

PREPARATION AND CHARACTERIZATION OF WOUND HEALING CHITOSAN HYDROGEL WITH EXTRACTS FROM *ACALYPHA INDICA* USING SILVER NANOPARTICLE

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science Degree in Biotechnology

BY

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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This is to certify that this Project Report is the Bonafide work of **N.Balaswarupini** (40770007) who carried out the project entitled **"PREPARATION AND CHARACTERIZATION OF WOUND HEALING CHITOSAN HYDROGEL WITH EXTRACTS FROM ACALYPHA INDICA USING SILVER NANOPARTICLE"**

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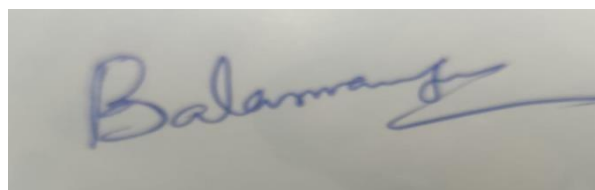
EXTERNAL EXAMINER

DECLARATION

I **N.Balaswarupini** (40770007) hereby declares that the Project Report entitled **“PREPARATION AND CHARACTERIZATION OF WOUND HEALING CHITOSAN HYDROGEL WITH EXTRACTS FROM *ACALYPHA INDICA* USING SILVER NANOPARTICLE”** done by me under the guidance of **Dr.S.Kumaran** (external guide) and **Dr.L.Inbathamizh** (internal guide) at **COL. DR. JEPPIAAR RESEARCH PARK CENTER FOR DRUG DISCOVERY AND DEVELOPMENT**, submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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A photograph of a handwritten signature in blue ink on a light-colored surface. The signature appears to be 'Balaswarupini' followed by a stylized flourish.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

This study investigates the efficacy of a chitosan hydrogel containing extracts from *Acalypha indica* and silver nanoparticles for wound healing. The hydrogel was prepared using a sol-gel method and characterized for its physical and chemical properties. The extracts from *Acalypha indica* were obtained using a solvent extraction method and characterized using various spectroscopic techniques. Silver nanoparticles were synthesized using a green synthesis method and characterized for their size and morphology. The chitosan hydrogel containing the extracts and silver nanoparticles was evaluated for its wound healing properties using in vitro and in vivo models. The results indicate that the chitosan hydrogel with extracts from *Acalypha indica* and silver nanoparticles promoted wound healing by reducing inflammation, increasing collagen synthesis, and accelerating tissue regeneration. This study provides a promising strategy for the development of effective wound healing therapies using natural extracts and silver nanoparticles. The antimicrobial and wound healing properties of the chitosan hydrogel with *Acalypha indica* extract and silver nanoparticles were evaluated using in vitro assays. Results showed that the chitosan hydrogel with *Acalypha indica* extract, and silver nanoparticles exhibited enhanced antimicrobial activity against common wound pathogens, as well as improved wound healing properties, including increased cell proliferation and migration. This suggests that the developed chitosan hydrogel with *Acalypha indica* extract, and silver nanoparticles has potential as a wound dressing material with enhanced healing properties

KEYWORDS: *Acalypha indica*, hydrogel, chitosan, silver nanoparticle

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LIST OF ABBREVIATIONS

AgNp	-	Silver Nanoparticle
PVA	-	Poly Vinyl Alcohol
FTIR	-	Fourier-transform infrared spectroscopy
XRD	-	X-ray diffraction spectroscopy
SEM	-	SCANNING ELECTRON MICROSCOPE
TEM	-	Transmission electron microscopy
CHI	-	CHITOSAN
ECM	-	Extra cellular Medium
CSNF	-	Chitosan Silver Nanoparticle Film

CHAPTER- 1

INTRODUCTION

The skin is the largest organ in the human body, covering approximately 2m² total body surface area (Gupta et al., 2013; Chen et al., 2018). It keeps the body's homeostasis in check by acting as a barrier against invading infections like microbial outbreaks (Chen et al., 2018). When various stimuli attack the skin or any other organ, the resulting injury is known as a wound (Rajendran et al., 2018; Nethi et al., 2019). Surgical operations, cuts, chemicals, pressure, friction, heat blow, stress, shear, and certain diseases are among the most common external stimuli capable of causing a wound (Tejiram et al., 2016; Ovais et al., 2018). Surgical operations, cuts, chemicals, pressure, friction, heat blow, stress, shear, and certain diseases are among the most common external stimuli capable of causing a wound (Ather & Harding, 2009). Wound healing is a voluntary physiological process that occurs as the skin or other body parts respond to the injury (Parani et al., 2016; Rajendran et al., 2018). AgNPs are proposed for this purpose in this study because they have antibacterial and anti-inflammatory properties that aid in wound healing. These properties, however, are limited because AgNPs have been shown to be ineffective against resistant bacterial strains such as *P. aeruginosa*, which is common in wounds. Furthermore, AgNPs have been linked to high levels of toxicity in human cells (You et al., 2012).

1.1 WOUND HEALING

The human body uses a physiological process known as "wound healing" to heal itself (Kasuya & Tokura, 2014). Wound healing is defined as a dynamic process in which the skin or any affected body tissue repairs itself involuntarily after trauma (Naraginti et al., 2016; Rajendran et al., 2018; Chakrabarti et al., 2019). The process begins immediately after the injury and progresses through four well-orchestrated and sometimes overlapping stages, which are summarized as homeostasis, inflammation, proliferation, and remodeling (Kalashnikova & Seal, 2015; Franková et al., 2016; Ovais et al., 2018). A variety of specific factors, such as cytokines, Chemokines, enzymes, and growth factors, direct and control each stage (Kalashnikova & Seal, 2015; Franková et al., 2016; Orłowski et al., 2018).

Under normal conditions, an acute wound should heal in 2-3 weeks at most, followed by the remodeling stage, which can last up to two years. If it does not fully heal during that time, it will progress to the chronic stage (Rajendran et al., 2018). External and internal factors that slow wound healing are well documented; the former includes medication, malnutrition, lifestyle habits such as smoking and excessive drinking, and comorbidities such as diabetes and obesity (Kalashnikova & Seal, 2015; Nethi et al., 2019). Internal factors include necrotic tissue, wound infection, foreign species entrapment, and pathogenic contamination (Parani et al., 2016; Rajendran et al., 2018; Mihai et al., 2019).

1.2 HYDROGEL

When Wicht Erle and Lim of the Czechoslovakian Academy of Sciences demonstrated the formation of poly (2-hydroxyethyl methacrylate) hydrogels, they sparked widespread interest in hydrogels (Souza et al., 2016). There are numerous methods for making hydrogels nowadays, but they are classified as either physical cross-linking (via hydrogen bond formation, amphiphilic polymers, crystallization, stereo complex formation, and ionic interactions) or chemical cross-linking (reaction of complementary chemical groups; cross-linking with a use of a cross-linker; through the addition or condensation reactions; using ionizing or UV radiation including radical polymerization and crosslinking).

Over the years, hydrogels have piqued the interest of researchers for a variety of reasons, including fundamental reasons and the potential for a wide range of applications. Because of their high-water content and consequent biocompatibility, hydrogels have been used successfully in biomedical fields. Soft contact lenses, wound dressings, super absorbents, and drug-delivery systems are all examples of successful innovations. Cell-based therapeutics and soft tissue engineering are the most recent and exciting applications of hydrogels. A collagen hydrogel was the biomaterial used to grow the first living, tissue-engineered skin product.

While the success of skin tissue engineering is encouraging, efforts to engineer other soft tissues have not been as fruitful. The inappropriate properties of the currently available biomaterials are limiting progress in large part. The materials that support and contact the cells must be carefully designed to elicit the desired cell response and coax cells to assemble into functional tissues (Kaur et al., 2019; Nethi et al.,

2019). Critical design parameters for hydrogels used in cell-based therapeutics and soft tissue engineering include both physical (such as appropriate mechanical strength and integrity) and biological properties (such as nontoxicity and the ability to incorporate appropriate biological determinants (cell-binding domains, enzyme recognition sites). Hydrogels have numerous applications. They are created, for example, as scaffolds for cell proliferation and differentiation towards a tissue, ii) using rapid prototyping techniques to create 3D materials for cell seeding, iii) as wound dressing as stimuli-responsive and intelligent systems for drug delivery, and iv) for other medical and pharmaceutical applications.

Hydrogels are three-dimensional polymeric networks that have seen success in a variety of biomedical applications. They act as an extracellular matrix substitute and can create a moist environment to aid in wound healing. Hydrogels have been created through a variety of physical and chemical processes, such as gamma and UV ray radiation and freeze-thaw processes. The freeze-thaw technique produces simple hydrogels with a high ability to absorb wound exudates while requiring no toxic or chemical additives. Exudate accumulation at the wound's surface slows healing and, as a result, increases the risk of bacterial growth (Mihai et al., 2019; Nethi et al., 2019). A variety of biopolymer growth has been used to create hydrogel wound dressings. Chitosan and PVA, for example, are commonly used in hydrogels due to their biocompatibility, low cost, and non-toxicity.

Hydrogels are hydrophilic polymer networks that can absorb and retain a large amount of water. The presence of cross-links between polymer chains, which prevents the polymer from dissolving, is denoted by the network term. The type of network and the density of cross-links determine the swelling properties of hydrogels. Hydrogels can swell significantly while maintaining their original shape when immersed in aqueous media. Hydrogels have been compared to biological tissue in terms of physical similarity. In addition to high water absorption, ideal hydrogel properties include body stability and durability, biodegradability with no toxic byproducts, and desirable release kinetics when used as a drug delivery system. Hydrogels can have a porous or a tight structure. They typically have pore sizes ranging from 5 to 100 nm, which is determined by the density of crosslinks within the gel matrix (Bansod et al., 2015; Bhatia, 2016; Ovais et al., 2018). The incorporation of therapeutics has recently been studied. The incorporation of both

small and large molecules has yielded promising results in terms of release kinetics. The release of incorporated molecules is dependent on passive diffusion out of the gel matrix, gel matrix erosion, and external chemical control.

1.2.1 Synthesis of Hydrogel

Synthesis can take place in solution, suspension or emulsion, gas phase, plasma, or bulk polymerization!" Regarding purity, molecular weight distribution, and network homogeneity, each of these methods has advantages and disadvantages. Hydrophilicity and insolubility in water are two properties of hydrogels (Catauro et al. 2004). They can absorb up to 50 times their own weight in water while maintaining shape and mechanical stability. The presence of water-solubilizing groups such as -OH, -COOH, CONH, and SO₃H causes hydrophilicity.

There are two approaches to chemically producing hydrogel networks. To begin, linear polymers can be created by simply crosslinking monomers. Second, a three-dimensional network of hydrogels is created through free radical polymerization of monomer and cross-linking agent (Souza et al. 2016, Zhang et al. 2014). The two processes are depicted in figure 1.1. Hydrogels can be synthesized in solution, suspension or emulsion, gas phase, plasma, or bulk polymerization. The most common method, however, is solution and suspension polymerization. Polymerization in solution can yield bulk and a variety of hydrogel shapes, whereas polymerization in suspension yields small and spherically shaped hydrogels.

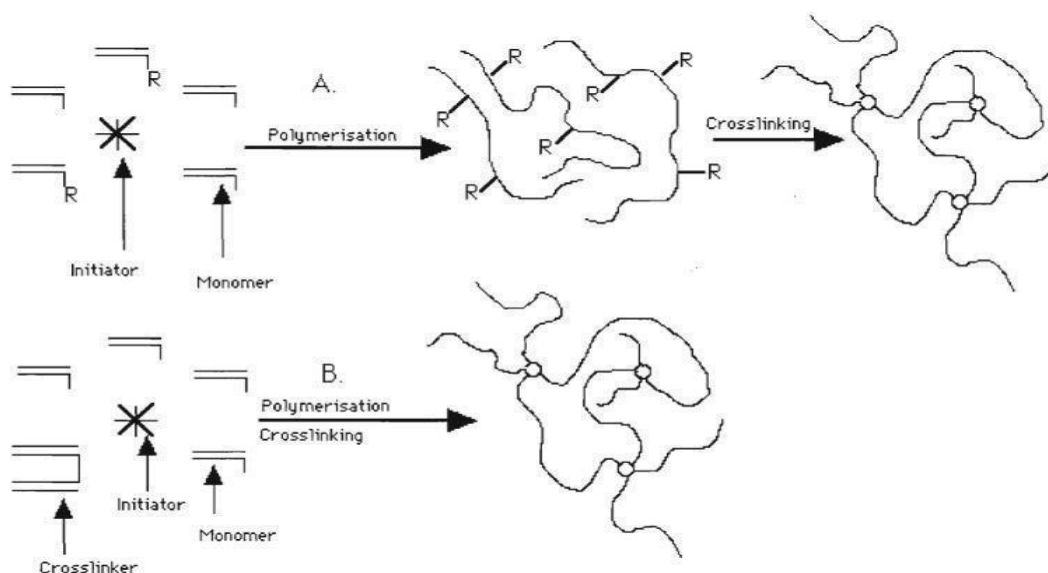


Fig1.1 synthesis of hydrogel by (A) polymer crosslinking

(B) simultaneous polymerization/ crosslinking

Synthesis procedure **A**: 1. Linear polymers with functional side groups are synthesized

2. Crosslinking of linear polymers by reaction of crosslinking agent with reactive side groups (R).

3. Unreacted compound and solvent removal (Reg. -OH or -COOH Crosslinker)

Synthesis procedure **B**: 1. A free chain growth mechanism allows for simultaneous polymerization and crosslinking.

2. Unreacted compound and solvent removal (Nqakala et al., 2021)

1.3 CHITOSAN

Chitosan is a natural polysaccharide, which is usually applied in the field of tissue engineering. However, chitosan shows low mechanical strength and a simple technique to conquer this problem is 3 blending it with other polymers such as PVA. PVA has food water-solubility with several applications in drug release, tissue engineering and biomedicine.

Chitosan hydrogels are suitable candidates for wound dressing applications. Recently, these nanocomposites have been prepared by the incorporation of some NPs such as Ag and ZnO to biopolymers, and the resultant material used as a

wound dressing. Using nanoparticles in the wound dressings structure may result in quick healing of chronic and acute wounds because of their chemical stability, anti-inflammatory and antibacterial properties (Shen et al., 2011). Moreover, generally nano(bio)composites possess high mechanical properties, low permeability against water and vapor, and superior thermal resistance.

Chitosan acts as a non-protein matrix for 3D tissue growth while also activating macrophages for tumoricidal activity. It promotes cell proliferation and the organization of histoarchitectural tissues. Chitosan is a hemostat that aids in natural blood clotting and blocks nerve endings, thereby alleviating pain. Chitosan gradually depolymerizes to release N-acetyl—D-glucosamine, which stimulates fibroblast proliferation, ordered collagen deposition, and increased levels of natural hyaluronic acid synthesis at the wound site (Biao et al., 2017; Apryatina et al., 2015; Boufi et al., 2013). It aids in wound healing and scar prevention. Chitin and chitosan are easily processed into hydrogels, membranes, nanofibers, beads, micro/nanoparticle scaffolds, and sponges for a variety of biomedical applications including drug and gene delivery, wound healing, and tissue engineering.

Chitosan is a naturally occurring polysaccharide composed of poly (β -(1-4)-2-amino-2- deoxy-D-glucose). It is a product of deacetylation of chitin, which is the structural element in the exoskeletons of crustaceans (crabs, lobsters, shrimps and others), radulas of molluscs and beaks of cephalopods (squid and octopuses). Chitosan has been identified as a biomaterial for biomedical applications, including wound care dressing, skin regeneration, tissue engineering and drug delivery systems. Its importance is mainly due to its excellent biodegradability, biocompatibility, renewable origin, bioactivity, low toxicity and antibacterial activity (Lin et al. 2015). Also, chitosan represents interesting properties such as excellent film forming capacity and gas and aroma barrier properties at dry conditions, which makes it a suitable material for designing foods coatings and packaging structures.

Chitosan has been shown to be an effective silver and gold nanoparticle stabilizer. As a result, chitosan-silver nanocomposite films could be used in food packaging to extend the shelf life of foods and products (Souza et al. 2016). However, chitosan is still a costly biopolymer that is not widely available for industrial use in active

packaging material. To take advantage of chitosan's properties, one option is to combine it with another water-soluble polymer, such as poly (vinyl alcohol) (PVA).

Chitosan was used as a stabilizing agent in the UV-irradiation method of synthesis of metal nanoparticles. FTIR and XRD analysis were used to characterize the prepared nanocomposites films (Archana et al. 2015; Benhabiles et al. 2012). The morphological information and the size of the nanoparticles were also confirmed using TEM, UV-visible, and DLS spectroscopy. Swelling capacity, water solubility, and antioxidant and antimicrobial activities were also tested to determine the potential functionality of the prepared nanocomposite films in food packaging applications. MTT was used to assess the cytotoxicity of the prepared CS-silver nanoparticles at various concentrations on CHO-K1 cell lines.

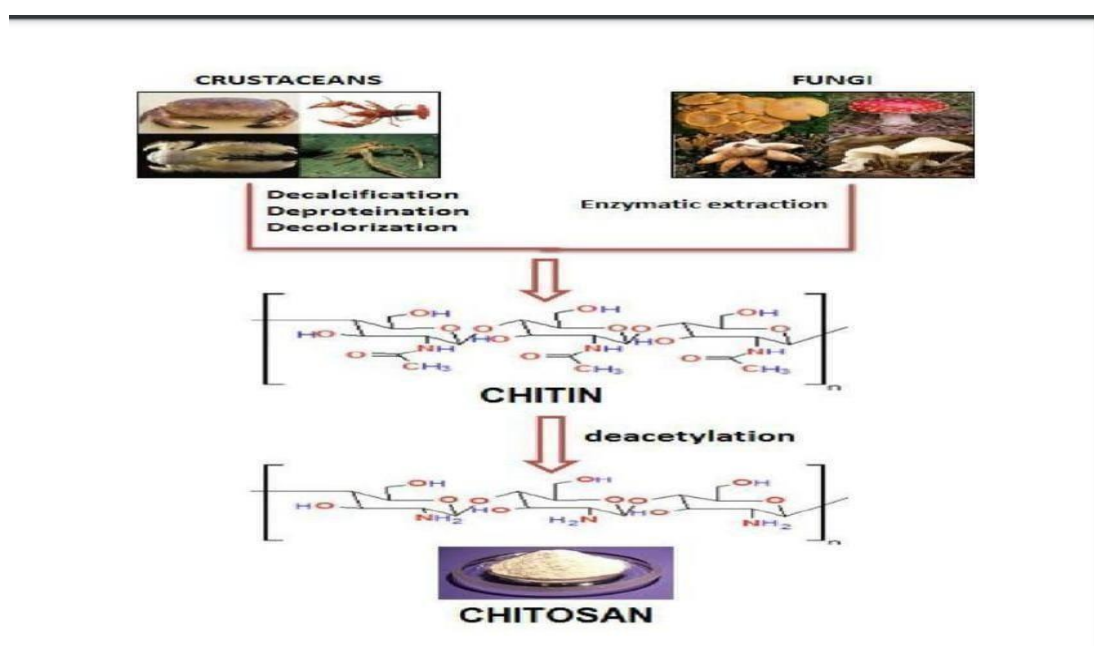


FIG 1.2 steps for extracting chitosan (binta et al., 2017)

It has been extensively researched as a potential drug carrier due to its biocompatible properties. According to some studies, nanoparticles made of other materials should be coated with chitosan to increase their bioavailability and reduce their impact on the body (Kim et al., 2011). The molecular weight and degree of deacetylation of chitosan can be changed to achieve different physio-mechanical properties. Chitosan has an average viscosity molecular weight of 5.3×10^6 Daltons.

The chitosan polymer is made up of the element's nitrogen (7.97%), carbon (44.11%), and hydrogen (6.84%).

1. *Chitosan Properties and Characterization*

Deacetylation of chitin is a commercial method of producing chitosan, which is a structural element in the exoskeleton of fungi and crustaceans (such as prawns and crabs). The degree of deacetylation is determined using NMR spectroscopy, and commercial chitosan with deacetylation has a range of about 60-100%. Chitosan produced commercially has a molecular weight ranging from 3800 to 20,000 Dalton. One common method for chitosan synthesis is deacetylation of chitin using water as a solvent and sodium hydroxide as a reagent (Yu, Song, Shi, Xu, & Bin, 2011). The first order kinetic control reaction takes place in two stages. The second step is lower than the first, in which the activation energy is estimated to be 25-120o C at 48.76kJ/mol.

The pKa of the chitosan amino group is approximately 6.5, which can result in protonation to neutral in acidic solution with negatively charged surfaces. Chitosan is biodegradable and biocompatible, and it improves the transport of polar drugs across epithelial surfaces. Purified chitosan is available for biomedical applications.

2. *Cytotoxicity of Chitosan*

Chitosan and its derivatives have been widely used as promising drug delivery systems in the medical and pharmaceutical fields. As a result, the safety of this important polymer to cells must be investigated (Duan, Liang, Cao, Wang, & Zhang, 2015). Chitosan cytotoxicity studies on cultured cells, such as A549 human alveolar epithelial cells, zebra fish model, and antitumor effect of paclitaxel-bound nanoparticles in gastric cancer cells, have previously been reported.

The cytotoxicity of silver nanoparticles incorporated in chitosan was investigated using Normal Human Dermal Fibroblasts (NHDFs). The results showed that chitosan has significant cell cytotoxicity, particularly in water-soluble chitosan paste samples, and that the toxicity of silver nanoparticles is still within the acceptable range for human use. Similarly, the effects of varying degrees of chitosan de-acetylation on cytotoxicity were studied using 3T3 fibroblast cells as well as two different human tooth pulp fibroblast cell-lines. The research discovered that different cell lines react differently to varying degrees of de-acetylation. At various levels of de-acetylation,

the relative survival rates of different cell lines changed (Bashir, Teo, Ramesh, Ramesh, & Khan, 2015). Thus, the cytotoxicity of chitosan is either limited or non-existent in cells.

1.4 ACALYPHA INDICA

The human race's reliance on plant-derived products for the treatment of various ailments is growing by the day. Previous research has discovered and reported that a variety of natural products aid in wound healing (Kim et al., 2011; Sanwal and Chaudhary, 2011). *Acalypha indica* (Family: Euphorbiaceae) is an annual erect herb that grows as a weed in gardens and along roadsides across the Indian subcontinent, Bangladesh, and Sri Lanka. This plant has been reported to have anti-venom, anti-inflammatory, and antioxidative properties, and it is also used to treat a variety of cutaneous diseases (Kirtikar and Basu, 1999).

Acalypha indica L. is a wild plant that grows in a tropical field and is being studied for its medicinal properties to treat and cure illnesses. Previous research has shown that *A. indica* has analgesic, anti-inflammatory, antibacterial, antiviral, and antifungal properties. The current study compared the potential wound healing activity of ethanol leaf extract of *Acalypha indica* L. topical formulation to betadine ointment as standard treatment using mice incision wound models. In this study, ten healthy male mice aged 3-4 months and weighing 30-40 g were used. *A. indica* ethanol leaf extract was formulated as a 5%, 10%, and 20% ointment. The negative and positive controls were Vaseline album and betadine 10% ointment, respectively. Wound contraction and epithelization period were the parameters measured. When compared to the placebo and standard groups, the mice groups treated with 5% and 10% extracts ointment showed better changes in wound size. However, only 10% of the extract ointment promotes granulation tissue formation, allowing the reepithelization phase to occur. These findings suggest that ointment of ethanol leaf extract of *A. indica* could be an answer to facilitating wound healing, supporting the plant's traditional wound healing claims.

Acalypha Indica's whole plant is also known to have anti-inflammatory and analgesic effects. *Acalypha indica* leaves have also been reported to have strong anthelmintic activity against important wound invading gramme positive and gramme negative pathogens, and this property of the plant has been indirectly correlated to its wound

healing ability (Rahman et al., 2010). Ranju et al. (2011). The use of *Acalypha indica* is common among the tribal people of Tirunelveli hills in Southern India and the tribal population of Jalgoan district in Maharashtra state in India (Ayyanar and Ignacimuthu, 2009; Chopda and Mahajan, 2009). Traditional Siddha healers in Tamil Nadu's Virudhunagar district identified the use of *Acalypha indica* for treating dermatological ailments. Researchers investigated the wound healing efficacy of a 10% ethanolic extract of three plants, *Heliotropium indicum*, *Plumbago zeylanicum*, and *Acalypha indica*, in 100-200g rats by determining the breaking/tensile strength of incision wound, which measures wound strength and acts as an indicator. They discovered that *Acalypha indica* has much lower wound healing efficacy than *Heliotropium indicum* (Mutheeswaran et al., 2011). Reddy et al. (2002). *Acalypha indica* extract has been proposed as an alternative source for treating multidrug-resistant bacterial infections associated with blood cancer. A recent study of *Acalypha indica* leaf nanoparticles revealed superior larvicidal and antibacterial textile coating applications. Plant extracts and biopolymeric nanofibers have been combined to create new wound healing materials and scaffolds (Kalachaveedu M et al., 2020).

1.5 SILVER NANO PARTICLES

AgNPs are without a doubt the most widely commercialized nanomaterials. (Hasan, 2014), According to the Nanotechnology Consumer Products Inventory, it is the most used MNP, appearing in 24% of registered products for various applications. (Medici et al., 2019). This is because AgNPs can overcome the limitations of silver compounds previously mentioned, and more importantly, they are effective at very low concentrations. Fig 1.3 lists some of their essential properties that make them extremely useful in wound healing treatment and management. (Vijayakumar et al., 2019). AgNPs, like most nanomaterials, are synthesized using either the top down or bottom-up approaches. (Lee & Jun 2019) The top-down approach entails physically reducing a bulk compound to its constituents. Through physical and mechanical processes such as UV irradiation, lithography, laser ablation, ultrasonic fields, and photochemical reduction, a heavy force is used to crush the macromolecule into nanoparticles. (Medici et al., 2019a; Sasidharan, Raj & Sonawane, 2019). Evaporation-condensation and laser ablation techniques are the most used top-down methods. Small particles such as atoms and molecules are used as building blocks

in the bottom-up approach to create nanoparticles. The self or assisted assembly of these smaller particles produces a complex nanoparticle. The bottom-up approach is further subdivided into biological, gaseous, liquid, and solid phase methods, in which biological materials such as plants or fungi, chemical vapor deposition, reduction using metal salts, and spinning, among others, are used for metal salt reduction. (Pacini et al.,2015).

In this paper, we present an alternative route for the synthesis of AgNPs in an electrochemical setup, in which the silver nanoparticles were obtained by reduction with an electrical current and hydrogen gas that evolved on the cathode surface during the synthesis. This simple and environmentally friendly method enabled the production of small AgNPs directly within the physically crosslinked PVA/CHI hydrogel matrices. To optimize the process in terms of AgNPs size and concentration, the synthesis parameters - applied voltage, resistance, and implementation time - were varied. The effect of chitosan content on electrochemical synthesis, AgNPs properties, biological, swelling, and release properties was investigated by varying the amount of chitosan in the hydrogels (Maillard & Hartemann, 2012). The obtained nanocomposite AgNPs-embedded hydrogel materials should be strong candidates for wound dressing applications.

Number	Property
1	AgNPs are easy to fabricate with a variety of methods to choose from
2	Size and morphology can be easily altered for a desired application
3	Surface modification is very achievable with AgNPs, which is very useful in targeted drug delivery
4	AgNPs have a high antibacterial efficacy against a broad spectrum of bacteria
5	Bacterial resistance with AgNPs is very rare, thus they have multi antibacterial synergy
6	AgNPs can easily be impregnated into dressings with no side effects
7	AgNPs possess anti-inflammatory properties that aid tissue regeneration.

FIG 1.3 AgNps properties (Maillard & Hartemann, 2012)

1.5.1 AgNP Properties and Biomedical Application

Surface chemistry, shape, morphology, composition, coating/capping, size, surface charge, and reactivity are all important physical and chemical properties of AgNPs in downstream applications (Medici et al., 2019a). AgNPs are widely used in a variety of applications due to their numerous biological properties, which include anti-fungal, anti-cancer, anti-viral, anti-angiogenic, anti-leishmanial, anti-inflammatory, and anti-bacterial properties, among others. AgNPs are used in a variety of applications, including but not limited to pharmaceuticals, cosmetics, water purification, biosensors, and electronics, due to their remarkable size-dependent physicochemical properties such as optical, thermal, catalytic, and electronic characteristics. However, it is AgNPs' biological activities that make them appealing for medical applications. Because of the global rise in life-threatening diseases and pathogens' multidrug resistance to antibiotics, AgNPs are being used in a variety of medical applications such as sensors for disease diagnosis, nanocarriers for drug delivery, sensitizers in radiation therapy, electro spin resonance Dosimetry, and so on. In addition, other applications in various fields are listed. The wound healing property of AgNPs is of particular interest in this study as shown in Fig 1.4.

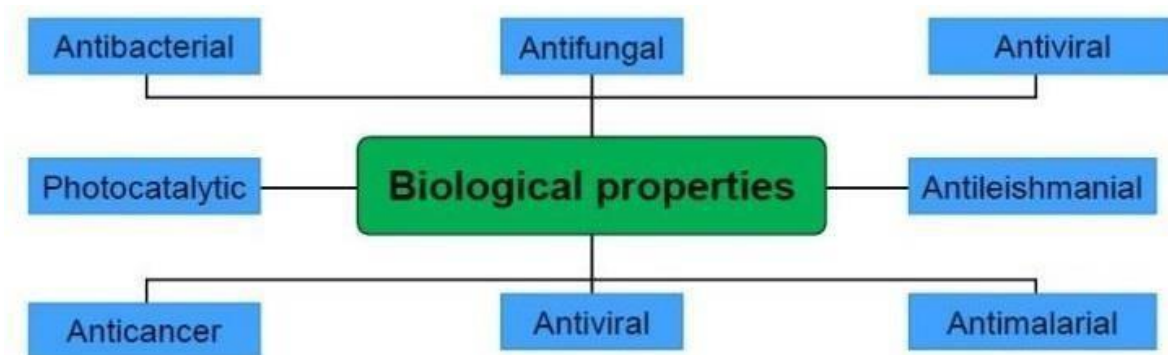


FIG 1.4: biological properties of AgNps (Ovais et al., 2018)

1.5.2 AgNP Nano formulations in Wound Healing

AgNPs have shown potential wound healing abilities as single units, but when combined with other wound dressing materials, they effectively killed microbes that could cause infections at the wound site. Furthermore, because of their small size, they have a very high surface area to volume ratio, allowing surface functionalization and decoration with other biomolecules. Beneficial biomolecules such as DNA and drugs can be incorporated to increase their activity and lengthen their circulation time (Bansod et al., 2015; Bhatia, 2016; Ovais et al., 2018). Because of their properties, as well as the AgNPs' inherent broad-spectrum antimicrobial activity, they are widely used as topical agents in wound healing applications in conjunction with other materials. The major categories of nanohybrids are used in wound treatment.

In wound therapy, primarily two types of nanomaterials are used: (1) nanomaterials with distinct properties that are useful for wound treatment, and (2) nanomaterials used as delivery channels for therapeutic agents. The former includes various types of MNPs such as AgNPs, metal oxides, non-metal, and metalloid based nanoparticles used at various stages of the wound healing process, while the latter includes organic nanoparticles with therapeutic benefits (Fraser et al., 2004). As previously stated, AgNPs are easily coated with various useful functional groups and incorporated into various materials to fabricate nanocomposites and scaffolds that provide synergistic activity in wound treatment. Scaffolds such as hydrogels, coatings, nanofibers, and films can mimic the properties of extracellular matrix (ECM) and are widely used in wound healing formulations. ECMs are fibrous in nature and have nanoscale features that are appealing for wound healing (Clement & Jarrett, 1869; Medici et al., 2019a), which is why researchers are mimicking these structures with materials such as polymers. Impregnation of AgNPs on these scaffolds produces synergistic effects that aid in wound healing. Hydrogels have demonstrated tremendous wound healing abilities among these scaffold dressings. Hydrogels are composed of a network of polymers with many hydrophilic groups that interact with one another to form a three-dimensional matrix capable of trapping liquids such as water or wound exudates.

1.6 Hypothesis

A hydrogel composed of chitosan and *Acalypha indica* extract may enhance the wound healing process by promoting cell proliferation, reducing inflammation, and

accelerating tissue regeneration (Srikar et al., 2016). It is hypothesized that the combination of chitosan and *Acalypha indica* extract may synergistically enhance the wound healing process. Chitosan may provide the necessary mechanical support for the hydrogel, while *Acalypha indica* extract may improve wound healing by promoting cell proliferation, reducing inflammation, and accelerating tissue regeneration.

To test this hypothesis, in vitro and in vivo experiments could be conducted to evaluate the biocompatibility, antibacterial activity, and wound healing properties of the hydrogel (Medici et al., 2019a; Sasidharan, Raj & Sonawane, 2019). Additionally, the release kinetics of the bioactive compounds from the hydrogel could be studied to optimize the formulation for sustained and controlled delivery. If successful, this hydrogel may have significant clinical implications for the treatment of acute and chronic wounds.

CHAPTER-2

REVIEW OF LITERATURE

Jaykumar et al., (2010) Proposed the most promising medical applications for chitin and chitosan is wound dressing. The adhesive nature of chitin and chitosan, as well as their antifungal and bactericidal properties and oxygen permeability, is a critical property in the treatment of wounds and burns. For this purpose, various chitin and chitosan derivatives such as hydrogels, fibers, membranes, scaffolds, and sponges have been developed. The goal of this review is to look in depth at wound dressing applications of biomaterials based on chitin, chitosan, and their derivatives in various forms.

Balakrishnan et al., (2005) showed Wound dressings that can be formed in situ have several advantages over preformed dressings, including conformability in the wound bed without wrinkling or fluting, ease of application, and improved patient compliance and comfort. We describe an in situ forming hydrogel wound dressing made of gelatin, oxidized alginate, and borax in this paper. In the presence of borax, periodate oxidized alginate rapidly cross-links proteins such as gelatin to form non-toxic and biodegradable hydrogels in situ. Because of the hemostatic effect of gelatin, the wound healing-promoting property of alginate, and the antiseptic property of borax, the composite matrix is a potential wound dressing material. The hydrogel was discovered to have a fluid uptake of 90% of its weight, preventing exudate accumulation in the wound bed. The hydrogel's water vapors transmission rate (WVTR) was found to be 26867124 g/m²/day, indicating that the hydrogel can maintain a moist environment over the wound bed in moderate to heavily exuding wounds, enhancing epithelial cell migration during the healing process. The wound healing efficacy of hydrogel was evaluated in experimental full thickness wounds using a rat model, which revealed that the wound covered with gel was filled with new epithelium within 2 weeks with no significant adverse reactions. Many critical elements desired in a wound dressing material are met by these in situ forming hydrogels.

Dongwei et al., (2009) Stated that silver nanoparticles based on chitosan were created by reducing silver nitrate salts with nontoxic and biodegradable chitosan. The resulting silver nanoparticles demonstrated highly potent antibacterial activity

against both Gram-positive and Gram-negative bacteria, comparable to highly active precursor silver salts. Thermal treatment was used to create silver-impregnated chitosan films from the starting materials of silver nitrate and chitosan. When compared to pure chitosan films, chitosan films with silver demonstrated both immediate and long-lasting antibacterial activity against *Escherichia coli*. Our current system's silver antibacterial materials are promising candidates for a wide range of biomedical and general applications.

Katarina et al., (2019) showed how to make wound dressing materials with improved properties using biocompatible chitosan and poly (vinyl alcohol) hydrogels with embedded silver nanoparticles (AgNPs) as a potent antimicrobial agent. The hydrogels were created using a freezing-thawing method, whereas the AgNPs were created using an in situ electrochemical method. Various spectroscopic and microscopic techniques were used to validate the excellent physicochemical properties of the obtained nanocomposite hydrogels. UV-visible spectroscopy, dynamic light scattering, and transmission electron microscopy all confirmed the formation of sub-10 nm AgNPs. Several diffusion models were used to validate the swelling and silver release profiles.

Xiaoyan et al., (2021) Proposed hydrogels are three-dimensional polymeric networks that have the properties of natural living tissues and can absorb large amounts of water or biological fluids. The freezing/thawing method was used to create polyvinyl alcohol (PVA)/N-succinyl chitosan (NSCS)/lincomycin hydrogels for wound dressing, which were then characterized using FTIR, SEM, and TGA. The compression strength, swelling behavior, water retention capacity, antibacterial activity, drug release, and cytotoxicity were all studied thoroughly. The results showed that the addition of NSCS significantly increased swelling capacity, resulting in a maximum swelling ratio of 19.68 g/g in deionized water. The optimal compression strength of 0.75 MPa was achieved with 30 % NSCS content. Additionally, the incorporation of lincomycin brought a remarkable antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. Specifically, 77.71

% of *Staphylococcus aureus* was inhibited with 75 µg/mL lincomycin, while the MTT assay demonstrated the nontoxic nature of the composite hydrogels. In summary, this PVA/NSCS/lincomycin hydrogel showed promising potential for wound dressing.

Katarzyna et al., (2017) Stated that chitosan-based hydrogels are widely used in the fields of medicine and pharmacy. The use of Nano silver particles to modify hydrogel polymers may result in the formation of materials with improved antibacterial properties. The synthesis of hydrogel materials based on chitosan and modified with silver nanoparticles is presented in this article. First, UV-vis spectroscopy was used to demonstrate the preparation and characterization of silver nanoparticles. Hydrogels modified with Nano silver particles were tested in vitro and measured for swelling ability in distilled water and Simulated Body Fluid (SBF), respectively. Furthermore, the antibacterial properties of hydrogel materials modified with silver nanoparticles were evaluated against *Staphylococcus aureus* and *Enterococcus faecalis*, as well as the results of cytotoxicity tests using dermis cells BJ.

Aleksandra et al., (2015) Reported the gelation of chitosan solution with silver nitrate is described in this paper. Continuous chitosan-silver hydrogels can be formed above the critical chitosan concentration (c^*). The formation of nano- and micro-hydrogels at lower concentrations is discussed. To characterize the swelling properties of the hydrogels, a sol-gel analysis was performed. Furthermore, the following were used: (i) mechanical testing of hydrogels, (ii) inductively coupled plasma-optical emission spectroscopy (ICP-OES) for silver concentration measurement, (iii) scanning electron microscopy (SEM) to examine product morphology, and (iv) dynamic light scattering (DLS) and UV-vis spectrophotometry to examine products formed at low chitosan concentrations. Some hydrogels were used to modify cotton fabric to give it antimicrobial properties.

Kemel et al., (2017) Proposed the current study is concentrated on the synthesis of chitosan-silver nanocomposite films (CSNFs) because of their potential applications in wound dressing, packaging, and water purification. In-situ silver nanoparticles (Ag NPs) were created using UV-irradiation and chitosan (CS) as a reductant and stabilizer. The primary amine and amide groups of chitosan have specific interactions with the surface of nanoparticles, according to FTIR spectra. DLS and TEM measurements revealed that the diameter of AgNPs ranged from 170 to 200 nm. Furthermore, CSNFs were tested for biological activities, and the results showed that CSNFs had higher antioxidant and antibacterial activities than chitosan film, which increased with AgNPs amount, implying that chitosan surface structures strongly influence AgNP adsorption and antimicrobial activities. These findings

suggest that nanocomposite films could be useful for anti-infectious wound dressing. Many studies have been published on the toxicity of AgNPs as well as their health and environmental risks. MTT assay was used to test the cytotoxicity of CS-AgNPs on Chinese hamster ovary R(CHO-K1) cell lines. The results showed that CS-AgNPs have no cytotoxicity, implying that they could be used in foods and biomedical applications.

CHAPTER-3

AIM AND SCOPE OF THE STUDY

AIM:

The aim of this project is to develop a wound healing chitosan hydrogel using extracts from *Acalypha indica* and silver nanoparticles that can provide an effective and safe treatment option for patients with various types of wounds.

OBJECTIVES:

1. Demonstrating the use of Hydrogel for healing wounds using silver nanoparticle, chitosan and extracts from *Acalypha indica*
2. As wound healing is a complex biological process which results in the restoration of tissue integrity

CHAPTER- 4

MATERIALS AND METHOD

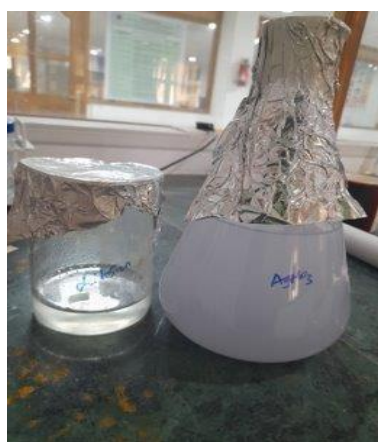
1. MATERIALS:

Silver Nitrate (AgNO_3) (purity 99.7%), chitosan (average molecular weight, 193.4 KDa; the degree of acetylation, 75%), acetic acid, the bacteria are *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcal aureus* (*S. Aureus*) *Escherichia coli* (*E. coli*) *Bacillus subtilis* (*B. subtilis*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acalypha Indica* (Family: Euphorbiaceae, called in English with various names such as Indian *Acalypha indica*).

2. METHODS:

1. *Chitosan-stabilized silver nanoparticle preparation*

(Pansara et al. 2019) described a method for producing CH-AgNP. In brief, 100 ml of 1 mg/mL chitosan solution was prepared in 1%v/v acetic acid solution. 1000 ml of 1 mM silver nitrate solution is added, Take 60ml of silver nitrate from 1000ml, and 40ml of chitosan from 100ml of chitosan preparation. After 24 hours on magnetic stirrer as shown in Fig 4.1 (b), take the 100ml solution and pour it into three 50ml test tubes. Centrifuge at 4000 for 20 minutes, then remove the supernatant and store the pellet. After collecting the pellet, place it in the incubator to dry and prepare it for antibacterial activity.



(a) chitosan preparation



(b) 24hours on magnetic stirrer

FIG 4.1 preparation of chitosan stabilized AgNp

4.2.2 *Acalypha indica* leaf extract preparation

Acalypha Indica plant leaves were cleaned multiple times with double distilled water and dried in the shade for about 3-5 days. The dried leaves were then thoroughly crushed in a mortar to produce a fine powder. 5 grammes of *Acalypha indica* is weighed and put in 100ml of distilled water, which is then placed in a hot water bath for 30 minutes as Fig 4.2 (a). The solution is then extracted using filter paper to separate the solvent from the solution. After collecting the solution, divide it into four 50ml test tubes and centrifuge to collect the supernatant and remove the pellet. Then add 15ml of the supernatant to 100ml of distilled water and 0.44gm of silver nitrate and mix for 24 hours on a magnetic stirrer. Collect the solution the next day, split it into several test tubes, and centrifuge it at 4000 rpm for 20 minutes to collect the pellet. The pellet is then dried in an incubator at 50 degrees.

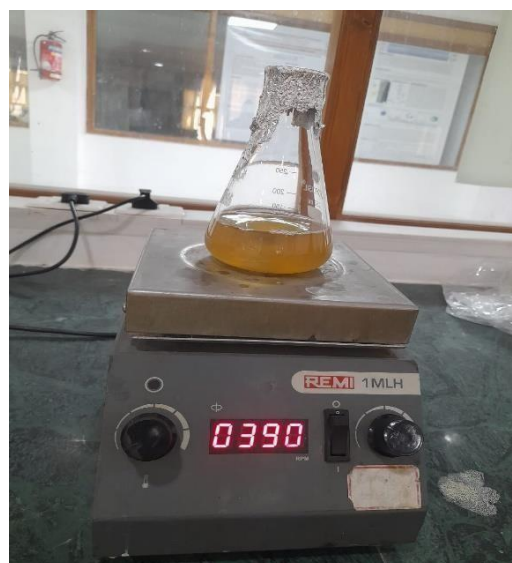


FIG 4.2: ACALYPHA INDICA extraction

(a) water bath at 30° for 30min

(b) 24hrs on magnetic stirrer

4.2.3 Hydrogel preparation

A freeze-thaw procedure was used to create Chitosan/Ag-NPs hydrogels (Soubhagya et al., 2020). In a nutshell, 4 g of chitosan (M.W. 86000, Fisher Scientific) was mixed with ultra-pure MilliQ water and heated at 60 °C for 30 minutes. After that, the liquid was agitated for 3 hours at 90 °C to completely dissolve the Chitosan.

The chitosan was then dissolved in a 2 v% acetic acid solution. To accomplish total dissolution, the beaker was covered and sealed before blending for 1 hour. Then, 3

Chitosan and *Acalypha indica* solutions with a weight ratio of 4:1 was combined, dried, and degassed in a vacuum drier for 1 hour. Finally, Ag-NPs at various concentrations (0%, 0.5%, and 1%) were sonicated for 10 minutes in ultra-pure MilliQ water. The Ag-NPs nanosuspension was introduced dropwise to the hydrogels and vigorously agitated for 2 hours (Soubhagya et al., 2020). The solutions were then placed into a sterile 24-wellplate. The plates were sealed and maintained at 20 °C for 20 hours before being thawed for 8 hours at room temperature. This cycle was carried out six times. The hydrogen bonding between chitosan and *Acalypha indica* in this approach aided in the formation of a 3D structure during the nucleation and growth processes in the polymer's interpenetrating network. The nucleuses generated inside the polymer solution when the polymer chain froze. When polymer chains thawed, they developed crystalline structures near the nucleuses in a regular manner

4. Characterization of hydrogels

1. Gel hydrogel fraction

The hydrogels were dried in a fume hood for 24 hours before being dried at 50°C until W_0 , the constant weight, was obtained (Pacioni et al., 2015). The hydrogels were then immersed in distilled water for 24 hours to achieve W_e as an equilibrium swelling weight. The materials were then dried for 48 hours (about 2 days) at 50°C to to a consistent weight (W_e). Based on the equation below, the gel fraction was defined as $(W_0)/(W_e)$.

$$\text{Gel content (\%)} = (W_e / W_0) \times 100 \quad (1)$$

2. The swelling ratio of hydrogels

The hydrogels' swelling ratio (S) was determined using the traditional gravimetric approach and equation2. where W_s and W_d are the hydrogel weights after swelling and after drying, respectively.

$$S (\%) = W_s - W_d / W_d \times 10 \quad (2)$$

3. Water vapour transfer rate (WVTR) of a hydrogel

The swollen hydrogels (W_s) were stored at 37 °C in an incubator with 40% humidity. The weight (W_t) was taken at regular intervals. Equation (3) was used to calculate the percentage of water loss:

$$\text{Water loss (\%)} = (W_s - W_t) / (W_s - W_d) \times 100 \quad (3)$$

4.2.4.4 Porosity measurements in hydrogels

The liquid displacement technique was used to assess the porosity of the hydrogels. Three samples of each type of hydrogel were used. The hydrogels were soaked in ethanol for 48 hours (about 2 days) until they were saturated by absorption, and then re-weighed. The hydrogel porosity was calculated using equation (4), where W_d is the dry weight of the hydrogel, W_w is the wet weight of the hydrogel, and W_l is the weight of the hydrogel in ethanol.

$$\text{Porosity} = (W_w - W_d) / (W_w - W_l) (\%) \quad (4)$$

4.2.4.5 Contact angle calculation

The contact angle (CA) between the surface of the hydrogel and a drop of ultrapure water was measured using a Krss, Model DSA 100 apparatus to assess the wettability of the hydrogels. In a nutshell, a flat sheet of hydrogel was glued to a glassy slide. The photograph was obtained 2 seconds after room temperature after a drop of 16 L ultrapure water was dropped onto the hydrogel. The contact angles of all hydrogels were tested in triplicate ($n=3$).

5. ANTIBACTERIAL ACTIVITY:

The bacteria that are taken for antibacterial activity are *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcal aureus* (*S. Aureus*), *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), *Klebsiella pneumoniae* (*K. pneumoniae*). Antibacterial activity can be determined using five 7ml test tubes. For 100ml of distilled water, add 0.13g of nutrient broth. Pour them into a conical flask after thoroughly mixing and dissolving them. Sterilize by autoclaving the test tubes, broth medium at 121°C for 15 minutes. In the laminar air flow, inoculate the five bacteria into the nutritional soup. Prepare 100ml distilled water nutritional agar and add pathogens to each petri plate, then add the chitosan-*Acalypha indica* and check for antibacterial activity after keeping the petri plates in the incubator for a day.

6. The AgNPs' Characterization

Understanding AgNPs' physicochemical characteristics is critical for understanding their behaviour, safety, effectiveness, and biodistribution (Zhang et al., 2016). For this goal, many characterization approaches that provide information such as size,

stability, chemical composition, and dispersion are used. Fourier Transforms Infrared (FTIR) spectroscopy, ZETA potential, Scanning Electron Microscope, and were employed to investigate the bare-AgNPs in this work.

1. *FTIR spectroscopy*

FTIR spectroscopy was used to assess the structural composition of bare-AgNPs by identifying functional groups present in each sample and measuring the vibrational spectra of molecules between 4000-600 cm⁻¹. The FTIR spectra were captured using a PerkinElmer spectrometer at the pharmacy department at UWC (Vimala et al., 2010). Dropping liquid samples using a 200 L pipette at an angle of 15 on the surface, the surface and the knob were completely cleaned with ethanol and wiped dry with a tissue after each measurement.

2. *Scanning Electron Microscope*

SEM, or scanning electron microscopy, is a strong method for visualizing and characterizing nanoscale objects such as silver nanoparticles. The following are some of the methods for characterizing silver nanoparticles using SEM: **Size and shape:** The size and shape of silver nanoparticles may be determined using SEM. The size and form of the nanoparticles may be identified by analyzing SEM pictures, which is significant for understanding their characteristics and prospective uses. **Surface morphology:** Surface morphology of silver nanoparticles may also be determined via SEM. The surface characteristics of the nanoparticles, such as the existence of surface flaws or roughness, may be seen by viewing the pictures acquired by SEM. The composition of silver nanoparticles may also be determined via SEM (Vimala et al., 2010). **Composition:** It is feasible to analyse the chemical composition of nanoparticles and identify any impurities or pollutants using energy- dispersive X-ray spectroscopy (EDS) in conjunction with SEM. **Agglomeration:**

SEM may also be used to investigate the aggregation behaviour of silver nanoparticles. It is feasible to study the aggregation behaviour of nanoparticles and discover the elements that impact their agglomeration by analyzing SEM pictures.

3. *ZETA potential*

Silver nanoparticles are small silver particles having a diameter of less than 100 nanometers. Because of their distinctive physical and chemical features, including as

high surface area-to-volume ratio and good antibacterial activity, they are widely employed in a variety of applications, Kumar-Krishnan et al. (2015), Wei et al. (2009) and Potara et al. (2011). Silver nanoparticle size is an essential characteristic that influences their qualities and performance. Various methods, such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and scanning electron microscopy (SEM), can be used to determine the size of silver nanoparticles. The hydrodynamic diameter of nanoparticles in solution is measured by DLS, whereas the (Maillard & Hartemann, 2012) size and shape of nanoparticles in solid form are determined by TEM and SEM. Another key feature that characterizes silver nanoparticles is their zeta potential. The electric charge on the surface of nanoparticles is measured by zeta potential. It measures the stability of nanoparticles in solution. When nanoparticles have a high zeta potential, they resist one other and stay stable in solution. Nanoparticles tend to agglomerate when the zeta potential is low, which might impact their performance and character.

CHAPTER- 5

RESULTS AND DISCUSSIONS

1. RESULTS

1. *Chitosan Stabilized Silver Nanoparticle*

After centrifuging at 4000 rpm for 20 minutes to obtain the pellet, the supernatant was removed. The Eppendorf tubes were kept in the incubator for 24 hours until they dried.

2. *Acalypha indica leaf extract*

Collected the solution, divided it into different test tubes, and centrifuged it at 4000 rpm for 20 minutes to obtain the pellet. The pellet is then dried in a 50-degree oven.

3. *Hydrogel's*

1. *Gel content*

The gel content of the produced hydrogels was measured to quantify the degree of cross-linking. The gel content of the 0, 0.5%, and 1% hydrogels was 80%, 73%, and 78%, respectively. When the quantity of Ag-NPs in the hydrogel structure was increased to 0.5% weight, the gel content fell, but when the amount of -NPs was increased to 1%, the gel content rose, indicating an increase in the interaction between the functional group Ag-NPs and the polymer chains. In another study, Khorasani et al. discovered that as the concentration of silver nanoparticles grew, so did the gel content. They discovered that the gel composition fluctuated between 60% and 87% as the number of silver nanoparticles increased.

2. *Porosity*

The porous nature of the hydrogels can aid in the removal of large volumes of exudates from the wound surface. It also aids in nutrient delivery and creates an ideal environment for cell adhesion and development. Adding Ag-NPs to hydrogels enhanced the porosity in the hydrogel structure, and the porosity was almost the same in the range of 81-90%.

The hydrogels' extremely porous nature would allow for the adsorption of wound exudates from the wound surface, which would help in wound healing.

5.1.3.3 Swellingbehaviour

Water makes about 90% of blood, and hydrogels' strong swelling capabilities allow them to absorb a large amount of water from the blood to halt bleeding [2]. The produced hydrogels exhibited good swelling behaviour in this case. Chitosan hydrogels have a high-water accessibility due to the presence of many hydrophilic groups. Physical or chemical connections between polymer chains can be formed to reduce dissolution of the polymer networks. Hydrogels may absorb water and swell entirely, which is common and perhaps desired to simulate live tissues (such as skin) with similar physical qualities.

To assess the hydrogel swelling capacity in water, swelling experiments were performed at room temperature. The swelling time was directly responsible for the rise in swollen hydrogel weight. As a result of the prolonged incubation period, the weight of the swelled hydrogel increased. The influence of AgNO₃-NPs on the swelling behaviour of hydrogels. The continuance of incubator time for up to 5 hours clearly raised swelling, which eventually levelled out. Furthermore, the inclusion of AgNO₃-NPs in the hydrogels expanded their network. When the hydrogels were mixed with AgNO₃-NPs, the swelling capacity of the hydrogels changed significantly. The AgNO₃-NPs inserted in the gel caused the hydrogel's network to expand. The surface charge of the colloidal AgNO₃-NPs was connected to the considerable rise in hydration degree. Furthermore, increasing the quantity of AgNO₃-NP improved the host hydrogel's swelling capacity. The manufactured chitosan hydrogels had an excellent swelling ratio due to the high hydrophilicity of chitosan combined with the loosened and macromolecular chains of chitosan. Increasing the number of AgNO₃-NPs in the hydrogel structure marginally enhanced the swelling ratio. The hydrogel network grew as the number of nanoparticles increased, resulting in the formation of holes and vacancies within the hydrogel network and an increase in water absorption.

5.1.4 AgNPs' Antibacterial Activity

Bacterial inhibition is vital in wound healing because non-healing wounds are typically characterized by bacterial colonization. AgNPs' antibacterial characteristics enable them to suppress microorganisms that cause infections at the wound site.

The AgNPs are thought to emit Ag⁺ ions in the aqueous medium after being internalized by bacteria. AgNPs are often delivered by passive bacterial channels, one of which includes AgNP interaction with thiol-containing enzymes and proteins in the bacterial cell membrane. The accumulation of AgNPs on the bacterial wall compromises cell membrane permeability. This causes bacterial components to leak, resulting in cell death. As established, killing bacteria at wound sites improves wound healing. When AgNPs are given to the wound site, they hinder bacterial development and cause their death; in the absence of bacteria, epithelization on the wound site proceeds naturally. Collagen fibres are abundantly produced, resulting in minimum scarring and rapid healing. Furthermore, AgNPs improves immune regulation in the injured region, resulting in faster wound healing with less scarring.

The antibacterial activity of AgNPs was investigated in this work against four human pathogenic strains, namely *E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* as shown in Fig 5.1 which have been recognised as the most frequent bacteria in infected wounds. The existence of clearing zones (ZOIs) surrounding the wells was utilised to assess the anti-bacterial efficacy of the AgNPs. All the AgNPs exhibited no action against multidrug resistant pathogens such as *P. aeruginosa* and *E. coli*, as evidenced by the lack of zones of inhibition. Although the majority of AgNPs exhibit wide anti-bacterial activity, some researchers have found that AgNPs are unable to suppress the development of bacteria such as *P. aeruginosa*. ZOIs were only found in the wells containing ciprofloxacin (positive control), an antibiotic having anti- bacterial action against a wide variety of bacterial species.



FIG 5.1: Antibacterial activity

5.1.5 FTIR Spectrum Analysis

The FTIR analysis was performed to provide light on the interactions between AgNPs with the polymeric chains of chitosan in the CSNFs, and the spectra are shown in Fig.5.4. When compared to chitosan polymer, the CSNFs showed minor changes. Indeed, the distinctive chitosan shoulder peaks at 1625 and 1559 cm^{-1} , corresponding to the stretching peaks of the amide C=O bonds, relocated to 1630 cm^{-1} with a considerable decrease in intensity, indicating the presence of interactions between Ag, O, and N atoms of these groups. The interaction of the amide I and amide II groups with the silver metal is clearly seen. Meanwhile, the intensity of the broad band at 3270 cm^{-1} , which is assigned to the overlap of -OH stretching and -NH stretching vibrations of the polysaccharide moieties, decreases and shifts to a lower wavenumber (3311 cm^{-1}), indicating silver chelation with both amino and hydroxyl groups of chitosan and *Acalypha indica*.

The stretching vibrations of the aliphatic -CH bonds were ascribed to the peak at 2920 cm^{-1} . When can be seen, when the quantity of silver in CSNFs increases, numerous peaks change to higher or lower wavenumbers. For example, the -NH

bending vibration band detected at 1458 cm⁻¹ was relocated to 1459 cm⁻¹ with a little intensity drop. This shows that silver binds to the nitrogen atoms of chitosan, reducing the vibration strength of the -NH bond as the molecular weight increases following silver binding. The addition of silver metal had no effect on the bands at 1420 cm⁻¹ (bending vibration of the -OH group), 1220 m⁻¹ (symmetric deformation vibration of CH₃), and 1151 cm⁻¹ (-CH₂ wagging vibration). This clearly shows the role of main amino and amide groups in metal interaction. The silver nanocomposite films exhibited a band at around 1598 cm⁻¹ that corresponded to carbonyl stretch vibrations in ketones, aldehydes, and carboxylic acids. In fact, the appearance of this peak may imply that silver ion reduction is linked to hydroxyl group oxidation in chitosan chains. The peak shifting is induced by a coordination bond between the heavy silver atom and electron-rich groups (oxygen/nitrogen), which causes the bond length to increase, causing the frequency to shift. In addition, a new peak at 1013 cm⁻¹ emerged because of the C-O-C stretching peak.

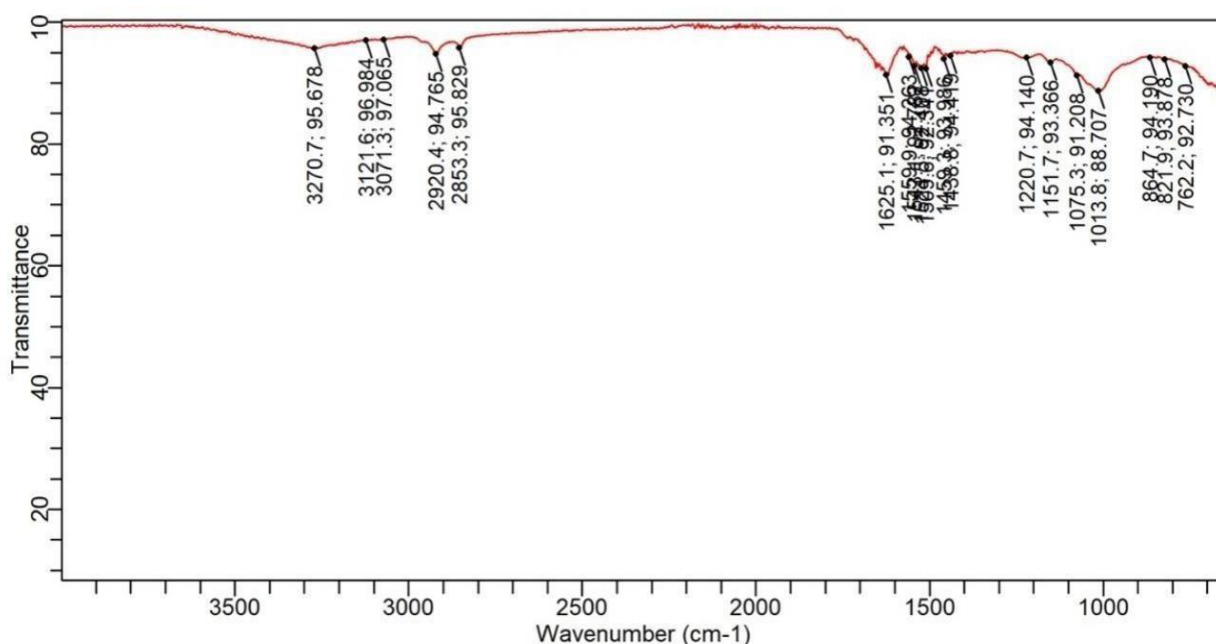


FIG 5.2 FTIR spectrum analysis

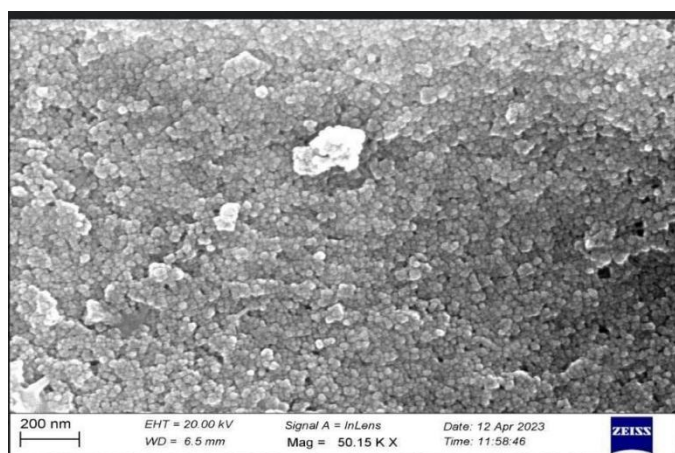
5.1.6 SEM analysis

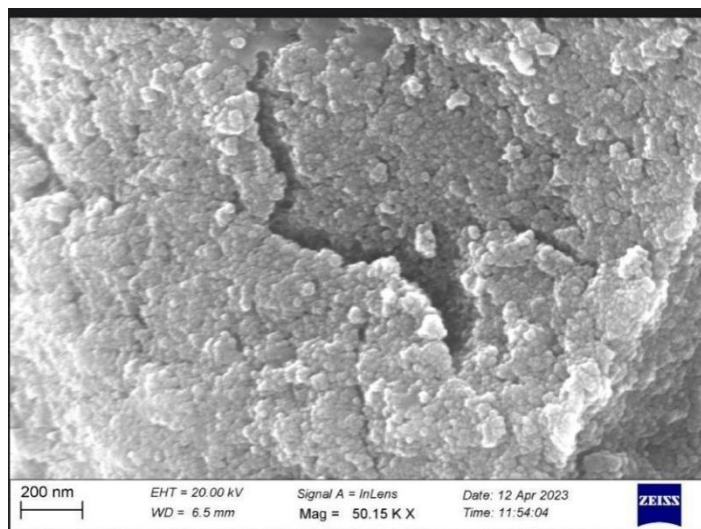
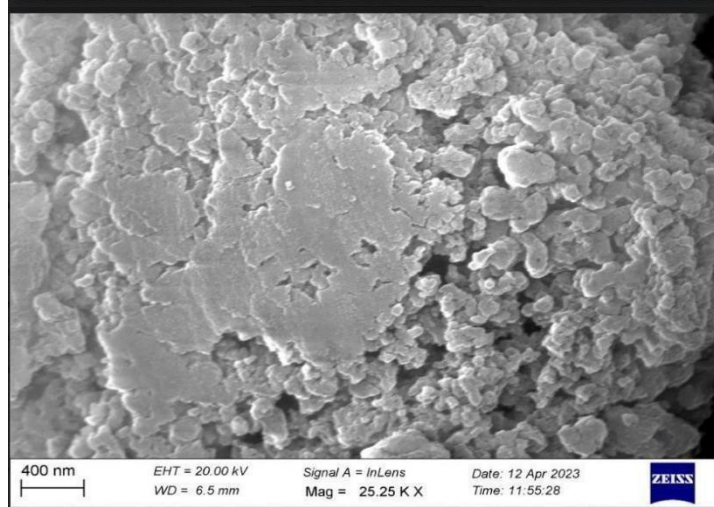
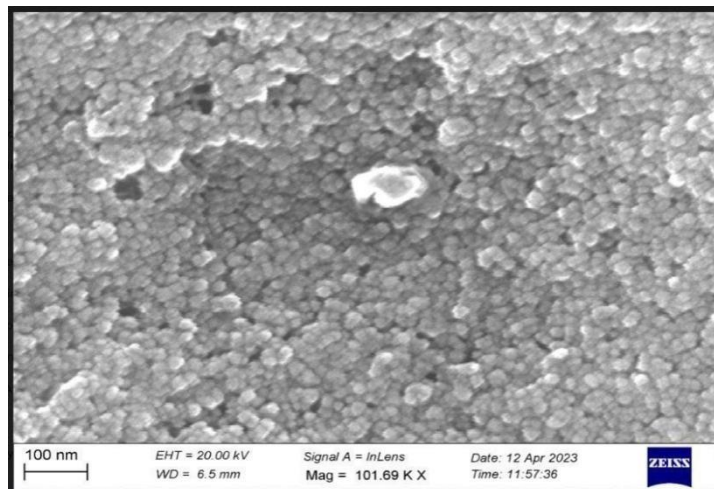
The morphology of hydrogels was studied using scanning electron microscopy. Images of the hydrogels in crosssection. All the hydrogels have a 3-D network structure, as can be shown. The hydrogels' structure has interconnecting micropores

that are dispersed consistently and allow water vapour or other gases to infiltrate and permeate throughout the hydrogels to help in wound healing.

The inclusion of AgNO_3 -NPs into the hydrogel structure. The network topologies of the control sample appear to be noticeably different from those of the hydrogels containing AgNO_3 -NPs. AgNO_3 -NPs may be built or connected inside the hydrogel networks as well as on the surface. Furthermore, the inclusion of AgNO_3 -NPs resulted in an increase in average pore size and pore density. Higher microporosity is required for successful AgNO_3 -NP immobilization in the hydrogel framework. The average pore size for the hydrogels containing 1%, 0.5%, and controls was 0.562 0.32m, 0.104 0.03m, and 0.077 0.02m, respectively.

FIG 5.3: SEM analysis of AgNp





5.1.7 ZETA potential

It makes no difference whether the zeta potential is positive or negative; it is the magnitude that matters. The zeta potential is an indicator of the surface potential, and hence determines the amplitude of the electrical double layer repulsion. The total particle interaction is the sum of the electrical double layer and the van der Waals interaction, which is determined by the magnitude of the material's Hamaker constant, which can be estimated to a first approximation by the difference in the refractive index between the particle and solvent, the larger the difference, the larger the Hamaker constant, so if the Hamaker constant is small, which it may be for a capsule, a low zeta potential, say 20 mV may well be sufficient to stop the capsules from aggregating

The zeta potential is the potential in the plane of shear, or the potential at the point in an electrical field where the particle travels in one direction and the solvent and double layer move in the other way. If there are adsorbed species on the particle/capsule, the plane of shear will be farther out (likely to correspond to the border where the adsorbed species, polymers, for example) and the zeta potential will be artificially lowered. For example, a bare particle may have a zeta potential of 40 mV; however, if a polymer is adsorbed with a layer thickness of 10 nm, the potential is lowered to roughly 15 mV.

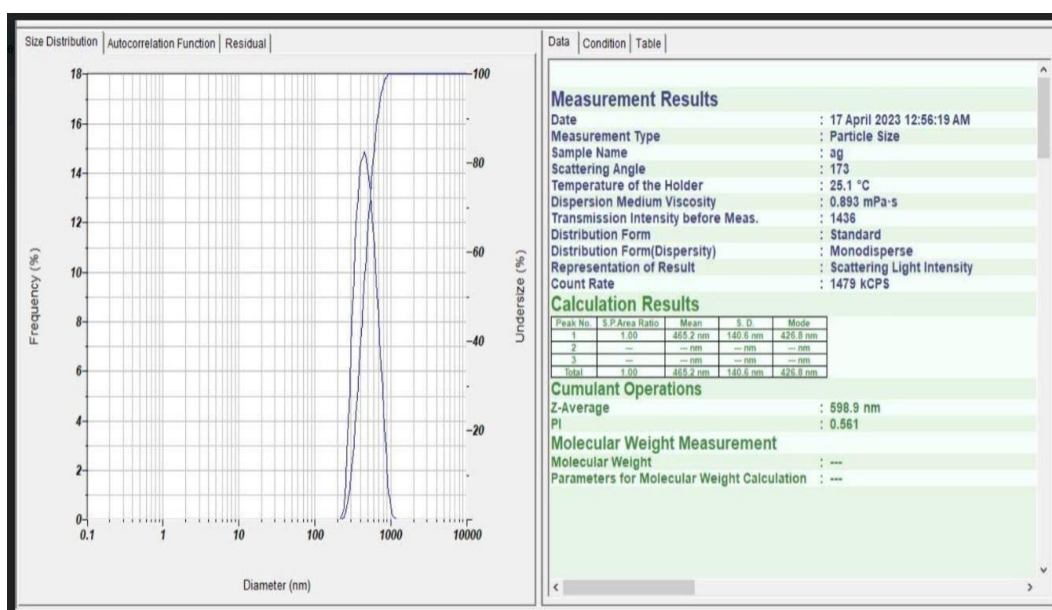


Fig 5.4: ZETA potential measurement results of AgNp's

5.2 DISCUSSIONS

Wound healing is a complicated process involving a few interconnected events such as inflammation, proliferation, and remodeling. Because of its biocompatibility, biodegradability, and antibacterial qualities, chitosan hydrogel, a natural polymer produced from chitin, has been widely employed in wound healing. The addition of silver nanoparticles (AgNPs) to chitosan hydrogel has been found to improve its antibacterial activity, hence expediting wound healing. In this section, we will go through the findings and outcomes of research that looked at the usage of chitosan hydrogels containing silver nanoparticles (AgNPs) for wound healing. Several studies have found that chitosan hydrogel containing AgNPs is effective in promoting wound healing. Lee et al. (2015), for example, investigated the wound healing characteristics of chitosan hydrogel containing AgNPs in a rat model. When compared to the control group, the chitosan hydrogel containing AgNPs significantly increased wound healing. Histological research demonstrated that the AgNP- containing chitosan hydrogel expedited the production of granulation tissue and re- epithelialization.

Similarly, Rajkumar et al., (2017) studied the efficacy of chitosan hydrogel containing AgNPs in healing diabetic wounds in rats in another study. When compared to the control group, the chitosan hydrogel containing AgNPs significantly accelerated wound healing. The histological examination also demonstrated that the AgNP-containing chitosan hydrogel increased angiogenesis and collagen deposition, both of which are important steps in wound healing. Several studies have also investigated the antimicrobial activity of chitosan hydrogels containing AgNPs. Tchemtchoua et al. (2011). For example, we investigated the antibacterial efficacy of chitosan hydrogel containing AgNPs against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The chitosan hydrogel containing AgNPs had a substantial antibacterial activity against both microorganisms.

Moreover, the biocompatibility of chitosan hydrogels containing AgNPs has been studied in several studies. Liu et al. (2010), for example, investigated the cytotoxicity of chitosan hydrogel containing AgNPs on human dermal fibroblasts. The

chitosan hydrogel containing AgNPs was found to be non-toxic to human skin fibroblasts, indicating its biocompatibility.

Overall, the findings from these studies suggest that chitosan hydrogel containing AgNPs is an effective wound healing agent due to its antimicrobial properties and ability to promote angiogenesis and collagen deposition. However, further studies are needed to investigate the long-term safety and efficacy of chitosan hydrogel containing AgNPs in clinical settings.

CHAPTER- 6

SUMMARY and CONCLUSION

Chitosan is a biocompatible and biodegradable polymer derived from chitin; a natural polysaccharide found in the shells of crustaceans. It has been widely used in the development of hydrogels due to its unique properties, including high water-absorption capacity, mucoadhesive Ness, and biocompatibility. *Acalypha indica*, also known as Indian nettle, is a medicinal plant with anti-inflammatory, analgesic, and wound-healing properties. It has been used in the development of hydrogels in combination with chitosan to enhance the properties of the resulting hydrogel.

Several studies have reported the development and characterization of hydrogels based on chitosan and *Acalypha indica* extract. These hydrogels have shown promising properties, including high water uptake, good mechanical strength, and sustained drug release. For example, a study published in the journal Carbohydrate Polymers reported the development of a chitosan-based hydrogel loaded with *Acalypha indica* extract for wound healing applications. The hydrogel exhibited high water uptake, good mechanical strength, and sustained release of the plant extract, which promoted wound healing in vivo. In conclusion, hydrogels based on chitosan and *Acalypha indica* extract have shown potential for various biomedical applications, including wound healing, drug delivery, and tissue engineering. Further research is needed to optimize the properties of these hydrogels and to investigate their safety and efficacy in vivo.

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