanks to all The **Staff Members**, of the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore, for lending a helping hand and invaluable guidance during the course of this thesis work.

I express my grateful thanks to **Ms. R. Shanthini,** Senior, for her incessant guidance and valuable advise leading to the successful completion of the thesis.

I whole-heartedly owes a special debt of gratitude to my beloved **parents** for their moral support, motivation and extreme care rendered during the course of the study.

It's my pleasure to thank all **my friends** for their constant encouragement in embellishing this study.

I acknowledge the contribution of all other unseen hands during the course of the study for help rendered in the successfull completion of the study.

Nandini.A

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1.0 INTRODUCTION

Wound healing is a multi-factorial physiological process. The complexity of this phenomenon makes it prone to several abnormality. Apart from cellular and biochemical components, several enzymatic pathways also become active during mend and help the tissue to heal. Natural and synthetic gel-like materials, films/membranes, composites, micro- /nano particulate systems have featured heavily in the progress of biomaterials for wound healing and other tissue-engineering purposes. Biocompatible and biodegradable polymer scaffold combined with cells or biological signals are being investigated as alternatives to traditional options for tissue re-enactment and transplantation. These approaches are already in clinical use as engineered tissues that enhance wound healing and skin rejuvenation (Cevher, 2011).

Plants are used for treatment of certain disorders from ancient days. Plants registered as a major source of medicinal provision and also many drugs derived from herbal plants. Around 25% drugs are derived by using plants. Due to less hygienic situation mostly in rural area wounds is common anarchy for skin problem. Boil, burn, wounds are unplanned physical injure of body by loss of skin. Wound healing process start from damage of skin. A complete wound healing process depends on degree of injury, human resistance capacity, infection potential of pathogens and early effective treatment procedure (Patel, 2014).

This study includes the actions of additional silver nanoparticles of *T. procumbens* on wound healing in an attempt to ascertain the exact nature of the active principle in bioactive film. *T. procumbens*, also known as 'Common button' or 'coat buttons' is a perennial plant from the Asteraceae family, native to Central and South America (Ravikumar *et al*., 2005).

Tridax procumbens Linn. a bounds in alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins. It is also rich in sodium, potassium and calcium. The plant is used to treat various ailments, such as bronchial catarrh, dysentery, diarrhoea, preventing hair loss, and to check haemorrhage from cuts. Its pharmacological studies have revealed some important medicinal properties like anti-inflammatory, hepato protective, wound-healing, immune-modulatory, antimicrobial, antiseptic, hypotensive and brady cardiac effects. Plants as being a safer source of nanoparticles synthesis, *T. procumbens* was selected for their variable phyto constituents and especially having high amount of ketones, phenols, alkanes, amine and lactones. All the available reports on *T. procumbens*, phyto constituents and pharmacological properties make this plant more acceptable for the present study.

Since ancient times, this species has been used in Ayurveda in India (Kethamakka and Deogade, 2014). Traditionally, this plant has been in use in India for wound healing and as an

anticoagulant, antifungal, and insect repellent. The juice extracted from the leaves is unswervingly applied on wounds. Its leaf extracts were used for contagious skin diseases in folk medicines. It is used in ayurvedic remedy for liver disorders, hepato protection, gastritis, and heartburn. *T. procumbens* is also used as treatment for boils, blisters, and cuts (Nallella, Sreeramulu *et al.,* 2013). Here the methanolic extract of this plant is used for preparing the silver nanoparticles.

Silver compounds and ions have been extensively employed for the treatment of several pathophysiological conditions, with special hygienic and healing purposes, due to their broad-spectrum antimicrobial activity (Ojeda-Martínez *et al.,* 2015).

Synthesis of nanoparticles from plant sources has proved to be an effective and alternate method for the novel production of nanoparticles. Synthesis of gold and silver nanoparticles can be achieved through various chemical and physical methods including the chemical reduction of silver ions in aqueous solutions, with or without stabilizing agents, thermal decomposition in inorganic solvent, chemical and photo reduction (Malik, 2014).

Natural polymers are widely used in the regenerative medicine field, for wounds and burns dressing because of their biocom-patibility, biodegradability dressing (Grumezescu *et al.,* 2014).

A flexible polymeric film, suitable for use as a wound dressing, wherein the polymer comprises at least 70 % by weight. Chitosan is a very promising biopolymer because it is environmentally friendly due to its biodegradability and has good film forming properties (Kanatt *et al.,* 2012).

Chitosan is a biodegradable, biocompatible, polycationic, naturally derived polymer with low immunogenicity and it is a linear polysaccharide which is made by treating the chitin shells of shrimp and other crustaceans with alkaline substances. Chitosan can be isolated directly from the cell walls of certain fungi, but is usually prepared from chitin. substance hydrolysis and enzymatic hydrolysis methods have been extensively used for the isolation of chitosan from marine crustacean shells, as they are quite inexpensive. In the chemical hydrolysis method, four main steps are involved in producing chitosan from marine crustacean shells. They are demineralization, deproteinization, discoloration, deacetylation (Venkatesan, and Kim, 2014).

Chitosan has a number of mercantile and possible biomedical uses. It can be used in cultivation as a seed cure and biopesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a chastising agent, also helping to check spoilage. In diligence, it can be used in a self healing polyurethane paint facade. In medication, it is useful

in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin. More controversially, chitosan has been asserted to have use in restraining fat absorption, which would make it useful for dieting. Chitosan's is used within some wound dressing to stop haemorrhage. It also appears to shrink the growth of bacteria and fungus. It's hemostatic agents are often chitosan salts made from mixing chitosan with an organic acid (such as succinic or lactic acid). The chitosan salts can be assorted with other materials to make them more absorbent (such as mixing with alginate), or to vary the rate of solubility and bio absorbability of the chitosan salt. The chitosan salts are biocompatible and biodegradable manufacture them useful as absorbable haemostats. It is being studied as a medication deliverance system. Chitosan and derivatives have been explored in the development of nanomaterials, bio adhesives and edible coatings for drug delivery and in medical devices (Salmean *et al.*, 2016).

Gelatin is a natural water soluble protein pigeonholed by the absence of an appreciable stink and the random configuration of polypeptide chains in aqueous solution. It is obtained from the partial hydrolysis of collagen; a fibrous protein mainly found in firm parts of vertebrate and invertebrate animals as bones, skins, connective tissues and tendons and its structure consists of rigid bar-like molecules that in order in fibres inter-connected by covalent bonds. Soluble gelatin is produced by the deterioration of the collagen triple-helix. Gelatin properties are influenced by two main factors: the characteristics of the preliminary collagen and the extraction procedure (Marina Ramos *et al.*, 2016).

The degree of collagen alteration into gelatin is reliant on the pre-treatment with warm-water extraction, temperature, pH, and extraction time. Interstitial collagen molecules are unruffled of three polypeptide α-chains intertwined and stabilized by hydrogen bonding and hydrophobic interactions. The destabilization is produced by betrayal hydrogen and covalent bonds as a result of the heat treatment, resulting in helix-to-coil transition and subsequent conversion into soluble gelatin. Formerly, the insoluble indigenous collagen must be pre-treated to break non-covalent bonds so as to disorganize the protein structure, thus producing adequate swelling and collagen solubilisation, apposite for extraction (Ramos *et al.*, 2016).

Some synthetic polymers from non-renewable sources are also biodegradable, such as polyvinyl alcohol (PVA). PVA is a mock, water soluble polymer with admirable film forming, emulsifying, and adhesive properties. It also imparts good tensile strength (TS) and biodegradability and hence has been used in many biomaterial applications. Chitosan contains free hydroxyl and amine groups, and is therefore miscible with PVA due to the formation of

hydrogen bonds. Polymer blending is one of the most effective methods to have new material with desired properties (Kanatt *et al.,* 2012).

Poly vinyl alcohol (PVA) is the only known carbon-carbon backbone polymer which is biodegradable and has gained immense attention due to the range of applications possible due to this property (Matsumura *et al.,* 1999).

It can also be easily blended with other natural polymers and also has the advantage of being polar and soluble in water. It can improve the mechanical properties of the biopolymer as it has high tensile strength and elasticity. Blending of PVA with biopolymers such as chitosan, starch and have been investigated by researchers (Ke and Sun, 2003; Kanatt *et al.,* 2012).

Gelatin is a fibrous protein which due its unique sequence of amino acids consisting of high content of proline, hydroxy proline and glycine that is available in nature and has film forming properties and can be blended with other polymers to improve its mechanical and barrier properties (Kanatt *et al.,* 2017)

Films formed by amalgamation of polymers usually results in modified physical and mechanical properties compared to films made of entity components. Since synthetic polymers are easily obtained and have low production cost, blending of natural and synthetic polymers improves the cost performance ratio of the resulting films. Blending of synthetic polymers, such as PVA with Chitosan have been reported to improve mechanical properties of Chitosan films. In terms of active agents that can be incorporated into films, plant extracts have received much attention as they contain high concentrations of phenolic compounds that possess strong antioxidant properties (Kanatt *et al.,* 2012).

Poly vinyl alcohol (PVA) is the only known carbon-carbon backbone polymer which is biodegradable and has gained immense attention due to the range of applications possible due to this property (Matsumura *et al*., 1999).

It can also be effortlessly blended with other acknowledged polymers and also has the lead of being polar and soluble in water. It can perk up the mechanical properties of the biopolymer as it has soaring tensile strength and elasticity. Amalgamation of poly vinyl alcohol with biopolymers such as chitosan, starch and have been investigated by researchers (Ke and Sun, 2003; Kanatt *et al*., 2012).

Gelatin is a fibrous protein which due its unique sequence of amino acids consisting of high content of proline, hydroxyl proline and glycine that is available in nature and has film forming properties and can be blended with other polymers to improve its mechanical and barrier properties (Kanatt *et al*., 2017).

Chitosan-membrane-based wound products have been investigated both in laboratory animals and humans, but are still at the early stage of development.

With this background information the present study was designed with the following objectives:

1. To prepare silver nanoparticles using *T.procumbens* leaf extract.
2. To prepare chitosan-gelatin-PVA composite crosslinked with formaldehyde and silver nanoparticles of *T.procumbens.*
3. To characterize the chitosan-gelatin-PVA (C-G-PVA) composites for moisture content, water absorption capacity, solubility of the film, swelling degree, Fourier Transformed Infrared Spectroscopy, 3D surface active analysis, Thermogravimetric Analysis and Scanning Electron Mcroscopy.

2.0 REVIEW AND LITERATURE

2.1 Wound healing

The body skin is a barrier to invasion of pathogenic microorganisms and loss of water, and prevents bleeding. Therefore, skin is a vital organ whose joined structure may be damaged or disrupted by physical, chemical, and biological agents. This damage is referred to as wound. Wound healing is an active process throughout which a series of connections between different cells, cytokine mediators, and extracellular matrix take place. Generally, wound healing is a continuous process including coagulation, inflammation, proliferation, and recover. Moreover, wound healing is a reconstructive process that takes place after the damage to skin and soft tissue. In fact, after the damage incidence, an inflammatory response is developed and subdermal cells begin to increase collagen and then epithelial tissue is gradually reconstructed (Samani *et al.,* 2016).

Wound healing is very important for survival mechanism, and it represents to maintain the normal structure and function of tissues. Wound healing is still a challenge for the pharmaceutical industry, despite being more complex nowadays. The 1–3% of drugs only used in western pharmacopeias for curing and healing of wounds. It is due to this challenge that medicinal plants possess enormous potential to come up with a widespread solution for the wound healing treatments (Krishnaveni, 2018)

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. It is reflected in a set of biochemical events in a closely organized cascade to repair damaged tissue. In the human adult, wound healing is often divided into three phases, i.e., inflammatory, proliferative, and remodeling phases. The inflammatory phase starts immediately after injury with the launch of hemostatic mechanisms to stop bleeding straight away (Maver *et al*., 2015).

2.2 *Tridax procumbens* Linn.

T. procumbens (Figure 1) is a species of flowering plant belonging to family asteraceae and is the most potent species among 30 species. It is best known as widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical and mild temperate regions worldwide. It is listed as a noxious weed in the United States and has a pest status. Some of the medicinally important species of the genus *Tridax* are: *T. angustifolia, T. serboana, T. bicolor, T. accedens, T. dubia, T. erecta and T. rosea* (Mir *et al.,* 2017)

Figure 1: *Tridax procumbens* Linn.

2.2.1 Taxonomic classification

Kingdom : Plantae
Division : Angiosperms
Class : Magnoliopsida
Order : Asterales
Family : Asteraceae
Genus : *Tridax*
Species : *Procumbens*

T. procumbens is employed as indigenous medicine for a variety of ailments. It has been extensively used in Indian traditional medicine for wound healing, as anticoagulant, antifungal and insect repellent, in diarrhea and dysentery. Leaf extracts are used to treat infectious skin diseases in folk medicines. It is also dispensed as 'Bhringraj' which is well known ayurvedic medicine for liver disorders. Antioxidant properties have been demonstrated, also hair growth promoting activity have been analyzed (Mir *et al.,* 2017).

2.3 Polymer

The word polymer is derived from Greek words, poly which means many and parts or units of high molecular mass. Each molecule consists of a very large number of single structural units joined together in a regular manner by covalent bonds. Polymers are the giant molecules of high molecular weight called macromolecules which are formed by linking together a large number of small molecules, called monomers. The process by which monomers combine to form polymer is known as polymerization. The polymerization is defined as a chemical reaction in which two or more substances combine together with or without evolution of water, heat or any other solvents to form a molecule of high molecular weight. The product obtained is called polymer and the starting material from which the polymers are made is called monomer. These polymers are found in nature generally from plants and animals sources. Examples are proteins, cellulose, starch, resins. Semi-synthetic polymers-These polymers are obtained from natural polymers by simple chemical treatment to change the physical properties of natural polymers like Starch, silicones (Babu *et al.,* 2016).

2.3.1 Synthetic polymers

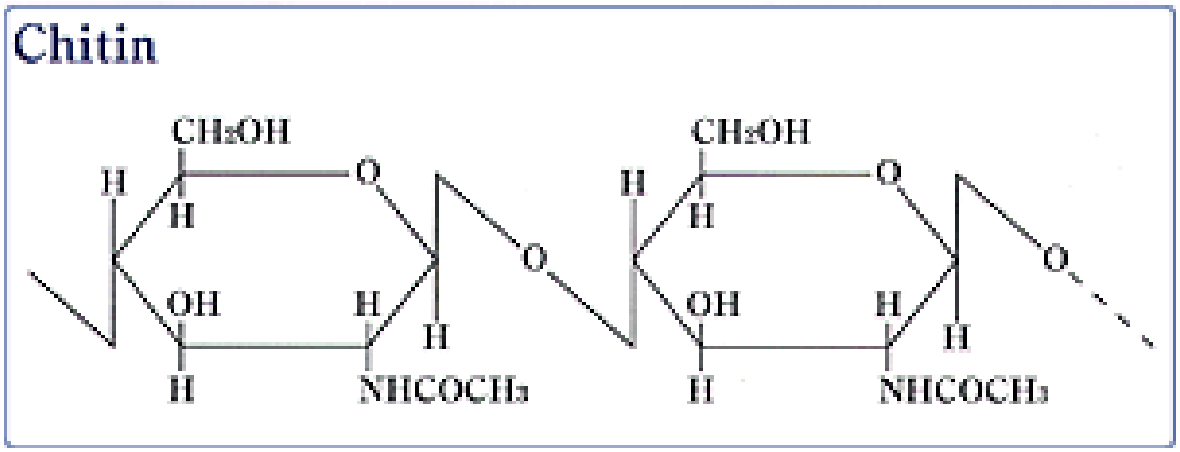
The fibers which are synthesized in laboratory by polymerization of simple chemical molecules are called synthetic polymers, example: Nylon, polyethene, polystyrene, synthetic rubber, PVC, Teflon, Low density polyethylene (LDPE), High density polyethylene (HDPE), thermoplastic polyurethanes etc (Kaushik *et al.*, 2016**).**

Biopolymers are efficient in the tissue repair processes only to a certain extent, being limited in interactions at the molecular level with wound pathogens. These natural macromolecules have attracted attention as dressing materials due to their biodegradability, where material degradation and the new tissue formation should be parallel processes (normally the case in the treatment of acute wounds). The situation with chronic wounds is more complicated due to low stability of these biomaterials in contact with the fluids containing elevated levels of hydrolytic enzymes; eg, lysozyme cleaves chitosan-based materials, whereas collagen is a natural substrate of several matrix metalloproteinases. Moreover, the use of biopolymers in wound management has not yet been clearly translated into a platform for wide-spread clinical use (Ghadi *et al.*, 2011).

2.3.2 Chitin

Chitin or poly (β-(1→4)-*N*-acetyl-D-glucosamine) is a natural polysaccharide of major importance, first identified in 1884 (Figure 2). This biopolymer is synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline microfibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. So far, the main commercial sources of chitin are crab and shrimp shells. In industrial processing, chitin is extracted by acid treatment to dissolve the calcium carbonate followed by alkaline solution to dissolve proteins. In addition, a decolorization step is often added in order to remove pigments and obtain a colorless pure chitin. All those treatments must be adapted to chitin source, owing to differences in the ultrastructure of the initial material (the extraction and pre-treatments of chitin will be described later), to produce first a high quality chitin, and then chitosan (after partial deacetylation). Chitin is infusible and sparingly soluble during transformation into different conformations of the initial material (the extraction and pre-treatments of chitin will be described later), to produce first a high quality chitin, and then chitosan(after partial deacetylation). Chitin is infusible and sparingly soluble during transformation into different conformations (Younes *et al.*, 2015).

Figure 2: Chitin



2.3.3 Isolation of chitin

Isolation of chitin from crustacean shell waste consists of two basic steps: (1) protein separation-deproteinization, and (2) calcium carbonate (and calcium phosphate) separation demineralization. These steps also can be conducted in a reverse order, i.e., demineralization, followed by deproteinization. Howcver, if protein recovery is an objective, its extraction before demineralization is preferred so as to maximize protein yield and quality. A simplified flow diagram of chitin processing is given in Figure 3 (Hong *et al.*, 2010).

2.3.4 Methods of Chitin Extraction

2.3.4.1 Deproteinisation

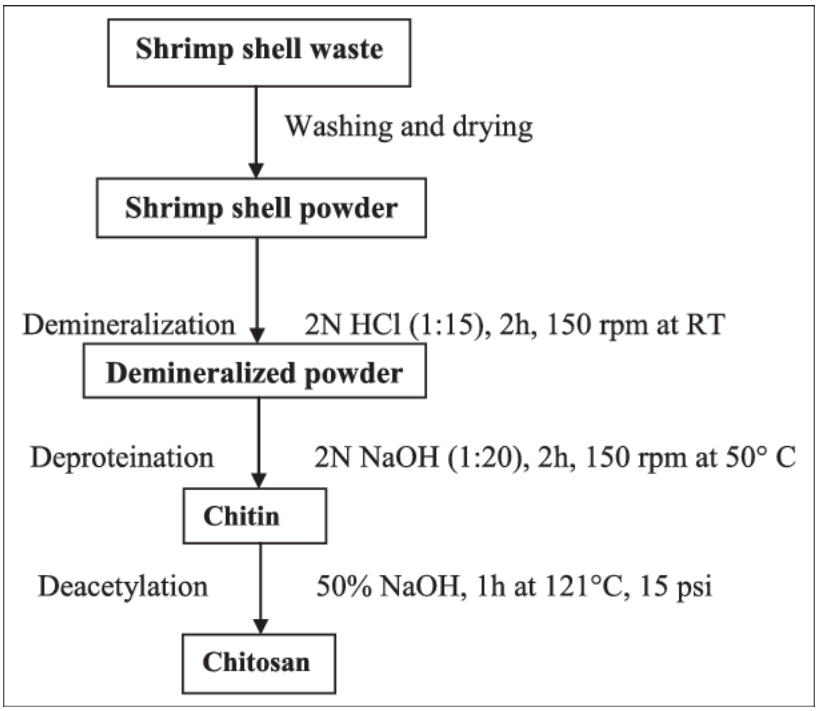
Deproteinisation is usually performed by alkaline treatment. Demineralisation is generally performed by acid treatment including HCl, HNO3, H2SO4, CH3COOH, and HCOOH; however, HCl seems to be the preferred reagent. It was shown that the order of the two steps may be reversed for shrimp waste containing large protein concentrations, which stem primarily from the skeletal tissue and to a lesser extent from the remaining muscle tissue (Arbia *et al.*, 2013).

2.3.4.2 Deacetylation

These are the heterogeneous deacetylation of solid chitin and the homogeneous deacetylation of pre-swollen chitin under vacuum (by reducing pressure) in an aqueous medium. Heterogeneous deacetylation, which is the preferred industrial treatment, involves preferential reaction in the amorphous regions of the polymer, leaving almost intact the intractable crystalline native regions in the parent chitin. Alternatively, homogeneous modification is conducted by use of moderately concentrated alkali (13% w/w) acting on pre-

swollen chitin to improve the interaction with the alkali and left to react at 25-40º C for 12-24 hours (Aranaz *et al.*, 2009*).*

Figure 3. Simplified flow diagram of chitin and chitosan



2.3.4.3 Demineralization

Demineralization is conventionally accomplished by extraction with dilute hydrochloric acid (up to 10%) at room temperature to dissolve the calcium carbonate as calcium chloride. Among such methods are those exceptions to the above are seen in the methods of where demineralization was accornplished with 90% formic acid and 22% HCI, respectively, at room temperature. The work of which demineralization was accomplished in 37% HCI at - 20° C, is also noted (Hong *et al.,* 2010).

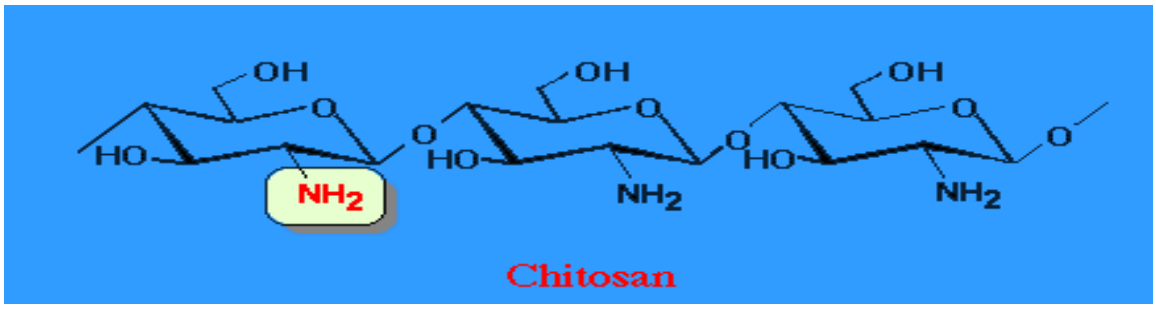
2.3.5 Chitosan

Chitosan (Ch) is a very promising biopolymer because it is environmentally friendly due to its biodegradability and has good film forming properties. In the food industry, Ch films promise immense potential to be used as active packaging material due to its antimicrobial activity, non-toxicity and low permeability to oxygen (Kanatt *et al.,* 2012).

Chitosan is a ß-1,4-linked polymer of glucosamine (2-amino-2-deoxy-b-d-glucose) and lesser amounts of *N*-acetylglucosamine. It is a derivative of chitin (poly-*N*-acetylglucosamine), which is the second most abundant biopolymer after cellulose. Over the last 200 years, the

study and application of chitosan has taken on many different forms. Researchers continue to build on the original finding of Bracannot, discovering new uses for chitin and chitosan as they find different forms of it in nature (Dai1 *et al.*, 2011*).*

Figure 4: Chitosan



2.3.5.1 Chitosan structure and its origin

Chitosan stimulates hemostasis and accelerate regeneration of tissues, therefore it found to be useful for wound healing management. For a material to be used for biomedical research, a natural product is preferred because these materials are more biocompatible than synthetic materials. Chitosan is an attractive material for a tissue engineering scaffold because it has structural similarities to glycosaminoglycan and is hydrophilic in nature. The monomeric unit of chitosan, N-acetyl glucosamine is important in healing of wound. Due to the biodegradability, biocompatibility, antimicrobial effect and its non-toxicity, much attention has been paid to chitosan for its biomedical applications. Chitosan stimulates hemostasis and accelerate regeneration of tissues, therefore it found to be useful for wound healing management **(Ahmed *et al.*, 2015).**

2.3.5.2 Extraction of Chitosan

Sodium hydroxide solution (12.5 N) and 50 g of sodium hydroxide was dissolved in 100 ml of distilled water are used for the deacetylation process. 50% of 10 ml NaOH to 0.1 g of chitin was added and boiled at 100° C for 5mins and this mixture was cooled for 10 mins and 5 ml of distilled water was added and incubated at room temperature for 24 hours and filtered using Whatman filter paper and washed with distilled water. The supernatant is dried in the hot air oven at 60° C and the chitosan was obtained and measured (Vignesh *et al.*, 2018).

2.3.5.3 Properties of chitin and chitosan

Both chitin-chitosan are biopolymers composed of glucosamine and N-acetylated glucosamine (2-acetylamino-2-deoxy-D-glucopyranose) units linked by (I--->4) glycosidic bonds. They are the major constituent of shells of arthropods such as crabs, shrimps, lobsters and insects and are also produced extracellularly by fungi and brown algae. Chitin is

extensively acetylated while chitosan is largely deacetylated. Chitin is insoluble in water and acid while chitosan is also relatively insoluble in water but soluble in acid. The enzymes involved in the formation and degradation of chitin have been identified. Chitin synthese polymerizes glucosamine to chitin and chitinase hydrolyzes chitin to monomers. Lectins that bind N-acetyl-D-glucosamine block synthase activity. A deacetylase transforms chitin to chitosan by hydrolyzing the acetamido groups of N-acetyl-glucosamine. This enzyme is a glycoprotein with high mannose content of approximately 30% by weight (Koide *et al.,* 1998).

2.4 Polyvinyl Alcohol (PVA)

Poly vinyl alcohol (PVA) is the only known carbon-carbon backbone polymer which is biodegradable and has gained immense attention due to the range of applications possible due to this property (Matsumura *et al.,* 1999).

It can also be easily blended with other natural polymers and also has the advantage of being polar and soluble in water. It can improve the mechanical properties of the biopolymer as it has high tensile strength and elasticity. Blending of PVA with biopolymers such as chitosan, starch and have been investigated by researchers (Sun, 2003; Kanatt *et al.,* 2012).

2.5 Gelatin

Gelatin is a fibrous protein which due its unique sequence of amino acids consisting of high content of proline, hydroxy proline and glycine that is available in nature and has film forming properties and can be blended with other polymers to improve its mechanical and barrier properties. Glycerol is a major by-product generated during the production of biodiesel and its use as a plasticizer in packaging films can increase the value addition. In recent years extensive studies have been carried out to improve the functional properties of food packaging material thereby improving the quality of the packed food. This has led to the development of active packaging and edible films. Bioactive compounds from natural sources have been frequently utilized in active packaging applications (Kanatt *et al.,* 2017).

Gelatin is a biocompatible polymer and is used for many biomedical applications, including wound dressings, and has haemostatic potential. The haemostatic action is based on platelet activation at the point of contact of blood with the gelatin, which activates the coagulation cascade. As a result of its gelatin properties, it can act as a binding agent and stops the low of blood into blood vessels by constricting the vessels. Clinical and in vivo studies have shown that gelatin is effective in wound healing. A topical application of gelatin to skin has proved to be effective in accelerating wound healing. Hydrogels can minimize hypoxia, which is a major problem in patients with burns, by providing a moist environment on the

surface of the wound. The haemostatic potential of both chitosan and gelatin provide better healing by initiating the blood-clotting cascade (Kumar *et al.*, 2014).

2.6 Characterization of bioactive film

2.6.1 Moisture content

Moisture content (MC%) of the membrane was determined by drying 3-cm 2 pieces of sample in an oven at 105° C for 24 h (Azad *et al*, 2004).

2.6.2 Water absorption capacity

It is of utmost importance, if they are used for biological applications and wound healing. It is used to measure the capacity of blank and drug loaded films to absorb wound exudates. Pre weighed, one inch film was placed in 15ml. of distilled water and the weight of the film was noted periodically at first hour, second hour, 3rd hour and 24th hour. Every time after noting the weight, the film was placed in fresh water. Water absorption capacity of the film was determined in triplicate and calculated by formula (Ahamed *et al.*, 2011*).*

2.6.3 Solubility of the film

The films were cut into 2cm × 2cm pieces for the determination of solubility and swelling degree. The pieces were dried at 105°C to constant weight to obtain the initial dry mass (*M1*). Then, they were placed in 100 ml beakers with 50 ml distilled water covered with plastic wraps and stored at 25°C for 24 h. Next, the films were dried superficially with filter papers and dried at 105°C to constant weight to obtain the final dry mass (*M2*) (Sun *et al.*, 2016).

2.6.4 Swelling degree

The films were put into 50 ml beakers with 30 ml distilled water for 24 hr at 25° C after weighing the films (*M1*). The wet films were then dried superficially with filter papers, followed by weighing the wet films (*M2*) (Chen *et al.*, 2016).

2.6.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes on the order of 10 µ3, which helps to perform difference spectroscopy, where one could distinguish the small absorption bands of functionally active residues from the large background absorption of the entire protein. FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academic and industrial research. Furthermore, FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between

enzyme and substrate during the catalytic process. Furthermore, it is a non-invasive technique. Finally, the advantages of FTIR spectrometers over dispersive ones are rapid data collection, strong signal, large signal-to-noise ratio, and less sample heat-up. Recently, further advancement has been made in an FTIR method called Attenuated Total Reflection (ATR)-FTIR spectroscopy. Using ATR-FTIR, we can determine the chemical properties on the polymer surface, and sample preparation is easy compared to conventional FTIR. Therefore, FTIR is a suitable, valuable, non-invasive, cost effective, and simple technique to identify the role of biological molecules in the reduction of silver nitrate to silver. FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio (Zhang *et al.,* 2016).

2.6.6 3D surface active analysis

3D Analyst Extension provides some powerful and impressive tools for analysis and display of 3D surfaces, as well as integration with traditional 2D raster and vector data sources. The 3D Analyst is an extension that adds support for 3D shapes, surface modeling, and real-time perspective viewing to ArcGIS. With it, you can create and visualize spatial data using a third dimension to provide insight, reveal trends, and solve problems. Here is a typical 3D perspective view of Pack Forest elevation with streams and roads. The view has a 3x vertical exaggeration applied, which makes topographic features more distinct. For skilled map readers, it is easy to visualize the relationship of topography, roads, and streams as a landscape system. However, reading a topographic map requires certain knowledge and experience that not everyone possesses. Even for experienced map readers, an image like this conveys far more intuitive information than a flat paper map (Ponnaian *et al.,* 2015).

2.6.7 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was based on the measurement of the temperature changes that occur in a substance, either by chemical reactions or physical changes. TGA is a technique by which a sample is weighed and continuously heated at a controlled rate. The weights of samples change when the temperature increases. The resulting curve is called thermogram. The thermogram provides information about the thermal stability and composition of the original sample, the composition and stability of intermediates and the waste composition. Each material has its corresponding thermogram allowing identification (Sharma *et al.,* 2017).

2.6.8 Scanning Electron Microscopy (SEM)

Recently, the field of nanoscience and nanotechnology has provided a driving force in the development of various high-resolution microscopy techniques in order to learn more about nano materials using a beam of highly energetic electrons to probe objects on a very fine scale. Among various electron microscopy techniques, SEM is a surface imaging method, fully

capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software. The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver powder morphology and also conduct chemical composition analysis. The limitation of SEM is that it is not able to resolve the internal structure, but it can provide valuable information regarding the purity and the degree of particle aggregation. The modern high-resolution SEM is able to identify the morphology of nanoparticles below the level of 10 nm (Liu *et al.*, 2016).

3.0 EXPERIMENTAL PROCEDURE

The experimental procedure adopted for the study "Wound healing potential of bioactive film incorporated with essential natural polymers and silver nanoparticles of *Tridax procumbens* Linn." is presented under THREE phases:

3.1 PHASE I

3.1.1 Collection of plant sample

3.1.2 Preparation of methanolic extract

3.1.3 Preparation of silver nanoparticles

3.1.4 Exposure to sunlight

3.1.5 Separation of silver nanoparticles

3.2 PHASE II

3.2.1 Preparation of chitosan solution

3.2.2 Preparation of gelatin-PVA solution

3.2.3 Preparation of chitosan – gelatin-PVA solution

3.2.4 Preparation of formaldehyde cross-linked chitosan-gelatin-PVA solution

3.2.5 Preparation of the bioactive film

3.3 PHASE III

3.3.1 Characterization of the bioactive film

I. Moisture content
II. Water absorption capacity
III. Solubility of the film
IV. Swelling degree
V. Fourier-Transformed Infrared spectroscopy
VI. 3D Surface active analysis
VII. Thermogravimetric analysis
VIII. Scanning electron microscopy

3.1 PHASE I

3.1.1 Collection of plant sample

The leaves of the plant sample were collected from the local areas of Coimbatore, Tamil Nadu.

3.1.2 Preparation of methanolic extract

Fresh leaves of *T. procumbens* were collected and cleaned to remove adhering dust particles, washed under running tap water, gently blotted dry using tissue paper. 10g of leaf sample was weighed, cut into small pieces and added to 100 ml of methanol. This was stored in dark with mild shaking for 24 hrs. The mixture was then filtered through Whatman No. 1 filter paper. The final extract was stored at 4º C for further experiments.

3.1.3 Preparation of silver nanoparticles

Silver nanoparticles were prepared from the methanolic extract of *T. procumbens* leaves. To 10 ml of the leaf extract 90 ml of 1mM silver nitrate solution was added (Donda *et al*., 2013). The extent of nanoparticles synthesis was monitored by measuring the absorbance at 400-600 nm (Donda *et al.,* 2013).

3.1.4 Exposure to sunlight

The methanolic extracts of *T. procumbens* with silver nitrate were exposed to sunlight for about 20 mins (Sulaiman *et al.,* 2013).

3.1.5 Separation of silver nanoparticles

To separate the synthesized silver nanoparticles, samples were centrifuged at 5000 rpm for 1 hr under refrigeration and wasted three times with deionized water. A dried powder of the silver nanoparticles was obtained by hot air oven drying. The process was repeated to obtain required amount of silver nanoparticles. The separated silver nanoparticles were stored for future experiments.

3.2 PHASE II

The bioactive film was prepared by the casting method (Madhavan *et al.,* 1999).

3.2.1 Preparation of chitosan solution

Chitosan solution was prepared by dissolving 3.0 g of chitosan in 1% aqueous acetic acid solution by stirring on a magnetic stirrer (500 rpm) at room temperature for 5 hrs.

3.2.2 Preparation of gelatin-PVA solution

2.0 g of polyvinyl alcohol (PVA) was dissolved in the 70 ml of the distilled water and heated for 5 mins and stirred well in magnetic stirrer at 45º C for 30 mins. 3.0 g of gelatin was dissolved in the hot PVA solution and stirred again in the magnetic stirrer for 15 mins.

3.2.3 Preparation of chitosan – gelatin-PVA solution

The chitosan solution and gelatin-PVA solution was mixed by stirring on the magnetic stirrer at 45ºC for 1 hr.

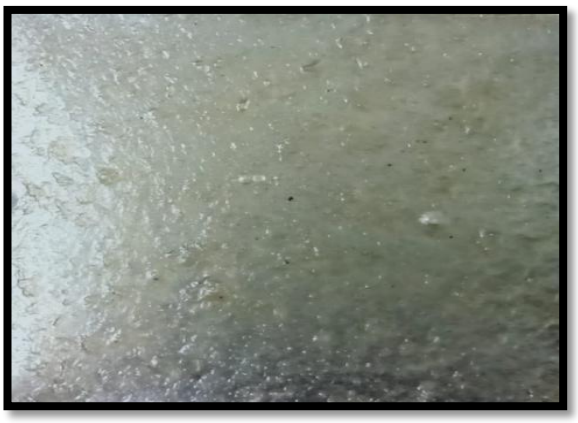
3.2.4 Preparation of formaldehyde cross-linked chitosan-gelatin-PVA solution

1.0 ml of ethylene glycol was added to the mixture and mixed well and the 0.35 ml of 25% formaldehyde was added. 30.0 mg of the silver nanoparticle synthesized from *T.procumbens* was added to this solution and degasified for 5 hrs to remove air bubbles.

3.2.5 Preparation of the bioactive film

The contents were then casted over on a polyethylene sheet and left for air drying to form a film. Drying may take 5-7 days.

Figure 5: C-G-PVA and silver nanoparticles of *T. procumbens* cross-linked with formaldehyde *composite film*



3.3 PHASE III

3.3.1 Characterization of the bioactive film

The film formed were characterized for the water absorption capacity, moisture content, solubility of the film, 3D surface active analysis, Thermogravimetric Analysis, Fourier-Transformed Infrared Spectroscopy and Scanned Electron Microscopy.

Tensile strength measures the ability of film to with stand rupture, mechanical pressures or the force required to break the film. Tensile strength of the blank films and optimized drug loaded film was determined by using the instron tensile testing machine at SDDC section in CLRI (Niyas Ahamed *et al.,* 2011). It is of utmost importance, if they are used for biological applications and wound healing. It is used to measure the capacity of film to absorb wound exudates. The initial weight of 1inch of dry film was noted. Then this film was placed in 15ml of distilled water taken in petriplate. The weight of the film was noted periodically at first hour, second hour, third hour and 24th hour. Every time after noting the weight, the film was placed in fresh water. (Hima Bindu *et al.,* 2011). Water absorption capacity of the film was calculated using a formula

$$\text{Water absorption capacity} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

The films were cut into 2cm×2cm pieces for the determination of solubility and swelling degree. The pieces were dried at 105°C to constant weight to obtain the initial dry mass (*M1*). Then, they were placed in 100 ml beakers with 50 ml distilled water covered with plastic wraps and stored at 25°C for 24 h. Next, the films were dried superficially with filter papers and dried at 105°C to constant weight to obtain the final dry mass (*M2*) (Sun *et al.,* 2017). Then, the solubility was calculated using the following equation:

$$\text{Film solubility} = \frac{1-M2}{M1} \times 100$$

The films were put into 50ml beakers with 30ml distilled water for 24h at 25°C after weighing the films (*M1*). The wet films were then dried superficially with filter papers, followed by weighing the wet films (*M2*). The swelling degree was calculated using the following equation:

$$\text{Film swelling degree} = \frac{M2-M1}{M1} \times 100$$

FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes on the order of 10^{-3}, which helps to perform difference spectroscopy, where one could distinguish the small absorption bands of functionally active residues from the large background absorption of the entire protein FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes on the order of 10^{-3}, which helps to perform difference spectroscopy, where one could distinguish the small absorption bands of functionally active residues from the large background absorption of the entire protein (Xi-Feng Zhang *et al.,* 2016).

The thermogravimetric analysis was carried out using GENER V4 IC POINT 2000 in nitrogen atmosphere at a heating rate of 10°C per minute. Primary weight change of these materials as a function of temperature was recorded using this study (Sharma *et al.,* 2017)**.**The morphological features of synthesized C-G-PVA with crosslinked with formaldehyde were studied by Scanning Electron Microscope. The film was coated using an ISU JFC-1100E under a high vacuum, 0.1 Torr, high voltage,1.2 KV and 50 mA (Liu *et al.,* 2016)

4.0 RESULTS AND DISCUSSION

The results of the present study entitled "The wound healing potential of bioactive film incorporated with essential natural polymers and silver nanoparticles isolated from *Tridax procumbens"* was carried out with the objective of producing a novel biomaterial and characterizing it for moisture content, water absorption capacity, solubility of the film, swelling degree, 3D surface analysis, Thermogravimetric Analysis, Fourier-Transformed Infrared Spectroscopy, Scanning Electron microscopy.

The different studies carried and the results obtained are discussed in this chapter as follows:

4.1 MOISTURE CONTENT

The moisture content of the film was presented in the table.

Table 1: Moisture Content of C-G-PVA silver nanoparticles of *T. procumbens* film

S.NO	SAMPLE	INITIAL WEIGHT (g)	FINAL WEIGHT(g)	RESULT
1	C-G-PVA	0.2113	0.1286	39.139

The above observation indicates that the moisture content of the film may be due to the lower bonding strength found in the composite.

(Taylor *et al.,* 1997) have reported that the moisture content estimates determined for film coated seeds were less than those made for the non-coated seeds, particularly with film coating. The lower moisture content values may be attributed to low water holding capacity of the coating. Perhaps the film material added dry weight to the seed without significantly contributing to its moisture content. If this were the case, film coating may have had a greater build-up of material than the other films.

(Hamilton *et al.,* 2014) have showed that the residual cavities may lead to reduced rain penetration and higher inner stretcher temperature resulting in lower moisture content, which in turn will result in lower local thermal conductivity.

From the above discussed film based moisture content, chitosan gelatin-PVA incorporated with silver nanoparticles of *T. procumbens* film has lower bonding strength found in the composite.

4.2 WATER ABSORPTION CAPACITY

Water absorption capacity of the C-G-PVA were determined and the results are presented in table.

Table 2: Water Absorption Capacity of C-G-PVA

silver nanoparticles of *T. procumbens* film

S.NO	SAMPLE	HOURS (TIME INTERVAL)	INITIAL WEIGHT (g)	FINAL WEIGHT(g)	RESULTS (g)
1	C-G-PVA	I Hour	0.1756	0.6856	290.43
		II Hour		0.7124	305.69
		III Hour		0.7220	311.16
		XIV Hour		0.8053	358.60

It can be observed from the tabulated values that C-G-PVA transgels have shown higher water absorption capacity and the film was stable in water even upto 24 hours. Chitosan, gelatin and PVA transgels were disintegrated within one hour.The initial weight (M_1) higher than compare to 24th hour time intervel water capacity (M_2) may due to the less interaction of the hydrophilic groups found on the backbone of complexes with cross linking agents formaldehyde.

(Pereda *et al.*, 2009) have repoted that the strong interactions between caseinate and chitosan in forming polyelectrolyte complex films lead to fewer sites in the polymer matrix at which water can be held, and they are responsible for the reduced final water content.

The chitosan gerlatin-PVA film composite have stronger interactions as suggested by Pereda.

4.3 SOLUBILITY OF THE FILM

The solubility of the film was determined and the results are presented in the table.

Table 3: Solubility of the film of C-G-PVA

silver nanoparticles of *T. procumbens* film

S.NO	SAMPLE	INITIAL WEIGHT (M2) (g)	FINAL WEIGHT (M2) (g)	RESULT (g)
1	C-G-PVA	0.2486	0.1655	33.427

The result reveals that the solubility of the film was decreased over the incubation period of time and the final result shows that the solubility of the film was found to be 33%.

(Shieh, 1999) have reported that the high permeability of smaller molecules such as He and H_2 arises from their high diffusivity, whereas a larger molecule such as CO_2 is highly permeable because of its high solubility in the film. The low permeability of N_2, on the other hand, is attributed to both a low diffusivity and a low solubility in the film.

(Norman *et al*., 1963) have reported that the solubility of zinc phosphate cement was generally greater in dilute acetic acid than either silicate or zinc oxide- eugenol cements. The silicate cements, however, were more soluble in citric acid. Zinc oxide- eugenol was less soluble in water than were the other materials.

From the above discussed solubility of the film, C-G-PVA was less permeable while comparing Shieh and Norman.

4.4 SWELLING DEGREE

The swelling degree of the film was determined and the results are presented in the table.

Table 4: Swelling degree of C-G-PVA and silver nanoparticles of *T. procumbens* film

S.NO	SAMPLE	INITIAL WEIGHT (M1) (g)	FINAL WEIGHT (M2) (g)	RESULT (g)
1	C-G-PVA	0.1890	0.7610	302.65

It was observed from the above tabulated values that the swelling degree of the film was increased during the incubation period of 24 hours and the results shows that the film has higher swelling capacity.

(Tanigami *et al.,* 1995) have reported that the swelling rate is an exponentially decreasing function of X. The degree of crystallinity must have varied during swelling depending on the solvent composition, swelling temperature and initial crystallinity. So the X, values indicated are thought to be higher than those of once fully swollen films.

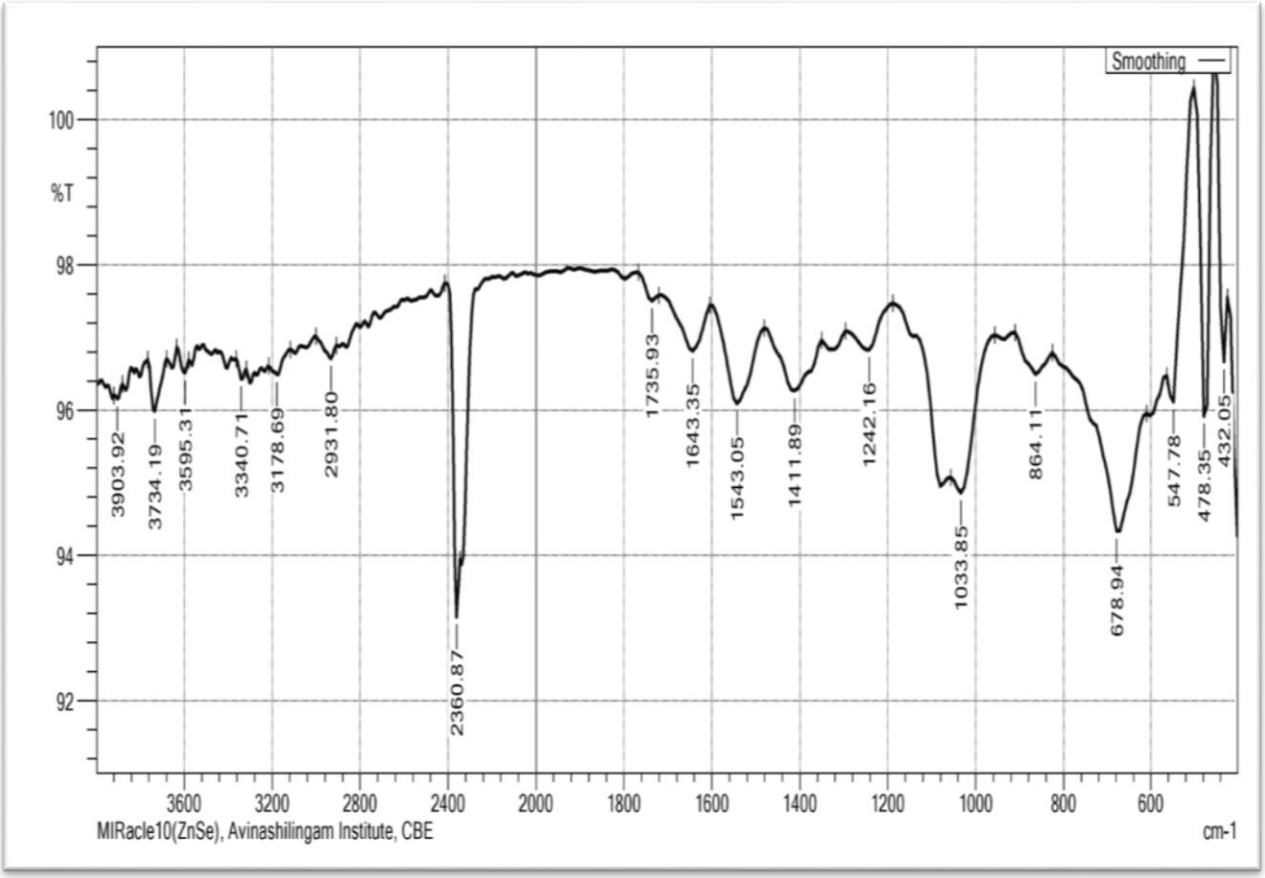
From the above discussed swelling degree of the chitosan composite reveals that the film have higher swelling degree property as indicated by Tanigami.

4.5 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

The Fourier Transform Infra-red (FTIR) spectrum of the film showed a absorption band between 2000 – 2500 cm-1.

Figure 6: FT-IR Spectrum of C-G-PVA

silver nanoparticles of *T. procumbens*



FTIR spectroscopy was performed to investigate the intermolecular interaction between C-G-PVA that was related to the physical and mechanical characters of the films, and the spectra are shown in Figure 6. The broad peaks 3340.71 and 3178.69 cm^{-1} were the stretching vibration of free hydroxyl group and it always overlaps with the stretching of N-H bonds in amino group. The peak at 2360.87 cm^{-1} were attributed to the vibration absorbance to $C0_2$ besides, the peak at 1411.89 cm^{-1} to phenol ring besides, the peak at 1033 absorbance of the C-O-C groups, the peaks at 1735.93 were the stretching vibration of C=O, the peaks at 678.94 cm^{-1} halogen compound (C-Br) respectively. It should be noted that there were obvious reflectance peaks for chitosan- gelatin at the wavelength where chitosan film showed characteristic peaks, suggesting that the effects of FTIR spectrum of PVP itself on that of chitosan could be excluded.

(Xie *et al.*, 1992) reported that the spectrum of the as-prepared film has strong absorption bands near 2100, 914, and 640 cm^{-1} that are associated with the stretching, bending, and wagging vibrations of - SiH,(x = l-3) groups, respectively. An Si-0-Si feature at 1100 cm^{-1} is also-evident which grows upon aging to become a dominant feature in the spectrum. After air exposure, new bands are observed at 2250, 2200, and 870 cm^{-1}

(Morent et al., 2008) have reported that the plasma treatment of PP leads to the incorporation of C–O, C O and O–C O groups. Therefore, it is believed that the broad OH peak is owing to the OH-groups of alcohols and carboxylic acids, while the C O peak confirms the presence of aldehydes, ketones and carboxylic acids on the surface.

(Gulmine et al., 2002) have reported that the represented graph shows the same absorptions, varying only in terms of intensity, which can be attributed to differences in film thickness. In the region 1300– 1400 cm^{-1} is displayed using an enlarged scale, and one can clearly see differences in the absorption pattern of the samples. Three bands assignable to CH2 and CH3 groups are present: band I at 1377 cm^{-1}, band II at 1366 cm^{-1} and band III at 1351 cm^{-1}.

4.6 3D SURFACE ACTIVE ANALYSIS

The 3D surface active analysis of the C-G-PVA composite film was done and the result was explained as below.

Table 5: 3D surface active analysis of C-G-PVA silver nanoparticles of *T. procumbens* film

	Cursor Left		Cursor Right		Cursor L-R		
	Avg Ht	Width	Avg Ht	Width	Step	Dist	Angle
1	463.18	7.990	458.99	7.904	-4.189	83.63	-2.868
2	466.19	7.990	462.86	7.904	-3.322	83.63	-2.274
3	467.34	7.990	466.51	7.904	-0.8315	83.63	-0.5696
Min	463.18	7.990	458.99	7.904	-4.189	83.63	-2.868
Max	467.34	7.990	466.51	7.904	-0.8315	83.63	-0.5696
Mean	465.57	7.990	462.79	7.904	-2.781	83.63	-1.904
SD	1.754	0	3.071	0	1.423	0	0.9740
Var%	0.377%	0%	0.664%	0%	-51.2%	0%	-51.2%

Figure 7: 3D surface active analysis of chitosan-gelatin-PVA silver nanoparticles of *T. procumbens* film

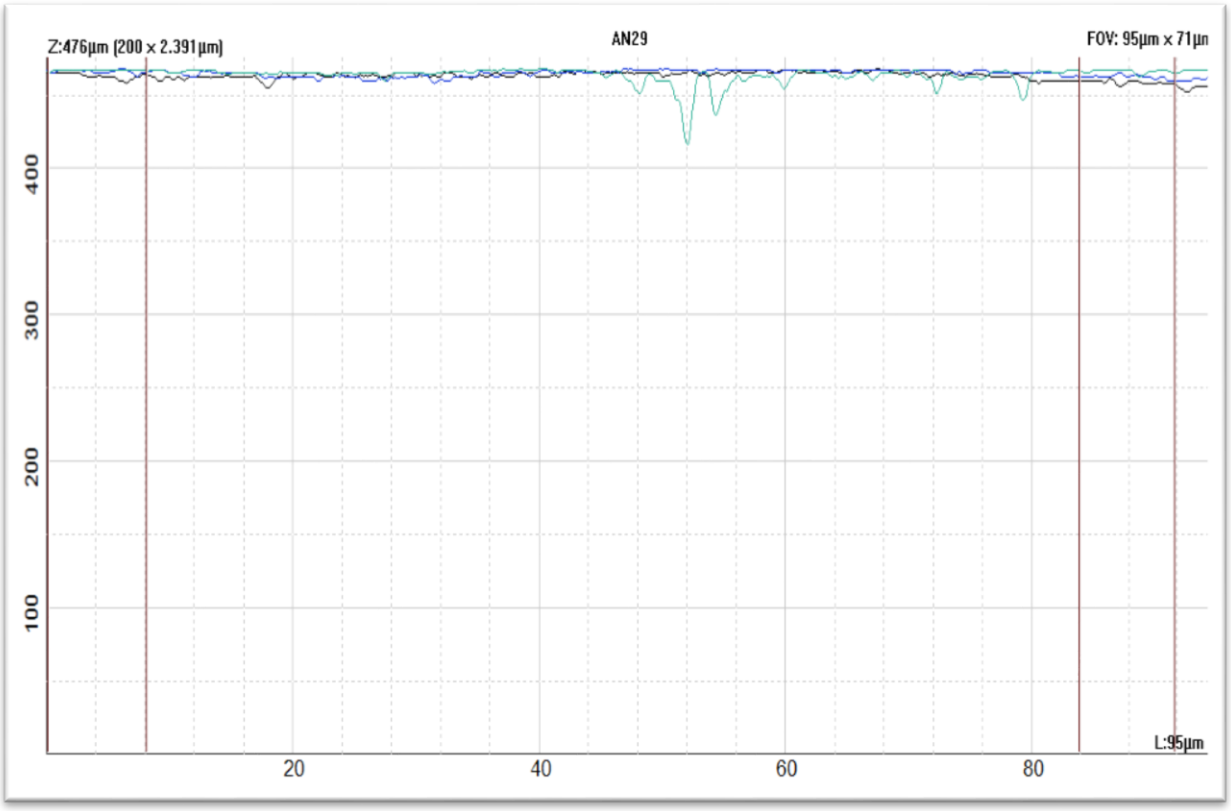


Table 6: Markers of 3D surface active analysis of C-G-PVA silver nanoparticles of *T. procumbens* film

	Markers 1-2							
	Ra	Rq	Rpv	Rp	Rv	Rsk	Rz	Rku
	2.068	2.618	14.25	5.030	9.219	-0.7640	7.688	3.822
	1.887	2.286	10.12	3.323	6.796	-0.5567	6.388	2.370
	3.126	5.865	52.49	5.003	47.49	-4.339	27.38	27.96
Min	1.887	2.286	10.12	3.323	6.796	-4.339	6.388	2.370
Max	3.126	5.865	52.49	5.030	47.49	-0.5567	27.38	27.96
Mean	2.361	3.590	25.62	4.452	21.17	-1.887	13.82	11.39
SD	0.5465	1.615	19.08	0.7985	18.64	1.736	9.602	11.74
Var%	23.1%	45.0%	74.5%	17.9%	88.0%	-92.0%	69.5%	103.1%

Figure 8: Markers of chitosan-gelatin-PVA silver nanoparticles of *T. procumbens* film

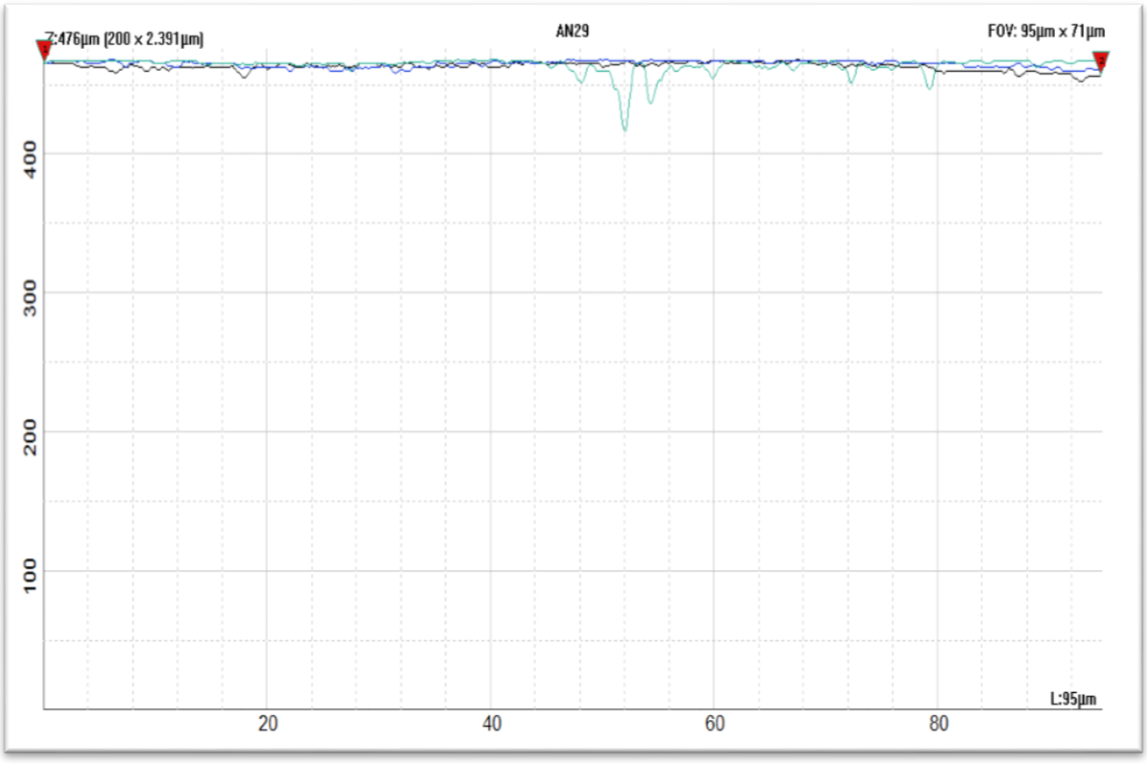


Figure 9: 3D surface active analysis of C-G-PVA silver nanoparticles of *T. procumbens* film

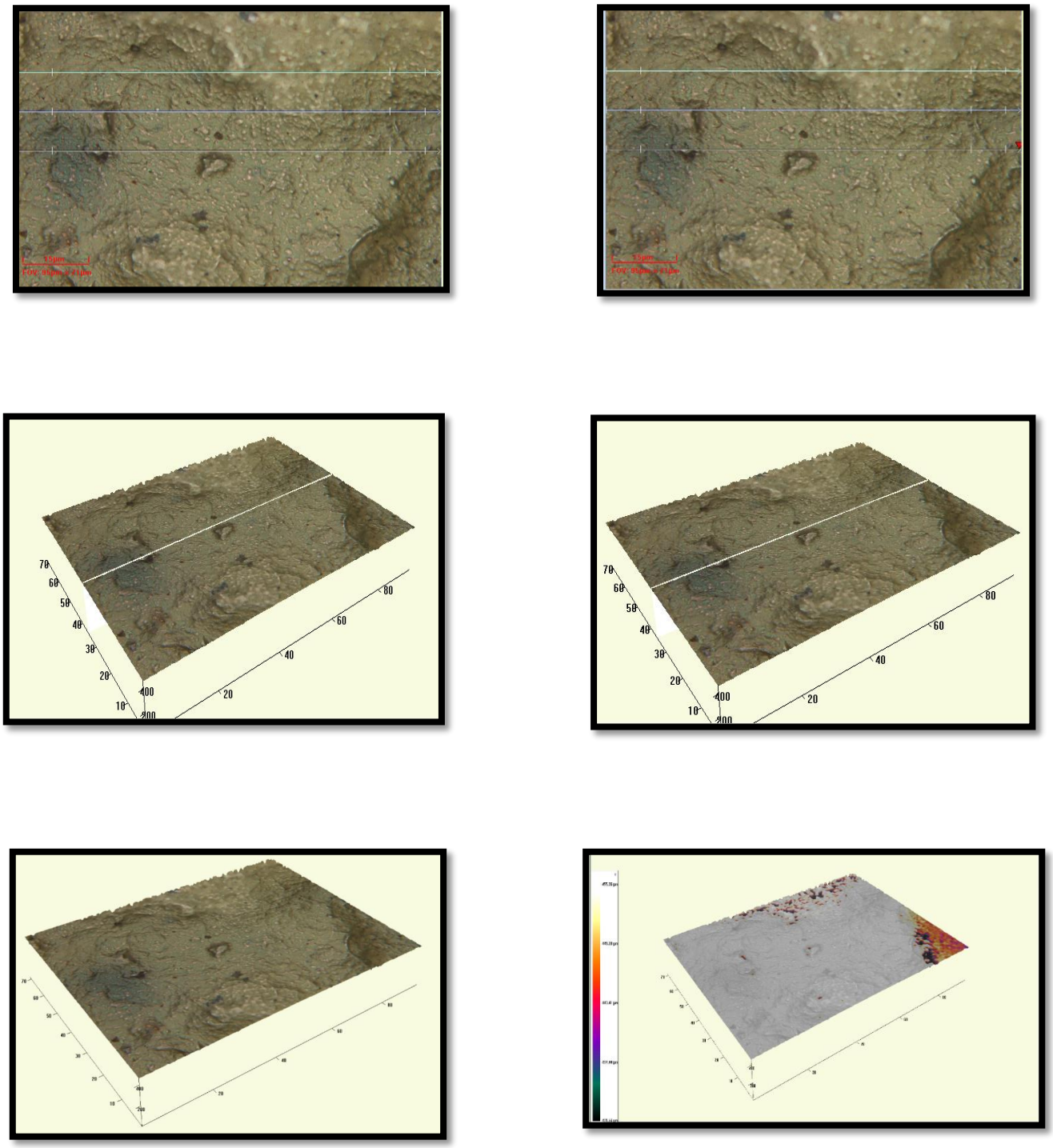
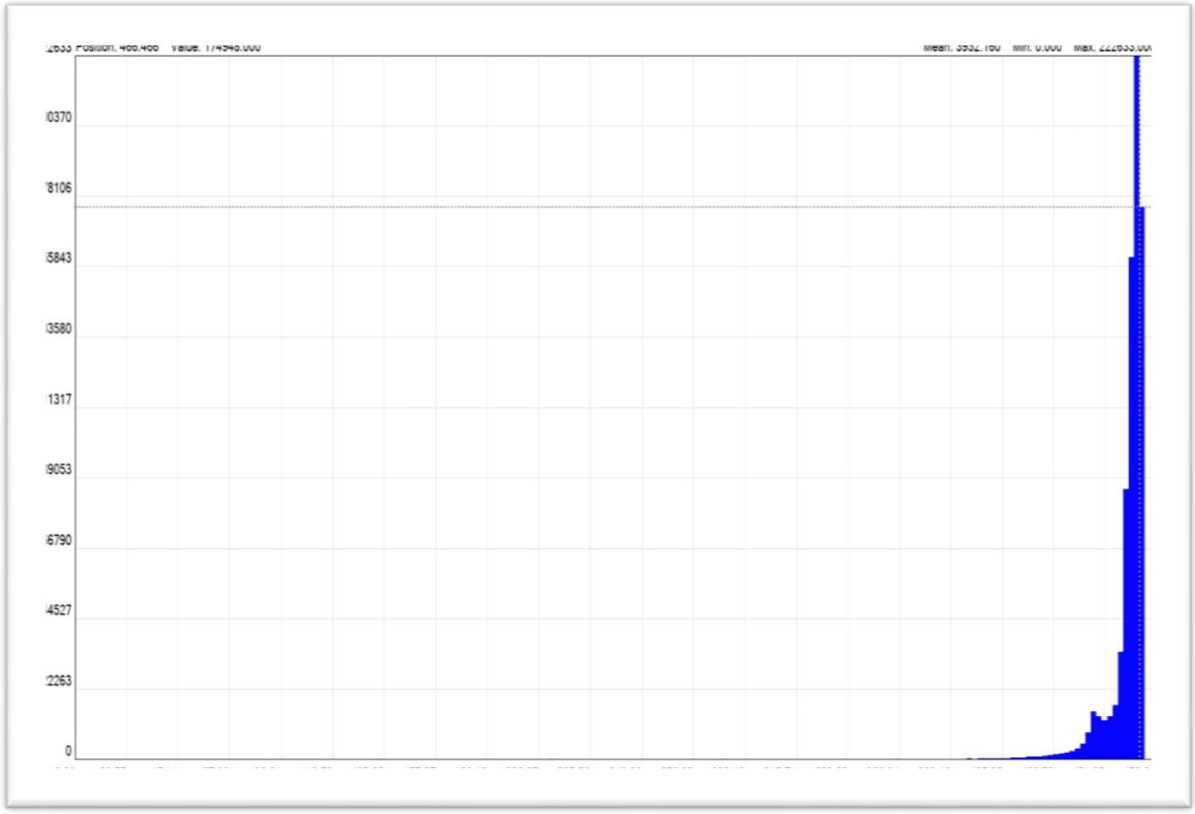


Figure 10: 3D analysis of chitosan-gelatin-PVA silver nanoparticles of *T. procumbens* film



3D surfaces of the C-G-PVA composite film was analyzed. Figure 8. reveals that the composite film has shows the percentage of contaminants and impurities inbetween 48 and 52 µm and the Figure 9. Shows the surface treatments to the depths of tens of microns at the same range. Depth analysis of the film which was shown in the images of Figure 10. Clearly represents the characterization of layer structure, buried feature and surface treatments that visualizes the indepth of the film. Figure 11. Represents that the film was compactible with retrospective 3D mappingof the depth profile.

(Xagas et al., 1999) have reported that the difference in surface parameters is clearly reflected in the photocatalytic behaviour. The photocatalytic performance of the TiO_2 films may be affected by the crystalline structure of TiO_2 (anatase has always shown a much higher activity than rutile, in the presence of O_2). However, in our case, this influence seems minor. On the contrary, the TR films which exhibited the best photocatalytic efficiency present rougher and more textured surface features and notably higher specific surface area.

(Peter de Groot, 2007) have reported that the instrument as described here operates with a narrowband light source, which can be a useful configuration at high magnifications using non achromatic optics. The basic concept extends to the more general case of non zero spectral bandwidth using the more extensive mathematical analysis. For highest accuracy, we often make use of this more detailed analysis even for narrow-bandwidth light sources where the interference phenomena are dominated by the objective NA but are nonetheless influenced by the spread in wavelengths.

4.7 Thermogavimetric Analysis (TGA)

Figure 11: Thermogavimetric Analysis of C-G-PVA silver nanoparticles of *T. procumbens* film

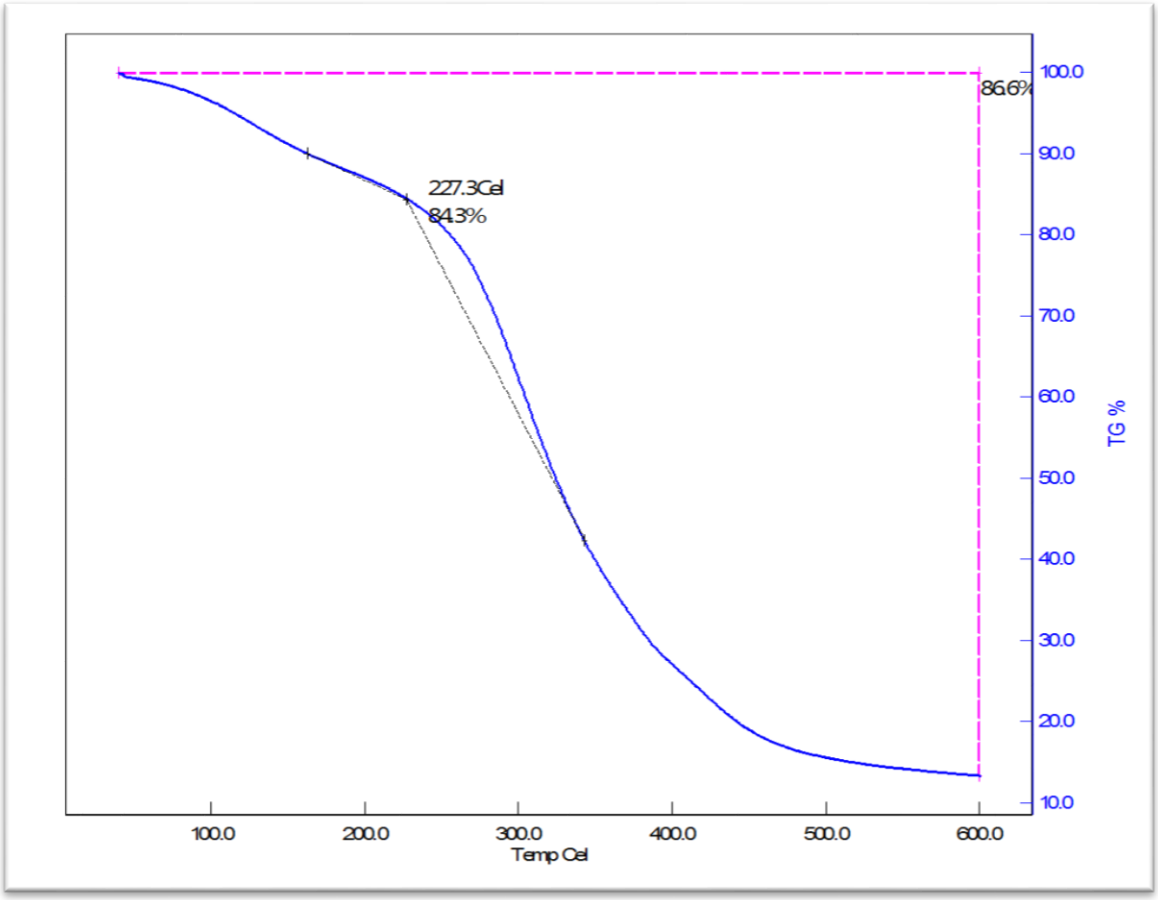
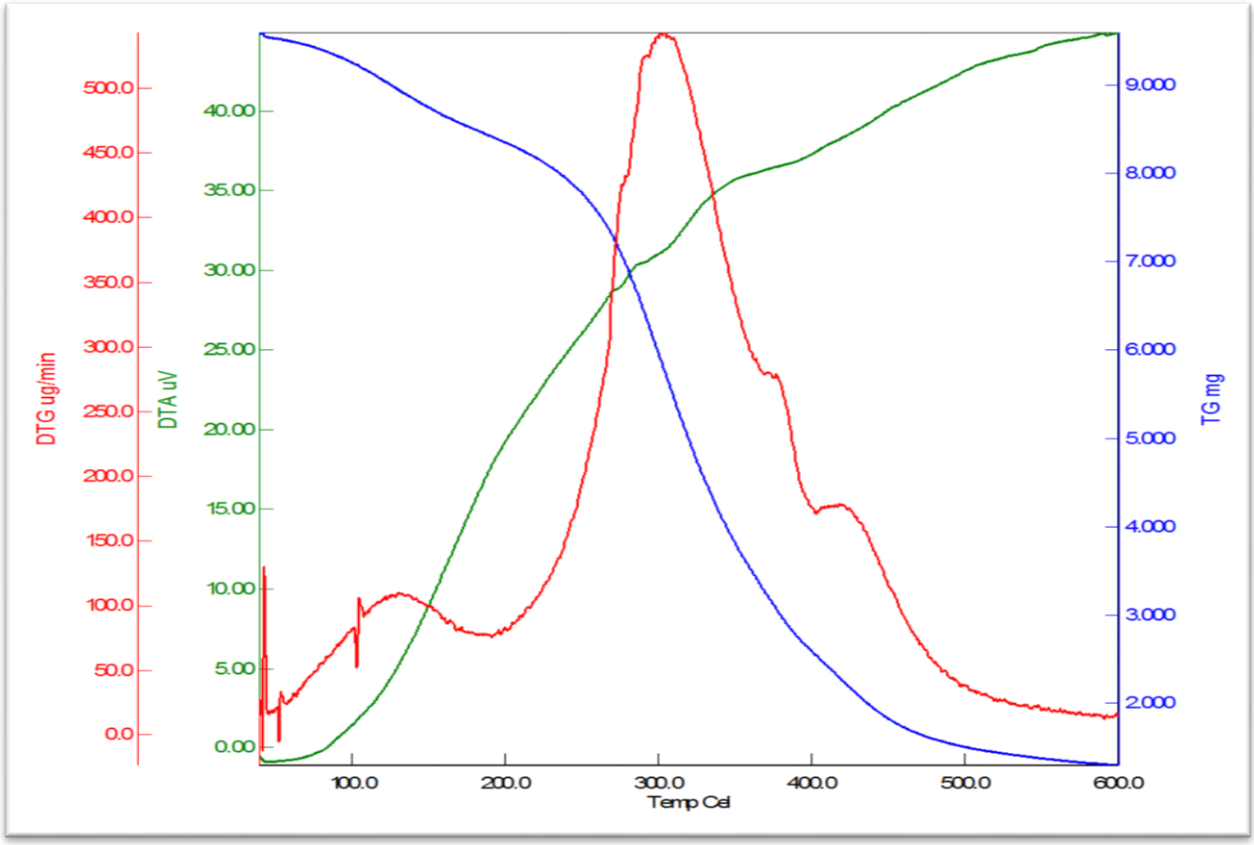


Figure 12: Thermogavimetric Analysis of chitosan-gelatin-PVA silver nanoparticles of *T. procumbens* film



Thermogravimetric analysis of the film composite was done for absoption, adsorption and desorption and also to measure temperature over time. The consistent with the higher amorphous phase content of DTAuV with a mass-gain rate of 84.3%/227.3C. The mass gain rate was evaluated as the slope of the TGA trace at the mean temperature of each region. So the data was sufficient to draw a conclusion regarding the effect of flow rate on oxidation rate.

(Motta *et al.*, 2002) have reported that the consistent with the higher amorphous phase content, the AC film in region I lost more water and much faster than ACMe OH and AC80, with a mass-loss rate of -0.11%/8C versus -0.07 and -0.05%/8C, respectively. The mass-loss rate was evaluated as the slope of the TGA trace at the mean temperature of each region, therefore expressed in wt.%/8C.

4.8 Scanning Electron Microscopy

The C-G-PVA composite was analyzed by Scanning Electron Microscopy and the result was reported below.

Figure 13: SEM images of surface morphology of the C-G-PVA silver nanoparticles of *T. procumbens* composite film

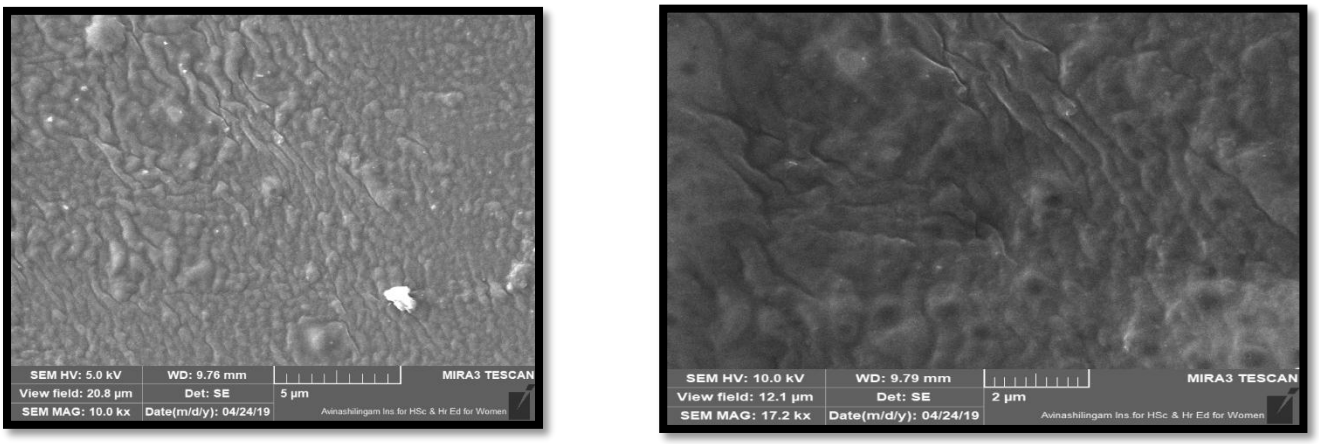
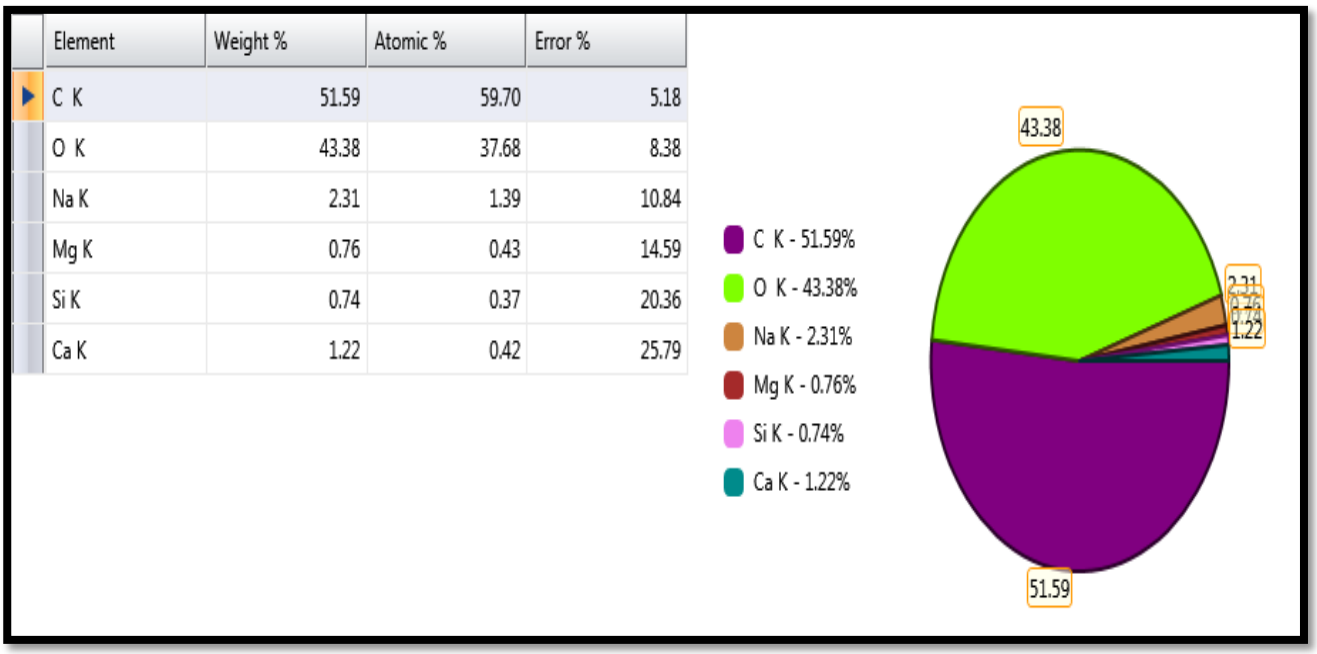


Figure 14: Elements present in the C-G-PVA silver nanoparticles of *T. procumbens* composite film analyzed by SEM

Figure 15: Elemental representation in the C-G-PVA silver nanoparticles of *T. procumbens* composite film analyzed by SEM



Element	Weight %	Atomic %	Error %
C K	51.59	59.70	5.18
O K	43.38	37.68	8.38
Na K	2.31	1.39	10.84
Mg K	0.76	0.43	14.59
Si K	0.74	0.37	20.36
Ca K	1.22	0.42	25.79

Scanning Electron microscopy of cross-linked C-G-PVA silver nanoparticles of *T. procumbens* was shown Figure 14. The results revealed that the cross-linked film composite was regular with randomly distributed spiral like structure. Many elements was present in the C-G-PVA composite film such as Na, Mg, Si, Ca that possesses nutrient advantage of the film.

(Phisalaphong, 2008) have reported that the BCC and BC film refers to BC film with and without the addition of chitosan in culture medium, respectively. BCC-MW 30,000 and 80,000 referred to sample of BCC films with the supplement of chitosan ofMW 30,000 and 80,000, respectively. The image reveals the well-organized fibril networks of BC and BCC films. By adding 0.75% chitosan, the obtained films were significantly denser as demonstrated in BCC-MW 30,000 and BCC-MW 80,000. The apparent BCC films were thicker and denser related to the chitosan content.

5.0 SUMMARY AND CONCLUSION

The rapid development of fish industry in recent times have contributed a lot of wastes expecially exoskeleton wastes which are thrown as such, these wastes are biodegradable and can be recycled and used for various applications. One such was the chitosan which was purified from the shrimp shells and used as a natural polymer in preparing a bioactive film for wound healing.

Silver nanoparticles of *T. procumbens* was prepared and chitosan-gelatin-PVA crosslinked with formaldehyde and silver nanoparticles of *T. procumbens* and cast into film and then dried. The film was removed and denoted as C-G-PVA.

The film prepared was characterized for their moisture content, water absorption capacity, solubility of the film, swelling degree, Fourier Transformed Infrared Spectroscopy, 3D surface active analysis, Thermogravimetric Analysis and Scanning Electron Mcroscopy. Each characterization reveals that the film was highly permeable and the film prepared was only a blend and there was no chemical reaction between the individuals of the composite.

From these studies, it can be concluded that the crosslinked C-G-PVA composite have shown a better moisture content, water absorption capacity, solubility of the film and swelling degree while comparing other un-crosslinked flims of various sources. The FTIR, 3D surface active analysis, TGA and SEM study, it can be concluded that the chitosan, gelatin and PVA composite was more biodegradable, because of less thermosstability of the composites which in turn reflects its low mechanical property as evidenced by water absortion capacity and solubility of the film.

Altogether the present study demonstrated that the bioactive film impregnated with the silver nanoparticles of *T. procumbens* which have a good wound healing potential incorporated with chitosan, gelatin and polyvinyl alcohol cross-linked with formaldehyde possesses an effective study on wound healing. However, future studies have to be carried out to find out the efficacy of in vivo activity using animal models.

Recommendation for future study

1. Antimicrobial, antibacterial, antioxidant activity can be studied.
2. X-ray crystallography, Nuclear Magnetic Resonance spectroscopy studies can be carried out to study the internal structure of the composite.
3. Animal studies can be carried out to confirm the biochemical, toxicological, pharmacological and immunological properties of the films.

BIBLIOGRAPHY

1. Abdeen, Z. (2011) Swelling and Reswelling Characteristics of Cross-Linked Poly (vinyl alcohol)/Chitosan Hydrogel Film, *Journal of Dispersion Science and Technology*, 32:1337–1344.
2. Ahamed, N. M. I. and Sastry, T. P (2011) Wound Dressing Application Of Chitosan Based Bioactive Compounds, *International Journal Of Pharmacy & Life Sciences*, 2(8):991-996.
3. Ahamed, N. M. I. and Sastry, T. P (2011) An In Vivo Study On The Wound Healing Activity Of Cellulose – Chitosan Composite Incorporated With Silver Nano Particles In Albino Rats, *International Journal of Research in Ayurveda & Pharmacy*, 2(4):1203-1209.
4. Ahmed, S., Ahmad, M., Jayachandran, M., Qureshi, M. A., and Ikram, S., (2015) Chitosan Based Dressings for Wound Care, *Immunochemistry & Immunopathology*.
5. Ambrogi, V., Donnadio, A., Pietrella, D., Latterini, L., Proietti, F. A., Marmottini, F., Padeletti, G., Kaciulis, S., Giovagnolia, S. and Riccia, M. (2014) Chitosan Films Containing Mesoporous Sba-15 Supported Silver Nanoparticles For Wound Dressing, *Journal of Materials Chemistry B,* 2:6054–6063.
6. Aranaz, I., Mengíbar, M., Harris, R., Paños, I., Miralles, B., Acosta, N., Galed, G. and Heras A. (2009) Functional Characterization of Chitin and Chitosan, *Current Chemical Biology*, 3(2): 203- 230**.**
7. Arbia, W., Leil, A., Adour, L. and Amrane, A., *(2013)* Chitin Extraction from Crustacean Shells Using Biological Methods – A Review, *Food Technol, Biotechnol, 51*(1):12–25.
8. Azad, A. K., Sermsintham, N., Chandrkrachang, S. and Stevens, W. F. (2004) Chitosan Membrane as a Wound-Healing Dressing: Characterization and Clinical Application, *Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater*, 69B:216–222.
9. Babu, R., Connor, K. E. O. and Seeram, R. (2013) Current Progress On Bio-Based Polymers And Their Future Trends, *Progress in Biomaterials*, 2.
10. Bonard, J. M., Dean, K. A., Coll, B. F. and Klinke, C. (2002) Field Emission of Individual Carbon Nanotubes in the Scanning Electron Microscope, *Physical review letters*, 89:197602.
11. Caruso, F., Furlong, D. N., Ariga, K., Ichinose, I. and Kunitake, T. (1998) Characterization of Polyelectrolyte-Protein Multilayer Films by Atomic Force Microscopy, Scanning

Electron Microscopy and Fourier Transform Infrared Reflection-Absorption Spectroscopy, *American Chemical Society, Langmuir,* 14:4559-4565.

12. Cevher, E. and Sezer, A. D. (2011) Biopolymers as Wound Healing Materials: Challenges and New Strategies, *Biomaterials Applications for Nanomedicine, Prof. Rosario Pignatello (Ed.), InTech*.
13. Chen, J. Q., Yang, Y. B., Zhao, Y. L., Bai, J. W., Ding, W. Y., Zhou, Y. H., Chen, X. Y. and Li, Y. H. (2016) Sub-MICs of Azithromycin Decrease Biofilm Formation of Streptococcus suis and Increase Capsular Polysaccharide Content of S.suis, *Front Microbiol*, 7:1659.

14. Dai, T., Tanaka, M., Huang, Y. Y. and Hamblin, M. R., (2011) Chitosan Preparations For Wounds And Burns: Antimicrobial And Wound-Healing Effects, *Expert Review of Anti-infective Therapy*, 9(7):857-79.

15. Donda, M. R., Kudlea, K. R., Alwalaa, J., Miryalaa, A., Sreedharb, B. and Rudraa M. P. P. (2013) Synthesis Of Silver Nanoparticles Using Extracts Of Securinega Leucopyrus And Evaluation Of Its Antibacterial Activity, *International Journal of Current Science, 7:E 1-8.*
16. Estaca, J. G., de Lacey, A. L., Caballero, M. E. L., Guillén, M. C. G. and Montero, P. (2010) Biodegradable Gelatin - Chitosan Films Incorporated With Essential Oils As Antimicrobial Agents For Fish Preservation, *Food Microbiology,* 27:889-896.
17. Ghadi, R., Jain, A., Khan, W., Domb, A. J.,(2016) Micro particulate polymers and hydrogels for wound healing, *Wound Healing Biomaterials*, 6:203-225.
18. Groot, P. and Lega, Z. C. (2007) Angle-Resolved Three-Dimensional Analysis Of Surface Films By Coherence Scanning Interferometry, *Optics Letters,* 32(12):1638-1640.
19. Grumezescu, A. M. and Mogos¸ G. D. (2013) Natural And Synthetic Polymers For Wounds And Burns Dressing, *International Journal of Pharmaceutics, 463:127–136.*
20. Grumezescu, A.M. and Mogos, G. D. (2014) Natural And Synthetic Polymers For Wounds And Burns Dressing, *International Journal of Pharmaceutics*, 463(2).
21. Gulmine, J. V., Janissek, P. R., Heise, H. M. and Akcelrud, L. (2002) Polyethylene Characterization by FTIR, *Polymer Testing,* 21:557–563.

22. Hong, K. and Meyers, S. P. (2010) Preparation and Characterization of Chitin and Chitosan — A Review, *Journal of Aquatic Food Product Technology*, 4(2):27-52.
23. Hosseini, M. H., Razavi, S. H. and Mousavi, M. A. (2009) Antimicrobial, Physical And Mechanical Properties Of Chitosan-Based Films Incorporated With Thyme, Clove And Cinnamon Essential Oils, *Journal of Food Processing and Preservation,* 33:727–743.
24. Johnson, C. E., Hasting, M. A. S. and Weimer, W. A. (1990) Thermogravimetric Analysis of the Oxidation of CVD Diamond Films, *Journal of Materials Research,* 5(11):2320-2325.
25. Kanatt, S. R., Rao, M. S., Chawla, S. P., Sharma, A. (2012) Active Chitosan - Polyvinyl Alcohol Films With Natural Extracts, *Food Hydrocolloids,* 29:290-297.
26. Kanatt, S. R., Jethwa, T., Sawant, K., and Chawla, S. P. (2017) PVA-Gelatin Films Incorporated with Tomato Pulp: A Potential Primary Food Packaging Film, *International Journal of Current Microbiology and Applied Sciences,* 6(10):1428-1441.
27. Kaushik, A., Verma, G. and Ghosh, A. K. (2016) Nano-Interfaces Between Clay Platelets And Polyurethane Hard Segments In Spray Coated Automotive Nanocomposites, *Progress in Organic Coatings*, 99:282–294.
28. Ke, T. and Sun, X. S. (2003) Starch, Poly(lactic acid), and Poly(vinyl alcohol) Blends, *Journal of Polymers and the Environment* , 11(1):7–14.
29. Kethamakka, S. R. P. & Deogade, M. S. (2014) Jayanti veda (Tridax procumbens) - Unnoticed Medicinal plant by Ayurveda, *Journal of Indian System of Medicine*, 2:6-20.
30. Koide, S. S. (1998) Chitin-Chitosan Properties, Benefits And Risks, *Nutrition Research* 18(6):1091-1101.
31. Krishnaveni, B., Neeharika, V., Venkatesh, S., Padmavathy, R. and Reddy, B. M. (2009) Wound Healing Activity of Carallia brachiata Bark, *Indian Journal of Pharmaceutical Sciences*, 71(5):576–578.
32. Kumar, P. T. S., Praveen, G., Naduthottathil, M., Chennazhi, K. P., and Rangasamy, J. (2014) Flexible, Micro-Porous Chitosan–Gelatin Hydrogel, Nanofibrin Composite Bandages For Treating Burn Wounds, *The Royal Society of Chemistry Advances,* 110(4):65081–65087.

33. Lee, D. H. and Condrate, R. A. (1999) FTIR Spectral Characterization Of Thin Film Coatings Of Oleic Acid On Glasses: I. Coatings On Glasses From Ethyl Alcohol, *Journal Of Materials Science*, 34:139–146.
34. Li, F. G. N., Smith, A. Z. P., Biddulph, P., Hamilton, I. G., Lowe, R., Mavrogianni, A., Oikonomou, E., Raslan, R., Stamp, S., Stone, A., Summerfield, A. J., Veitch, D., Gori, V. and Oreszczyn, T. (2014) Solid-Wall U-Values: Heat Flux Measurements Compared With Standard Assumptions, *Building Research & Information,* 43(2):238-252.
35. Liu, Z. G., Zhang, X. F., Shen, W. and Gurunathan, S., (2016) Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches, *International Journals of Molecular Science*, 17(9), 1534.
36. Malik, C. P. and Kushwaha, H. M. (2014) Biosynthesis Of Silver Nanoparticles Using Fresh Extracts Of Tridax procumbers Linn, *Indian Journal of Experimental Biology*, 52:359-368.
37. Matsumura, S., Tomizawa, N., Toki, A., Nishikawa, K. and Toshima, K. (1999) Novel Poly(vinyl alcohol)-Degrading enzyme and the Degradation mechanism, *Macromolecules* 32:7753–7761.
38. Maver, T., Maver, U., Kleinschek, S., Smrke, D. M. and Kreft, S. (2015) A Review Of Herbal Medicines In Wound Healing, *International Journal of Dermatology*, 54(7):740-51.
39. Mir, M., Ali, M. N., Barakullah, A., Gulzar, A., Arshad, M., Fatima, S. and Asad, M. (2018) Synthetic Polymeric Biomaterials for Wound Healing: A Review, *Progress in Biomaterials,* 7:1-21.
40. Mir, S. A., Jan, Z., Mir, S., Dar, A. M. and Chitale, G. (2017) Concise Review on Biological Activity of Tridax procumbens Linn, *Organic Chemistry Current Research,* 6:177.
41. Morent, R., Geyter, N. D., Leys, C., Gengembre, L. and Payen, E. (2008) Comparison Between Xps And Ftir - Analysis Of Plasma-Treated Polypropylene Film Surfaces, *Surface and Interface Analysis,* 40:597-600.
42. Motta, A., Fambri, L. and Migliaresi, C. (2002) Regenerated Silk Fibroin Films:Thermal and Dynamic Mechanical Analysis, *Macromolecular Chemistry and Physics*, 203(10-11):1658-1665.

43. Nallella, Sreeramulu, Sateesh, S., Ragan, A. and Raju, V. S. (2013) Ethno-Botanico-Medicine For Common Human Ailments In Nalgonda And Warangal Districts Of Telangana, Andhra Pradesh, India, *Annals of Plant Sciences*, 2(7):220–9.6.
44. Nishiyama, H., Suga, M., Ogura, T., Maruyama, Y., Koizumi, M., Mio, K., Kitamura, S. and Sato, C. (2010) Reprint of : Atmospheric Scanning Electron Microscope Observes Cells And Tissues In Open Medium Through Silicon Nitride Film, *Journal of Structural Biology*, 172:191–202.
45. Norman, R. D., Swartz, M. L. and Phillips, R. W. (1963) Studies on Film Thickness, Solubility and Marginal Leakage of Dental Cements, *Journal of Dental Research*, 42(4): 950–958.
46. Ojagh, S. M., Rezaei, M., Razavi, S. H. and Hosseini, S. M. H. (2010) Development And Evaluation Of A Novel Biodegradable Film Made From Chitosan And Cinnamon Essential Oil With Low Affinity Toward Water, *Food Chemistry, 122:161–166.*
47. Ojeda-Martínez, M. L., Yanez-Sánchez, I., Ordoñez, C. V., Martinez-Palomar, M. M., Rodríguez, A. A., Gracia-Sánchez, M. A., González, F. R. and Galvez-Gastélum, F. J. (2015) Skin Wound Healing With Chitosan Thin Films Containing Supported Silver Nanospheres, *Journal Of Bioactive And Compatible Polymers, 30(6):1–16.*
48. Olefjord, I., Mathieu, H. J. and Marcus, P. (1990) Intercomparison of Surface Analysis of Thin Aluminium Oxide Films, *Surface and Interface Analysis*, 15:681–692.
49. Park, Y. D., Lee, H. S., Choi, Y. J., Kwak, D., Cho, J. H., Lee, S. and Cho, K. (2009) Solubility- Induced Ordered Polythiophene Precursors for High- Performance Organic Thin- Film Transistors, *Advanced Functional Materials*, 19:1200-1206.
50. Patel, D. K. (2014) Some Traditional Medicinal Plants Useful For Boil, Burn and For Wounds Healing, *Journal of Biodiversity & Endangered Species*, 2:133.
51. Patil, P. S. and Kadam, L. D. (2002) Preparation And Characterization Of Spray Pyrolyzed Nickel Oxide (Nio) Thin Films, *Applied Surface Science,* 199:211–221.
52. Pereda, M., Aranguren, M. I. and Marcovich, N. E. (2009) Water Vapor Absorption and Permeability of Films Based on Chitosan and Sodium Caseinate, *Journal of Applied Polymer Science*, 111:2777–2784.
53. Phisalaphong, M. and Jatupaiboon, N. (2008) Biosynthesis and Characterization of Bacteria Cellulose–Chitosan Film, *Carbohydrate Polymers*, 74:482–488.

54. Ponnaian, P. K., Oommen, R., Kannaiyan, S. K. C., Jayachandran, S., Natarajan, M., and Santhanam, A., (2015) Synthesis and Characterization of Silver Nanoparticles for Biological Applications, *J. Environ. Nanotechnol*, 4(1), 23-26.
55. Ramos, M., Valdes, A., Beltran, A. and Garrigos, M. C. (2016) Gelatin-Based Films and Coatings for Food Packaging Applications, *Coatings*, 6(4):41.
56. Ravikumar, V,. Shivashangari, K.S. and Devaki, T. (2005) Hepatoprotective activity of Tridax procumbens against d-galactosamine/lipopolysaccharide-induced Hepatitis in Rats, *Journal of Ethnopharmacology*, 101(1-3):55-60.
57. Salmean, G. G., Gutierrez, G. A., Jesus, V. C., Ortega, M. H., Carmona, L. V., Chamorro, G. (2016) Methods for Extraction, Isolation and Purification of C-phycocyanin: 50 years of Research in Review, *International Journal of Food and Nutritional Science*, 3.
58. Samani, P. and Bell, G. (2016) Experimental Evolution of the Grain of Metabolic Specialization in Yeast, *Ecology and Evolution*, 6(12):3912-3922.
59. Sengoza, B. and Agarb, E. (2007) Effect Of Asphalt Film Thickness On The Moisture Sensitivity Characteristics Of Hot-Mix Asphalt, *Building and Environment*, 42:3621–3628.
60. Sharma, P., Hu-Lieskovan, S., Wargo, J.A. and Ribas, A. (2017) Primary, Adaptive, And Acquired Resistance To Cancer Immunotherapy, *Cell*, 168(4):707–723.
61. Shieh, J. J. and Chung, T. S. (1999) Gas Permeability, Diffusivity, and Solubility of Poly (4-Vinylpyridine) Film, *Journal of Polymer Science: Part B:Polymer Physics*, 37:2851–2861.
62. Sun, S., Yang, S., Tan, G., Costanzo, M., Oughtred, R., Hirschman, J., Theesfeld, C., Bansal, P., Sahni, N., Yi, S., Yu, A., Tyagi, T., Tie, C., Hill, D., Vidal, M., Andrews, J., Boone, C., Dolinski, K., Roth, F. (2016) An Extended Set Of Yeast-Based Functional Assays Accurately Identifies Human Disease Mutations, *Genome Research*, 26(5).
63. Taddei, A. and Romero, A. J. R. (2000) Bioactivity studies of extracts from Tridax procumbens, *Phytomedicine, Urban & Fischer Verlag,* 7(3):235-238.
64. Taylor, A. G., Grabe, D. F. and Paine, D. H. (1997) Moisture Content and Water Activity Determination of Pelleted and Film-Coated Seeds, *Association Of Official Seed Analysts And The Society Of Commercial Seed Technologists,* 19(1):24-32.

65. Tetsuya Tanigami, T., Yano, K., Yamaura, K. and Matsuzawa, S. (1995) Anomalous Swelling Of Poly (Vinyl Alcohol) Film In Mixed Solvents Of Dimethylsulfoxide And Water, *Polymer*, 36(15):2941-2946.
66. Udupa, A. L., Kulkarni, D. R. and Udupa, S. L. (1995) Effect Of Trzdax Procumbens Extracts On Wound Healing, *International Journal of Pharmacognosy,* 33(1):37-40.
67. Venkatesan, J., Kim, S. K., Nithya. R., and Sudha, P. N. (2014) Role Of Alginate In Bone Tissue Engineering, *Advances in Food & Nutrition Research*, 73:45-57.
68. Vignesh, J. V., Stalin, S., Raj, M. B., Stanley, S. A., (2018) Studies On The Extraction And Characterization Of Chitin From The Peels Of Various Waste Sources, *European Journal of Biotechnology and Bioscience*, 6(2), 43-47.
69. Xagas, A. P., Androulaki, E., Hiskia, A. and Falaras, P. (1999) Preparation, Fractal Surface Morphology And Photocatalytic Properties Of Tio_2 Films, *Thin Solid Films,* 357:173-178.
70. Xie, Y. H., Wilson, W. L., Ross, F. M., Mucha, J. A., Fitzgerald, E. A., Macaulay, J. M. and Harris, T. D. (1992) Luminescence And Structural Study Of Porous Silicon Films. *Journal of Applied Physics,* 71:2403-2407.
71. Yao, K. D., Liu, J., Chenc, C. X., Lu, X. D., Tu, H. L. and Silva J. A. L. D. (1996) Swelling Behavior of Pectin/Chitosan Complex Films, *Journal of Applied Polymer Science,* 60:279-283.
72. Younes, I. and Rinaudo, M. (2015) Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications, *Marine drugs,* 13:1133-1174.