



**TRIBHUVAN UNIVERSITY
INSTITUTE OF ENGINEERING
PULCHOWK CAMPUS**

THESIS NO: 080MSMSE011

**PHYSIOCHEMICAL DEVELOPMENT AND MULTI-TECHNIQUE
CHARACTERIZATION OF PVA/GE BASED HYDROGEL INCORPORATING
NATURAL BIOACTIVE COMPOUNDS FOR POTENTIAL BURN WOUND
HEALING APPLICATION**

by

PRABHAKAR THAPA MAGAR

A THESIS

**SUBMITTED TO THE DEPARTMENT OF APPLIED SCIENCES AND CHEMICAL
ENGINEERING**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE IN MATERIAL SCIENCE AND
ENGINEERING**

DEPARTMENT OF APPLIED SCIENCES AND CHEMICAL ENGINEERING

LALITPUR, NEPAL

MAY 2026

COPYRIGHT

The author has agreed that the library, Department of Applied Sciences and Chemical Engineering, Pulchowk Campus, Institute of Engineering may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor(s) who supervised the work recorded herein or, in their absence, by the Head of the Department wherein the thesis was done. It is understood that recognition will be given to the author of this thesis and the Department of Applied Sciences and Chemical Engineering, Pulchowk Campus, Institute of Engineering for any use of the material of the thesis. Copying or publication or any other use of this thesis for financial gain without the approval of the Department of Applied Sciences and Chemical Engineering, Pulchowk Campus, Institute of Engineering, and the author's written permission is prohibited. Request for permission to copy or to make any other use of the material in this thesis in whole or in part should be addressed to:

Tribhuvan University
Institute of Engineering



Pulchowk Campus
Department of
Applied Sciences and Chemical Engineering

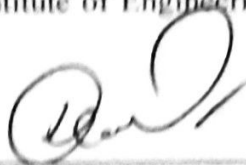
Head

Sahin

Department of Applied Sciences and Chemical Engineering
Institute of Engineering, Pulchowk, Campus
Pulchowk, Lalitpur, Nepal

BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL

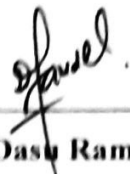
This thesis entitled "**Physicochemical Development and Multi-Technique Characterization of PVA/GE Based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Applications**" by Prabhakar Thapa Magar (Roll No. 080MSMSE011 and T.U. Registration 3-3-23-610-2023) in the partial fulfillment of the requirement for the Master of Science degrees in Material Science and Engineering. This report has been accepted and forwarded to the Controller of Examination, Institute of Engineering, Tribhuvan University, Nepal for the legal procedure.



Asst. Prof. Dr. Khem Raj Shrestha

Supervisor

Department of Applied Sciences and Chemical Engineering,
Pulchowk Campus, IOE, Tribhuvan University, Nepal



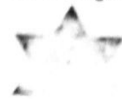
Asst. Prof. Dr. Dasu Ram Paudel

External Examiner

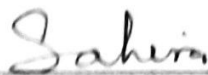
Department of Chemistry,

Tri-Chandra Multiple Campus, Tribhuvan University, Nepal

Tribhuvan University
Institute of Engineering



Pulchowk Campus
Department of Applied Sciences and Chemical Engineering



Prof. Dr. Sahira Joshi

Head of Department

Department of Applied Sciences and Chemical Engineering,
Pulchowk Campus, IOE, Tribhuvan University, Nepal

RECOMMENDATION

This is to recommend that Mr. Prabhakar Thapa Magar (Roll No 080MNSMF011, T.U. Registration No. 3-3-23-610-2023), has carried out the thesis work entitled "**Physicochemical Development and Multi-Technique Characterization of PVA/GE Based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Applications**" as part of the requirements for the Master of Science (M.Sc.) degree in Materials Science and Engineering under my supervision in the Department of Applied Sciences & Chemical Engineering, Pulchowk Campus, Institute of Engineering, Tribhuvan University, Kathmandu, Nepal.

He has fulfilled all the requirements laid down by the Institute of Engineering, Tribhuvan University, Nepal for the submission of the thesis work for the partial fulfillment of Master of Science (M.Sc.) degree in Materials Science and Engineering.



Supervisor

Asst. Prof. Dr. Khem Raj Shrestha

Department of Applied Sciences and Chemical Engineering

Pulchowk Campus, Institute of Engineering

Tribhuvan University

DECLARATION

This thesis entitled "**Physicochemical Development and Multi-Technique Characterization of PVA/GE Based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Applications**" is submitted to the Department of Applied Sciences & Chemical Engineering, Pulchowk Campus, Institute of Engineering, Tribhuvan University, Nepal for the partial fulfillment of the requirements for the Master of Science (M.Sc.) degree in Materials Science and Engineering. This thesis work is carried out by me under the supervision of Assistant Professor Dr. Khem Raj Shrestha, Department of Applied Sciences and Chemical Engineering, Institute of Engineering, Pulchowk Campus, Tribhuvan University, Nepal.

This work was done by me originally and has not been submitted earlier, for the award of any other degree.



Prabhakar Thapa Magar

Roll No. 080MSMSE011

T.U. Registration No. 3-3-23-610-2023

ACKNOWLEDGEMENTS

I am deeply grateful to my supervisor Assistant Professor Dr. khem Raj Shrestha from the Department of Applied Sciences and Chemical Engineering, Pulchowk Campus, Tribhuvan University; for his insightful suggestions, invaluable guidance and motivation for conducting and completion of my thesis work. I am also grateful to Professor Dr. Sahira Joshi, Head of Department, and Dr. Ganesh Kumar Shrestha, Program Coordinator, Department of Applied Sciences and Chemical Engineering, for their assistance and for creating a favorable academic atmosphere in which to conduct this research.

I would like to thank Nanomaterials Laboratory for providing the required facilities to conduct the experiment. I am particularly grateful to Amrit Campus for supporting the FTIR study and National Institute for Material Science (NIMS) in Japan for providing access to SEM. I would like to appreciate Nepal Academy of Science and Technology for their support in XRD.

I would like to express sincere appreciation to Dr. Chabi Lal Gnawali, Mr. Amrit Acharya, Dr. Tanka Mukhiya, Mrs. Purnima Mulmi and Dr. Deval Prasad Bhattarai for their invaluable support during the fabrication and characterization of the samples, which was critical to the success of this project. Furthermore, I'd also like to thank my friends, Mr. Sachin Ghimire, Mr. Ashman Karki, Ms. Bipana Ojha Khatri and Mr. Bibek Ghimire for their constructive feedback and technical assistance throughout this journey.

Finally, I express my deepest gratitude to my family for their unwavering support, encouragement, and inspiration. Their patience and belief in me have been a constant source of strength, and I owe the successful completion of this thesis to them.

STATEMENT OF ORIGINALITY AND AUTHENTICITY

I confirm that the dissertation I am submitting is an original and authentic piece of work written by myself that satisfies the University rules and regulations with respect to Plagiarism and Collusion. I further confirm that I have fully referenced and acknowledged all material incorporated as secondary resources.

I also certify that I have taken a copy of the dissertation which I will return until after the Board of Examiners has published the results and which I will make available on request in pursuance of any appropriate aspect of the marking and moderation of the work within the University Regulations.

I hereby declare that the thesis entitled "Physiochemical Development and Multi-Technique Characterization of PVA/GE Based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Application" is my original work.



Prabhakar Thapa Magar

TU Registration No.: 3-3-23-610-2023

M.Sc. Material Science & Engineering

5/14/2026

LIST OF ACRONYMS AND ABBREVIATION

PVA	Polyvinyl Alcohol
GE	Gelatin
ROS	Reactive Oxygen Species
3D	Three Dimensional
SEM	Scanning Electron Microscopy
UVS	Ultra Violet Visible Spectroscopy
XRD	X-ray Diffraction
BAH	Bioactive Hydrogel
AMA	Antimicrobial Activity
ADM	Agar Diffusion Method
SR	Swelling Ratio
ZOI	Zone of Inhibition
IOE	Institute of Engineering

LIST OF SYMBOLS

cm^{-1}	Per Centimeter
w/v	Weight by Volume
$^{\circ}\text{C}$	Degree Celsius
min	Minute
rpm	Rotation per minute
ml	Milliliter
g	Gram
nm	Nanometer
μm	Micrometer
mg	Milligram

ABSTRACT

The conventional hydrogels exhibit limitations in terms of biocompatibility, biodegradability, mechanical strength and potential toxicity. Due to the lack of well-characterized hydrogel scaffolds, recent approaches have focused on integrating hydrogels with extracts derived from locally available natural materials. The developed bioactive hydrogel was made by integrating polyvinyl alcohol and gelatin in the ratio of 92.5% of total volume of fabricated hydrogel with 7.5% of combination of other bioactive compounds like acemannan from aloe barbadensis miller, lycopene from Solanum lycopersicum and curcumin from Turmeric. The characterization of each extract was done through Fourier Transform Infrared Spectroscopy (FTIR) and UV-visible spectroscopy for the assessment of chemical bonding and identity. The developed biomaterials possess the high swelling capacity with structural integrity indicating the formation of bioactive hydrogel. The shifts observed in O-H and C=O peaks of bioactive hydrogel confirm the interaction between PVA, GE and extracts. The fabricated bioactive hydrogel has amorphous structure confirmed through X-ray diffraction (XRD). Scanning Electron Microscopy (SEM) images of fabricated hydrogels contain large no. of non-uniform pores with rough and irregular surface morphology. Agar Diffusion Methods revealed the antimicrobial activity of the fabricated bioactive hydrogels in almost all samples. The developed bioactive hydrogel demonstrated that the natural bioactive compounds are loaded in PVA-GE hydrogels with antimicrobial activity provides an emerging solution in burn wound management.

Keywords: hydrogel scaffold; PVA/Gelatin; Ace Manan; Curcumin; Lycopene

Table of Content

COPYRIGHT.....	2
BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL.....	3
RECOMMENDATION.....	4
DECLARATION.....	5
ACKNOWLEDGEMENTS.....	6
STATEMENT OF ORIGINALITY AND AUTHENTICITY.....	7
LIST OF ACRONYMS AND ABBREBIATION.....	8
LIST OF SYMBOLS.....	9
ABSTRACT.....	10
Table of Content.....	11
List of Table.....	13
List of Figure.....	14
CHAPTER 1: INTRODUCTION.....	15
1.1 Background.....	15
1.2 Hydrogels.....	16
1.3 Natural Bioactive Compounds.....	18
1.4 Various Characterization Techniques.....	21
1.5 Problem Statement.....	25
1.6 Objectives.....	26
1.7 Research Hypothesis.....	27
1.8 Scope of the study.....	27
CHAPTER 2: LITERATURE REVIEW.....	28
2.1 Definition of Skin.....	28
2.2 Types of Skin layer.....	28
3.3 Hydrogels as Bioactive Natural Biomaterials.....	29
CHAPTER 3: EXPERIMENTAL SECTION.....	31
3.1 Materials.....	31

3.1.1 Natural Polymers	31
3.1.2 Synthetic Polymers	31
3.1.3 Reagents	31
3.1.4 Equipment	31
3.2 Research Methodology	32
3.2.1 Phase 1: Preparation, Extraction and Purification of Extracts	32
3.2.1.1 Extraction of Acemannan from Aloe vera	32
3.2.1.2 Extraction of Curcumin from Curcuma longa	32
3.2.1.3 Extraction of Lycopene from Tomato	33
3.2.2 Phase 2: Fabrication of Bioactive Hydrogel	34
3.2.3 Phase 3: Physicochemical Characterization.....	35
3.2.3.1 Fourier Transform Infrared (FTIR) Spectroscopy	35
3.2.3.2 Ultraviolet Visible Spectroscopy (UVS).....	35
3.2.3.3 X-ray Diffraction (XRD)	35
3.2.3.4 Scanning Electron Microscopy (SEM)	35
3.2.3.5 Antimicrobial Activity (AMA)	36
3.2.3.6 Swelling Ratio (SR)	36
CHAPTER 4: RESULT AND DISCUSSION	37
4.1 FTIR Characterization of Individual Extracts.....	37
4.2 UV-Visible Spectroscopic Validation of Bioactive Extracts	38
4.3 XRD Analysis of Fabricated Bioactive Hydrogel.....	39
4.4 SEM Analysis of Fabricated Bioactive Hydrogel.....	40
4.5 Evidence of Bioactive Hydrogel Formations.....	42
4.6 Antimicrobial Activity of Fabricated Bioactive Hydrogel.....	42
CHAPTER 5: CONCLUSION AND RECOMMENDATION	47
5.1 Conclusion	47
5.2 LIMITATION.....	48
5.3 RECOMMENDATION.....	49
REFERENCES	50
APPENDICES	58

List of Table

Table 1: Identification of Lycopene, Acemannan and Curcumin extracts through FTIR Spectroscopy	37
Table 2: ZOI of Fabricated Hydrogel Samples Against <i>S. aureus</i>	43
Table 3: ZOI of Fabricated Hydrogel Samples Against <i>E. coli</i>	45

List of Figure

Figure 1:Extraction of Lycopene(L), Acemannan(A) & Curcumin(C)	33
Figure 2: Fabrication of Hydrogel before and after 24 hours air drying at room temperature	34
Figure 3: FTIR Spectra of Natural Bioactive Compounds and Fabricated Hydrogel.....	38
Figure 4: UV Spectra of Natural Bioactive Compounds	39
Figure 5: XRD Spectra of Fabricated Bioactive Hydrogels	40
Figure 6: SEM image of the fabricated bioactive hydrogel at 10 μm	41
Figure 7: SEM image of the fabricated bioactive hydrogel at 5 μm	41
Figure 8: Inhibition zones reflecting the activity of the prepared bioactive hydrogels against pathogens S.aureus and E. Coli.....	43

CHAPTER 1: INTRODUCTION

1.1 Background

The largest organ of the human body is skin that meet and fight directly against the external environmental, dehydration, various radiation, pathogenic invasion and wound injury. The anatomical and physiological part of this largest organ has vital role for the protection of internal organ of body for immune protection, thermoregulation, sensory perception and to maintain the balance of the body fluid. Epidermis, Dermis and Hypodermis are the basic three layers of skin as barrier to connect with various tissues, blood vessels, fibers and follicles. They provide the insulation and cushioning to the body. The injuries in the anatomical portion of any of these skin layers may lead to life threatening complications (Shpichka et al., 2019).

Among the various form of injuries in human body, burn injuries are serious injuries that are related with substantial morbidity and mortality. Burn injuries may happened due to radiation, heat, chemical or other by many reasons. These burn injuries not only affect the physical part of the body rather might cause the psychological trauma, longer hospital time period, pain, infection, disability and mortality (Burgess et al., 2022).

On the basis of depth & size of burn injuries, basically burn injuries are classified into First degree, Second degree, Third degree and Fourth degree. First Degree Burns only affect the outer layer of skin and epidermis & don't cause any scare whereas Second Degree Burns affect up to dermis layer and may cause the blister formation, pain and inflammation. Third Degree Burns destroy both epidermis and dermis while Fourth Degree Burns destroy the deeper tissues. The excessive fluid loss, bacterial infection, tissue necrosis and delay in healing happened in severe burns. Therefore, Effective Burn wound System is important for the rapid recovery and prevention of complications (Pappas-Taffer, 2024).

There are number of multiple overlapping biological stages starting from hemostasis, inflammation, proliferation and remodeling of tissues for the burn wound healing. The imbalance

in any of these biological stages may lead to delay in healing of burn wound or may cause severe injury for longer time period. Therefore, Various wound dressings such as bandages, cotton gauze and lint are used for the purpose of these burn wound management for many years. But these conventional dressings exhibit several limitations in terms of antimicrobial protection, permeability of oxygen, moisture retention and frequently adherence to newly formed tissues. These dressings are unable to maintain the ideal moist environment necessary for effective wound healing. As a result, there is need of sophisticated biomaterial like Hydrogels that are capable of accelerating the tissue generation by minimizing the infection and inflammation (Sikka et al., 2025).

1.2 Hydrogels

Hydrogels are soft, viscoelastic materials composed of three-dimensional crosslinked polymeric networks with the hydrophilic structures that enable them to retain the large amounts of water or biological fluids, which can be aqueous or non-aqueous maintaining structural integrity. The swelling capacity of these hydrogels are very high because of hydroxyls and carboxyl present in the hydrophilic functional groups (Sordi et al., 2021). There are various types of crosslinking methods to designed the hydrogel either from natural or synthetic polymers. As conventional hydrogels have limitation in terms of absorption capacity, porosity, biocompatibility, biodegradability, toxicity and mechanical strength, the recent hydrogels have better properties than these traditional hydrogels (Wanniarachchi et al., 2025). Due to these superior properties of recent hydrogels, these biomaterials have gained more popularity to treat the bund wound and tissue regeneration (Dodda et al., 2023).

As the traditional hydrogels are normally fabricated from synthetic polymers but due to limitation of these traditional hydrogels in terms of biocompatibility, toxicity and various factor, it has driven the hydrogel to be loaded with natural bioactive compounds (Kondej et al., 2024).

The modern Hydrogels are fabricated based on both natural and synthetic polymers in the field of tissue engineering for repairing and regenerating of tissue and organs (Brumberg et al., 2021). These Natural Based hydrogels offer several advantages in terms of biocompatibility,

degradability, low immunogenicity, vascularization inductivity and non-toxicity that provide the environment similar to soft tissues and allows diffusion of nutrients and cellular waste through its elastic network for the better tissue regeneration in burn wound areas (Zhao et al., 2023). Recent strategies have focused on composite hydrogels by combining both degradable as well as non-degradable natural and synthetic polymers with the tailored chemistries to create the bioactive systems with desired controlled functional properties. Studies with several hydrogel systems consisting of natural polymers such as gelatin and synthetic polymers such as polyvinyl alcohol offer biocompatibility, biodegradability and structural versatility.

Polyvinyl Alcohol (PVA) is one of the widely used synthetic polymers in fabricated hydrogel that offers high degree of swelling water with lower toxicity, chemical resistance, better biocompatibility and customized physical properties. PVA is a water-soluble polymer that are obtained by the hydrolysis of polyvinyl acetate. The strong hydrogen bonding interactions with excellent mechanical strength and swelling behavior is due to the presence of abundant hydroxyl groups in PVA. Due to the properties of PVA like elasticity, flexibility, permeability and transparency, it can be applied for the potential burn wound healing applications. However, it has limitations in cellular biomaterials interaction and therefore integrated with other tissues inducing biomaterials like Gelatin, chitosan, alginate, collagen or hyaluronic acid to accelerate the healing process (Rahman Khan & Rumon, 2025).

Among these biopolymers, Gelatin (GE) on the other hand has attracted considerable attention owing to its excellent biological characteristics and structural similarity to collagen which is a major component of the extracellular matrix. Gelatin is a natural polymer derived from the partial hydrolysis of collagen obtained from animal connective tissues, bones and skin with non-toxicity, excellent biocompatibility, biodegradability and highly water-soluble protein derived from the hydrolysis of collagen and low in cost. It has lower immunogenicity, proper solubility in aqueous system and solution gel transition at 30°C. It contains the amino acid sequences that enhances the good cell adhesion, proliferation and generation of tissues. It also inherent bioactive motifs that enhances cellular interaction for the enhancement of tissue repair processes. In addition, Gelatin can be integrated with the inclusion of the other materials to significantly alter the mechanical and biochemical properties (Pangesty et al., 2024).

The integration of PVA and GE are widely used for the preparation of hydrogel to improve biological performance by their biocompatibility, hydrophilicity, flexibility, cell affinity, semipermeable properties, higher surface area, structural integrity, non-toxicity and controlled biodegradability (Nguyen et al., 2016). In addition, Gelatin also help to improve the capacity of swelling and enhances the migration of fibroblasts and keratinocytes that are essential for the closure of burn wound and tissue regeneration. The synergistic integration of PVA and GE leads to composite hydrogels with proper balanced mechanical properties and prepare the proper biological environment for burn wound management (George et al., 2022).

However, PVA/GE based hydrogels often exhibit limited intrinsic biological activity. To enhance this biological activity for the more proper burn wound healing applications, the integration of natural bioactive compounds into hydrogel matrices has emerged as an effective strategy with the addition to polymer selection. Various Natural bioactive compounds are found in locally available natural herbs and plants which are antimicrobial in nature and possess the properties of tissue regeneration for the burn healing applications (Radeef & Kadhum Al-Mari, 2023).

Due to accumulation of wound exudates & damaged tissue barriers, the infected burn wounds are highly vulnerable to microbial colonization. The pathogens like *S. aureus*, *P. aeruginosa* & *E. coli* may cause the bacterial infection on the burn wound part leading to delay in wound healing and may lead to various complications. In order to treat these kind of wound infections, various antibiotics are commonly used but may have lots of side effect in future. Hence, excessive and long-time antibiotic usage lead to the emergence of multidrug resistant bacterial strains. Consequently, it has driven increase interest in the natural bioactive antimicrobial compounds that are safer and must sustainable alternatives for the potential burn wound healing applications (Dolgachev et al., 2016).

1.3 Natural Bioactive Compounds

To enhance their therapeutic properties, the incorporation of natural bioactive compounds extracted from medicinal plants such as turmeric, aloe vera and curcumin have demonstrated

promising potential burn wound healing applications. Polysaccharides and glycoproteins found in Aloe Vera enhance the fibroblast proliferation and synthesis of collagen whereas polyphenolic compound found in Curcumin possess the strong antioxidant and anti-inflammatory activity to enhance the burn wound healing. Similarly, tomato extracts contain the antioxidant compounds effective for the tissue regeneration of burn wound part (M. et al., 2022).

The integration of these natural extracts with PVA-GE offers several merits for the sustainable therapeutic release, reducing inflammation, increment of antimicrobial activity and acceleration for tissue regeneration. The Bioactive natural compounds act as crosslinking medium in the development of fabricated PVA-GE hydrogel (Zainuddin et al., 2025). All these extracts from Natural polymers have their own properties in terms of antioxidant delivery and antimicrobial activity for the treatment of burn healing site (Sepe et al., 2025) .

Curcumin is bioactive compounds found in *Curcuma longa* has garnered potential applications in burn wound healing due to its anti-inflammatory, antioxidant, antimicrobial and wound healing properties. Curcumin reduces the inflammation caused by inhibiting the production of pro-inflammatory cytokines and enzymes which are often elevated in burn injuries. It reduces the pain, swelling and the overall inflammatory response at the wound site. The burns generate the reactive oxygen species (ROS) which can cause the further damage of the tissue. Curcumin's antioxidant properties support to neutralize these ROS thereby minimizing the oxidative stress and promoting faster healing. It exhibits antimicrobial activity against range of pathogens that includes bacteria, virus & fungi. It is particularly beneficial in preventing infections in the burn wounds that can complicate and delay the healing process. It enhances the collagen production for the potential wound healing. These Collagen provides the structural integrity to the skin and helps in the formation of new tissue at the wound site. Curcumin promotes angiogenesis for the formation of new blood vessels that is essential for supplying oxygen and nutrients to the healing tissue. The formation of scar can be reduced by various factors and matrix related to curcumin that result to the better outcomes for the treatment of burn wound part and tissue regeneration (Palshetkar et al., 2024).

The polysaccharide that are found in the inner gel of Aloe vera leaves named acemannan possess the proper hydrophilicity with anti-inflammatory properties for the burn wound care management. acemannan, key component inside the Aloe vera mucilage reduces the pain and promotes the faster healing of burn wounds. It exhibits the antimicrobial effects against various bacteria, fungi and viruses which help to reduce the infections in burn wounds. The presence of compounds like anthraquinones and saponins contributes to this antimicrobial activity. The fibroblast activity is very important for the wound healing application and its contraction. It also accelerated the epithelization enhancing the rapid closure of burn wounds. Regular application of acemannan can help to reduce the scar formation by promoting balanced collagen synthesis and reducing excessive tissue formation. It assists in breaking down the mucilage matrix and facilitating the penetration of active compounds through the outer and inner layer of tissues of skin (Pal et al., 2024).

Lycopene, a non-provitamin carotenoid extracted and purified from the tomato that possess a strong antioxidant potency, antibacterial, antiapoptotic & anti-inflammatory which has many chemical functions such as an antioxidant scavenger, antihyperlipidemic agent and inhibitor of proinflammatory and pro thrombotic factor (Çevik et al., 2012). Lycopene metabolites promote the carotenoid in cells and hold great potential for the treatment of inflammatory diseases (Fang et al., 2024). Lycopene enhances collagen deposition, formation of granulation tissue, contraction of burn wound and epithelization in wound healing (Muhandri et al., 2024). The activity of lycopene enhances the fibroblast migration for significant burn wound healing (Annas et al., 2025).

These natural bioactive compounds were integrated with PVA-GE Hydrogel due to their ability to enhance the antimicrobials properties and non-toxic properties. PVA-GE hydrogel has been used in various studies for the vascular tissue engineering, drug delivery applications, burn wound healing, cell encapsulation, 3-D Scaffolds for the tissue engineering and various biomedical applications (Kim et al., 2018). The objective of this study is to provide the simple, easy, cost effective and natural bioactive hydrogel fabrication method for the tissue regeneration in burn wound parts and to assess the effect of curcumin, lycopene and acemannan on the physical and chemical composition of the fabricated bioactive hydrogels and enhancement of tissue regeneration potential. Further, these hydrogels can serve as potential carriers for the balanced release of natural bioactive compounds to the burn wound site by improving the bioavailability

and the minimization of systematic side effects. Overall, these natural bioactive compounds can interact synergistically with natural polymer matrices to change the physicochemical properties like porosity, swelling behavior and mechanical strength of hydrogel (Dhanaji et al., 2023).

1.4 Various Characterization Techniques

Physiochemical Characterization of Bioactive hydrogel is important to evaluate their potential application in burn wound management. Number of analytical and characterization techniques are employed to know the morphological, structural, thermal and biological properties of fabricated hydrogel systems.

Scanning Electron Microscopy (SEM) is performed to examine the surface morphology and internal microstructure of fabricate bioactive hydrogels. The physical properties were investigated using scanning electron microscopy (SEM) where the information of surface roughness, interconnected network structure, the observation of porosity and pore size distribution of the natural bioactive hydrogels can be done. The porous morphology is essential in burn wound management for the diffusion of oxygen, transportation of nutrient, cellular infiltration and exudate absorption. This is the method to achieve the high resolution for imaging and elemental analysis. The SEM images show the morphology of the bioactive sample and provide elemental composition and an indication of the uniformity of the sample s well as information on pore structure and particle size at micron level. The individual pores, the size and morphology of bioactive hydrogels and their composition can be observed through the SEM but only visibility on the surface of the sample (Datye & DeLaRiva, 2023).

The identification of the functional groups and evaluation of the molecular interactions in natural bioactive hydrogels were investigated using Fourier Transform Infrared (FTIR) spectroscopy.

The chemical bonding of various extracts as fingerprint can be done through the FTIR analysis.

In this context, the development and FTIR characterization of turmeric, curcumin, acemannan and PVA-GE hydrogel represents promising approach towards creating stable, bioactive and environmentally friendly biomaterial with potential application in burn would healing and

controlled drug delivery. FTIR are used to characterize the presence of various specific chemical groups in the natural bioactive hydrogel (Mansur et al., 2008).

The Ultraviolet-visible spectroscopy (UV-Vis) is done for the confirmation of incorporated natural bioactive compounds and for the evaluation of their optical properties within the hydrogel matrices. UV-Vis analysis can identify the characteristic absorption peaks corresponding to particular phytochemical constituents. The effective method of analysis used for the study of presence, stability and bioactivity of bioactive hydrogels based on their interaction with ultraviolet and visible light were investigated by UV-Visible spectroscopy. It provides the information about the components and concentrations of the bioactive hydrogels. The results of UV-Visible spectroscopy are shown as spectra in graphical format that offer a quantitative as well as visual representation of the spectra. Furthermore, UV-Vis is essential for the observation of the behavior of drug release and stability of natural bioactive compounds in hydrogel systems (Ghosh & Nandi, 2024).

The X-ray Diffraction (XRD) analysis further elucidates the internal structural of the natural bioactive hydrogels. XRD is generally performed to investigate the nature of biomaterials whether its amorphous or crystalline in nature. Alteration in diffraction patterns after the integration of GE and natural bioactive compounds can indicate the alternations in polymer chain organization and intermolecular interactions. The integration of these Gelatin and natural extracts to PVA which possess the semi crystalline properties result to the formation of the amorphous structure of the fabricated biomaterials. So that the peaks observed are not sharp rather it blunts confirming the dispersion of bioactive compounds inside the fabricated hydrogels. Due to these hydrophobic nature of the hydrogels, they exhibit the flexibility and swelling capacity of fluids. Crystallinity significantly affect the swelling behavior, degradation properties and mechanical strength (Kusjuriansah et al., 2023).

The antimicrobial evaluation of fabricated bioactive hydrogels is conducted by using agar diffusion methods (ADM) to examine the inhibitory effects against the pathogens. The agar diffusion method basically involves the measurement of zone of inhibition (ZOI) that appear on the surrounding of hydrogel samples placed on bacterial culture media. Large the inhibition zones

indicate the stronger the antimicrobial activity. The ADM for the analysis of antimicrobial properties of the fabricated bioactive hydrogels. *S. aureus* and *E. coli* represent the most common pathogens isolated from both acute and chronic injuries of various burn wound injuries and mainly focused in controlling their spread to avoid sepsis. Interestingly, the burn wound healing occurs more quickly with the help of dressing polymers such as natural bioactive hydrogels. Since last ten years, much attention was gained in the preparation and characterization of bioactive hydrogels which are considered as a starting point for the engineering antimicrobial activities. Indeed, in addition to their possible inherent antimicrobial agents that can be locally released over time either through noncovalent or covalent immobilization. The morphology, structure, bonding phenomena and potential antibacterial activity of natural bioactive hydrogels against the pathogens were assessed. The Antibacterial hydrogels are highly desirable to care the burn wound part by preventing the microbial contamination and reduce the infection related complications (Imtiaz et al., 2019).

This research focuses on the physiochemical development and multi-technique characterization of PVA/GE based hydrogel incorporated with natural bioactive compounds for the potential burn wound healing applications. The fabricated bioactive hydrogel aim to integrate the excellent mechanical and swelling properties of PVA with gelatin that possess the biodegrade with biological functionality and biocompatibility benefit of natural bioactive extracts. The morphological, structural and biological properties of the fabricated hydrogels are evaluated through comprehensive characterization using FTIR, XRD, UV spectroscopy, SEM and ADM.

The importance of this study lies in the fabrication of a cost effective, biocompatible, and non-toxic hydrogel for the treatment of burn wound healing with the minimization of infection risks. The use of antibiotics may reduce due to the use of natural bioactive compounds that offers additional therapeutic benefits. This study also contributes to enhance the field of proper biomaterials by exploring the naturally extracted therapeutic extracts incorporated within the networks of the hydrogel. The finding of this study lead to design and optimization of advanced bioactive hydrogel-based burn wound dressings for the biomedical and clinical applications.

With potential demand for the natural bioactive hydrogels, it continues to attract substantial attention from researchers, clinicians, biomedical engineer, material scientist and medical industries. The incorporation of natural bioactive compounds with smart and multifunctional hydrogels, growth factors, nanoparticles and stimuli responsive systems are the future developments to revolutionize the burn wound management and tissue regeneration applications. Therefore, the research on PVA/GE hydrogels integrated with bioactive compounds represents an essential step toward the fabrication of innovative and effective burn wound healing materials.

The successful development and multi technique characterization of such bioactive hydrogels may not only enhance the burn wound management but also provide new opportunities to know the release of drugs, tissue scaffolding, regenerative medicine and biosensing technologies. Consequently, knowing the physicochemical interactions, structural properties and biological behavior of the bioactive hydrogels is of the paramount importance for their successful clinical translation and commercialization.

1.5 Problem Statement

Burn wounds are the severe most traumatic and physically debilitating injuries affecting outer layer of skin to nearly every organ system which leads to significant morbidity and mortality. Burn Wounds are highly sensitive to dehydration, inflammation, microbial infection and delay in tissue regeneration. Early burn excision of wound and grafting of skin are common clinical practices that have significantly improved the outcomes for severe burn injured patients by reducing the mortality rate and days of hospital stay. But due to high infection risk, pain, hypertrophic scarring and slow wound healing continue to remain the major challenge in burn research and management (Wang et al., 2018). The conventional dressings are the most commonly used clinical dressing due to their low cost and simple manufacturing procedure. However, the conventional dressings such as cotton gauze often fail to provide the proper environment necessary for the effective tissue regeneration and infection control. The treatment also causes the scar formation despite the esthetic or functional changes. In addition, Removal of conventional dressings can damage the newly formed tissues and prolong the duration of healing. Burn wound management has evolved significantly from using conventional bandages to creating contemporary materials that promotes the healing, prevent infection and aid in tissue regeneration. Modern dressings are more effective than conventional dressings in terms of biocompatibility, degradability and moisture retention. These novel developments in dressing include pain relief and improved hypoxic or anaerobic environments. This leads to fabrication of hydrogels like polymers for the treatment of burn wound healing parts (Alberts et al., 2025). As these hydrogels are better than that of conventional dressings but still lacks the proper antimicrobial properties and properties of tissue regeneration (Stoica et al., 2020). To overcome this problem, the PVA-GE hydrogels are loaded with natural bioactive compounds for the potential burn wound healing application with tissue regeneration (Osmokrovic et al., 2025).

1.6 Objectives

General Objectives:

- To fabricate and characterize the natural bioactive PVA/GE hydrogel for potential burn wound healing applications.

Specific Objectives:

- To extract the natural bioactive compounds.
- To prepare the PVA/GE composite hydrogel.
- To integrate the extracts into the PVA/GE hydrogel matrix.
- To characterize the fabricated bioactive hydrogels using FTIR, XRD & SEM.
- To evaluate antibacterial activity using ADM.
- To investigate the physiochemical properties for potential wound healing applications.

1.7 Research Hypothesis

The integration of natural bioactive compounds into PVA/GE hydrogel matrix increases the physicochemical, antimicrobial and biological properties for the potential burn wound heal applications.

1.8 Scope of the study

This study focuses on the physicochemical development and various characterization of PVA/GE based hydrogel integrated with natural bioactive compounds for the potential application in the burn wound system. The research includes the proper selection of natural and synthetic polymers, fabrication of bioactive hydrogels and incorporation of a natural bioactive agent. Comprehensive characterization technique includes FTIR, XRD, SEM, UV-Visible spectroscopy and antimicrobial analysis using ADM. The characterization of bioactive hydrogels will be conducted to evaluate the properties such as swelling behavior, structure, surface morphology and functional groups. In addition, biological assessment like antimicrobial test and in vitro evaluation will be performed to examine the fabricated bioactive hydrogel's potentiality in promoting the burn wound healing. However, this study is limited only to lab scale preparation and in vitro assessment and does not include the in vivo analysis. The study mainly focuses on evaluating the properties of bioactive hydrogel loaded with natural bioactive compound relevant to potential burn wound healing applications.

CHAPTER 2: LITERATURE REVIEW

2.1 Definition of Skin

The skin is the largest organ of the human body which work as a protective barrier against the microbial pathogens, dehydration, ultraviolet radiation, environmental hazards and physical injuries. Its protective layer to regulate the body metabolism, immune responses, sensory perception and fluid balance. The anatomical portion of human skin consist of three layers: epidermis, dermis and hypodermis. All these layers have their own anatomical and physiological characterization important for the tissue integrity maintenance and body metabolism (Gusarova & Diomidova, 2025).

2.2 Types of Skin layer

The epidermis is the outermost layer of the skin that acts as the first line of defense against the external environmental factors. The first layer is composed of keratinocytes to produce the keratin protein to provide the skin strength and waterproofing. The epidermis layer obtains the nutrients from the dermal layer through the diffusion process. There are five sub layers within the epidermis. Among these five layers, stratum basale contains the actively dividing cells that is responsible for the continuous regeneration of epidermal tissues. The melanocytes of this sub layer release the melanin pigment for the protection of ultraviolet radiation. The stratum corneum, outer layer among these sub layers contains the dead keratinized cells act as a defense barrier against pathogens and fluid loss. Destroy of this epidermis may lead to infection and dehydration due to disturbance in skin barrier function. The first degree burns only affect this layer and can heal without scare (de Szalay & Wertz, 2023).

The dermis is the inner layer after the epidermis that provide the elasticity and structural support to skin. It contains fibers, blood vessels, nerves, hair follicles, sweat glands and fibroblasts. Papillary dermis and Reticular dermis are two sub layers in dermis. Papillary dermis consists of

connective tissues and capillaries. Reticular dermis contains dense collagenous fibers to provide the mechanical strength to layer. The collagen and elastin present in the dermis are important for the potential burn wound healing and tissue repairment. The blister formation, severe pain, inflammation and delay in healing may happened due to destruction in dermis. In addition, the deep burn dermis will result the destruction of blood vessels and nerves(Zhang et al., 2024).

The hypodermis is the innermost layer of the skin that contains the adipose tissues and connective tissues for the energy storage, cushioning and insulation. It consists the larger blood vessels and nerves to supply nutrients. The burn in these layers may lead to necrosis and systematic infections like complication (Mohamed & Hargest, 2022).

One of the most severe forms of the tissue injury are burn wounds with high risk of microbial infection, loss of fluid, electrolyte imbalance, tissue necrosis, scarring and slow healing process. Burns can occur due to chemicals, thermal exposure, radiation, friction and electricity. Among them, thermal burns are the most common type of burn due to hot liquids, exposure to flames, steam or any heated objects. Therefore, the severity of these burn wounds depends on the size, depth and location of tissue damage. To overcome this phenomenon, the effective wound management requires prevention of microbial contamination, sterilized environment and enhancement of tissue regeneration. Conventional dressing like gauze and cotton-based materials provide the basic protection but lock the proper biodegradability, biocompatibility and slow healing process. As a result, the advance wound care biomaterials with proper biocompatibility have gained the signification attention for the burn wound healing management (Żwieręło et al., 2023).

3.3 Hydrogels as Bioactive Natural Biomaterials

The hydrogels have emerged as promising biomaterials for burn wound management applications due to their hydrophilicity properties, three-dimensional structure, flexibility and biocompatibility. These hydrogels can copy the natural cellular matrix for the regeneration, proliferation and migration of the tissues. In addition, hydrogels are capable of absorbing the wound exudates by maintaining the moist environment for proper burn wound healing. Various natural polymers and

synthetic polymers including acemannan, lycopene, curcumin, GE and PVA have been researched for the fabrication of hydrogels. Natural polymers are non-toxic, higher biodegradability, biocompatibility and inherent biological activity comparison to conventional one (S. Sun & Chen, 2024).

Despite these benefits, Traditional hydrogels are the biomaterials that exhibit the limitation in terms of mechanical strength, degradation and uncontrolled release of drug. Due to this limitation, the proper therapeutic treatment of the burn wound parts becomes slow and infected many times where structural integrity and sustained therapeutic action are vital. To overcome this situation, researchers have explored the integration of the natural bioactive compounds into the hydrogel matrices to enhance the biocompatibility, biodegradability and other functional properties (Surowiecka et al., 2022).

The natural bioactive compounds such as curcumin, lycopene and acemananan have demonstrated the significant antimicrobial, anti-inflammatory, non-toxicity and antioxidant properties. These properties of extracts are highly beneficial in wound healing that help to prevent the infection, reduce the inflammation and accelerate the repairment and regeneration of tissue. However, the direct application of these natural bioactive compounds is often limited in terms of poor solubility, instability and rapid degradation in physiological conditions. Integrations with the PVA-GE hydrogels provides the protective environment to improve their stability and enables the controlled and sustained release (Fathi et al., 2022).

However, still there is need of further research to optimize the physiochemical properties of such bioactive hydrogels particularly in terms of biocompatibility, antimicrobial properties, anti-inflammatory, mechanical strength and porosity. Moreover, a comprehensive understanding of the bioactive hydrogel structure and its functional performance is still lacking. Therefore the natural bioactive compounds need to load in hydrogel for the proper healing in burn wound site (Mujahid et al., 2025).

CHAPTER 3: EXPERIMENTAL SECTION

3.1 Materials

3.1.1 Natural Polymers

The natural polymers used for the fabrication of bioactive hydrogels include the acemannan, lycopene and curcumin. These natural bioactive compounds were selected for their antimicrobial and healing properties. Similarly, GE was used to enhanced the biodegradability and biocompatibility properties of fabricated bioactive hydrogels (Yarahmadi et al., 2024).

3.1.2 Synthetic Polymers

PVA is a synthetic polymer used to develop the bioactive hydrogels for burn wound management. It is biocompatible, non-toxic and water soluble making it an attractive choice for the fabrication of bioactive hydrogels (Jiang et al., 2011).

3.1.3 Reagents

Acetone, Ethanol & Distilled water are used during the extraction of bioactive compounds and fabrication of bioactive hydrogels.

3.1.4 Equipment

Microbalance, Oven, Rotary Shaker, Centrifuge and Ultra Sonicator

3.2 Research Methodology

3.2.1 Phase 1: Preparation, Extraction and Purification of Extracts

3.2.1.1 Extraction of Acemannan from Aloe vera

The leaves of Aloe vera were collected and thoroughly washed with the distilled water to remove the surface impurities. The leaves were cut at the base of the plant and they were left upright for 30 min to drain the latex. The top, spines and peel were separated from the inner fillets using a scalpel. The fillet was homogenized using a blender and the obtained gel was lyophilized. The polysaccharide was prepared according to ultrasound-assisted extraction method with minor modification. Dried Aloe vera gel was dissolved in ultrapure water at a ratio of 1:100 (w/v) with stirring at 25°C for 2 hours. Subsequently, the solution underwent sonication at high intensity and low frequency (60 kHz, 550W) for 20 min at 25°C with cycles of 5 min on and 2 min off. The resulting solution was then centrifuged at 5000 rpm and 4°C for 30 min. The supernatant containing water soluble polysaccharides was pipetted into a triple volume of cold absolute ethanol leading to immediate precipitation of polysaccharides. The solution was stirred continuously for 45 min at 25°C then incubated overnight at 4°C in a sealed container. After centrifugation at 5000 rpm and 4°C for 30 min, the obtained pellet was dried in oven. The dried product was powdered and stores in airtight container in a dry place for further use (Iosageanu et al., 2024).

3.2.1.2 Extraction of Curcumin from Curcuma longa

The roots of Turmeric were collected and thoroughly washed with the distilled water to remove the surface impurities. Then dried for certain days and grinded through the Grinding Machine and the obtained powder turmeric was seized through Mesh Size 1 mm at 20°C. Then the solution of 20 ml of distilled water and 80 ml of ethanol is taken in beaker making the 100 ml solvent where the 2.5g turmeric powder were mixed and left for the sonication for 70 minute. After the sonication then the solution was centrifuged at 4000 rpm for 10 min and then filtration was done. The Solvent

in beaker were dried in oven for 12 hours at 50°C and the dried extracts were stored in a dark, airtight container to prevent the degradation for further use (Zemljič et al., 2014).

3.2.1.3 Extraction of Lycopene from Tomato

The fresh bright red fully mature tomatoes were washed, chopped and dried in oven for 24 hours. Then the dried tomatoes were grinded through Grinding Machine and the obtained red powder were collected. The 20g of the obtained red powder was taken in 250 ml of the conical flask. Then these samples were extracted overnight in the orbital shaker with the solvent mixture of 200ml of hexanes and acetone in the ratio of 75:25 respectively at room temperature. The solvent from extract were separated at 50°C in an oven leaving behind the crude extract only. The final product was stored in amber colored containers to protect it from light induced degradation at 4°C for the further use (Hussain et al., 2017).

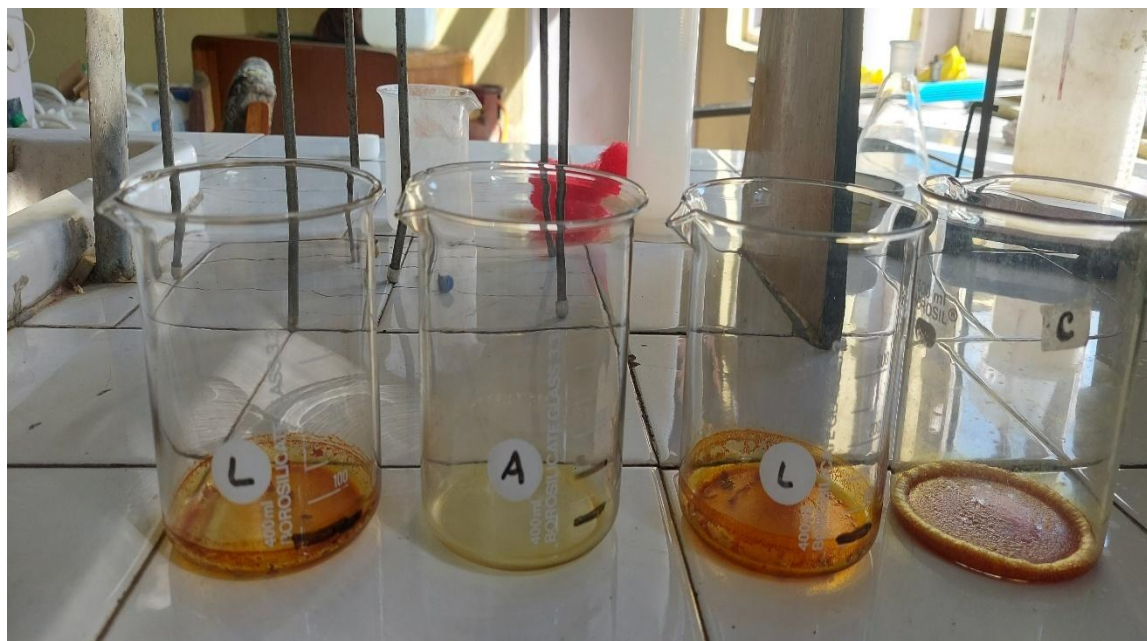


Figure 1: Extraction of Lycopene(L), Acemannan(A) & Curcumin(C)

3.2.2 Phase 2: Fabrication of Bioactive Hydrogel

A precise amount of PVA (4g) was slowly added to 40 ml of distilled water and the solution was kept at 25°C for 2 hour and the solution stirred at 90°C for 30 min. The precise amount of the gelatin was added to 0.6 % base on the weight of PVA. Then the solution stirred at 600 rpm for 4 hours at 80°C. Then again centrifugation for 10 min at 3000 rpm to remove the trapped gas. Then The fabricated PVA-GE hydrogels are integrated with the curcumin, acemanan & lycopene. Then the solution was left for the air drying at room condition overnight and the final bioactive hydrogels were stored in refrigeration for the further analysis (M. Sun et al., 2022).

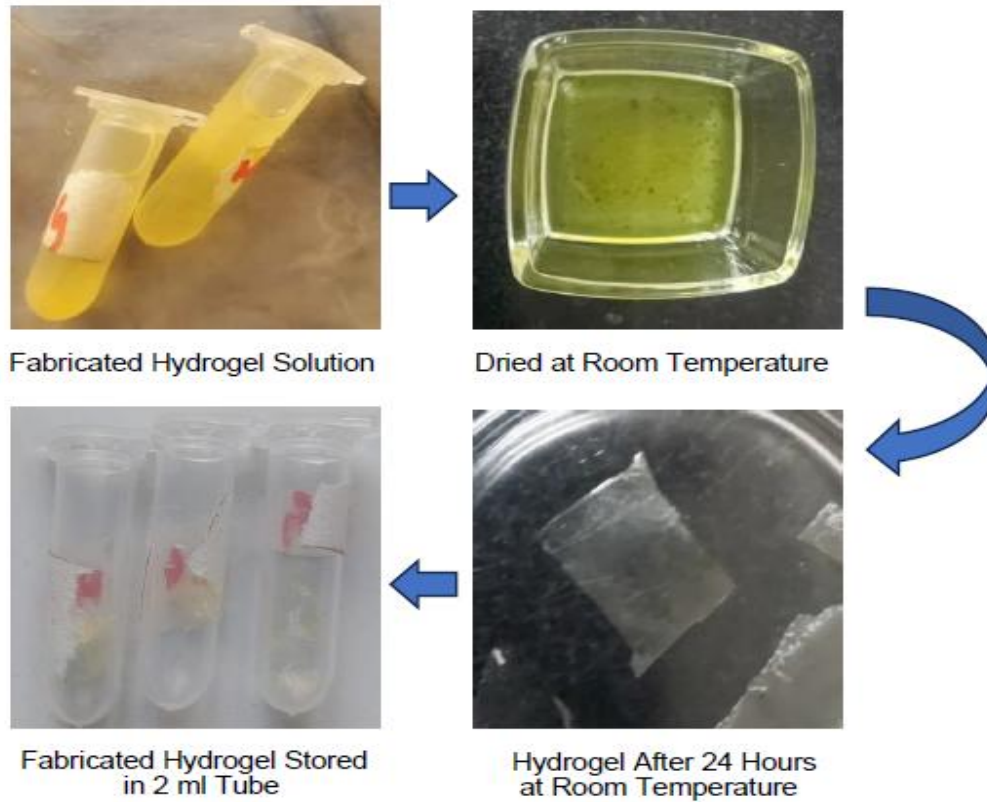


Figure 2: Fabrication of Hydrogel before and after 24 hours air drying at room temperature

3.2.3 Phase 3: Physicochemical Characterization

3.2.3.1 Fourier Transform Infrared (FTIR) Spectroscopy

To identify the chemical bonds and functional groups present in the compounds, FTIR spectra of all bioactive extracts and the fabricated hydrogel were recorded using a Nicolet 4700 spectrometer (Thermo Electron Corp., USA).

3.2.3.2 Ultraviolet Visible Spectroscopy (UVS)

The identification of presence of each extraction and its purity was done through UV Spectroscopy (Simadzu Corp., Japan) by observing the maximum amplitude of wavelength applying both rapid and non-destructive methods.

3.2.3.3 X-ray Diffraction (XRD)

The crystalline nature of the fabricated hydrogel whether crystalline, semi-crystalline or amorphous was analyzed using an X-ray diffractometer. The XRD patterns were evaluated based on peak position, intensity and sharpness (Elsayed et al., 2023).

3.2.3.4 Scanning Electron Microscopy (SEM)

The surface morphology, porosity and the distribution of cavities in the fabricated hydrogel were examined using a Scanning Electron Microscope (SEM) at an acceleration certain voltage. Samples were prepared prior to SEM observation. PVA-GE bioactive hydrogels were developed as stated above, dehydrated in ethanol series. Dried PVA -GE hydrogels were mounted in the sample holder and sputter coated with thin platinum layer and viewed under scanning electron microscope (Abou-Okeil et al., 2024).

3.2.3.5 Antimicrobial Activity (AMA)

The ZOI was the main parameter to know the antimicrobial activity of pathogens by applying both gram positive and gram-negative bacteria in the fabricated bioactive hydrogels (Regina Leong et al., 2023).

3.2.3.6 Swelling Ratio (SR)

The swelling behavior of the hydrogels were determined by immersing the dried bioactive hydrogels in the distilled water at room temperature for 24 hours. The initial weight of dried bioactive hydrogels was measured and then the water swollen bioactive hydrogels are measured at predetermined time intervals of 1, 2, 4, 12 and 14 hours.

The standard equation of SR as follows:

Bioactive hydrogels swelling ratio (%):

$$\frac{W_s - W_o}{W_o} * 100$$

Where W_s is the weight of swelling hydrogel and W_o is the initial weight of dry hydrogel

(Ashtaputrey & Ashtaputrey, 2018)

CHAPTER 4: RESULT AND DISCUSSION

4.1 FTIR Characterization of Individual Extracts

Table 1: Identification of Lycopene, Acemannan and Curcumin extracts through FTIR Spectroscopy

Ex.	Wave No. cm^{-1} (reference)	Sample	Functional Group	Intensity
Ly.	1500-1520 (Nur et al., 2022)	1517	C=C stretch	High
Ac.	1720-1740 (Seeleamngam et al., n.d.)	1717	C=O stretch	High
Cu.	1600-1625 (Shannon et al., 2022)	1592	C=O stretch	High

The chemical composition of each extract of natural material was obtained as Table 1 where the sample wave numbers are compared to wavenumber from literature as shown above.

The characterization peak values corresponding to stretching vibrations of different functional groups were observed at 1517 cm^{-1} for lycopene, 1717 cm^{-1} for acemannan and 1592 cm^{-1} for curcumin within the range of 4000-400 cm^{-1} . These results confirm the presence of key functional groups associated with each bioactive compound.

The identification of the fingerprint regions and functional groups of bioactive extracts and gelatin in the fabricated bioactive hydrogel was carried out using FTIR spectroscopy. Furthermore, the structural integrity, viscosity and biodegradability of the hydrogel due to presence of gelatin are strongly influenced by the ratio of polyvinyl alcohol to gelatin used during the fabrication (Jaipan et al., 2017).

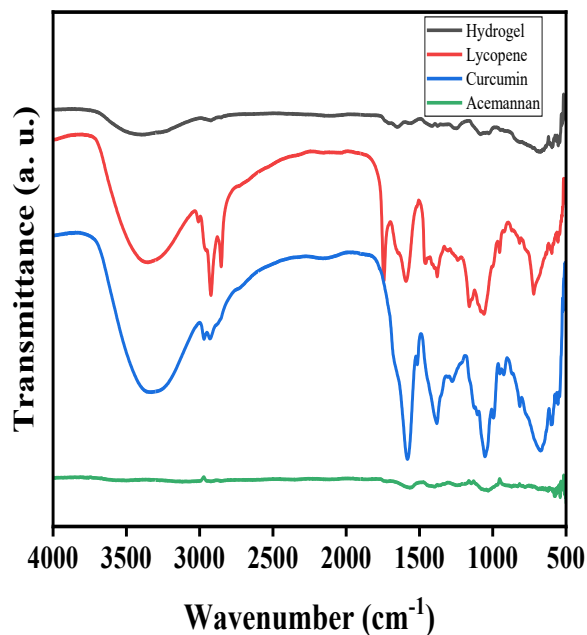


Figure 3: FTIR Spectra of Natural Bioactive Compounds and Fabricated Hydrogel

4.2 UV-Visible Spectroscopic Validation of Bioactive Extracts

The UV-Visible spectroscopic analysis was carried out to validate the presence and purity of the extracted bioactive compounds. The maximum absorbance of lycopene was observed at 453 nm confirming its successful extraction and suitability for enhancing antioxidant properties. This absorbance peak also enables quantitative estimation of lycopene concentration. Curcumin exhibited a maximum absorbance at 418 nm which confirms its purity and effective extraction prior to incorporation into the hydrogel system. In contrast, acemannan does not possess strong chromophores in the visible region; however, it shows characteristic absorption in the ultraviolet region around 209 nm due to the presence of hydroxyl and acetyl functional groups. All UV-Visible spectroscopic measurements were performed using a SHIMADZU UV-2600i spectrophotometer at the Nanomaterials Laboratory, Pulchowk Campus, Institute of Engineering, Tribhuvan University, Nepal.

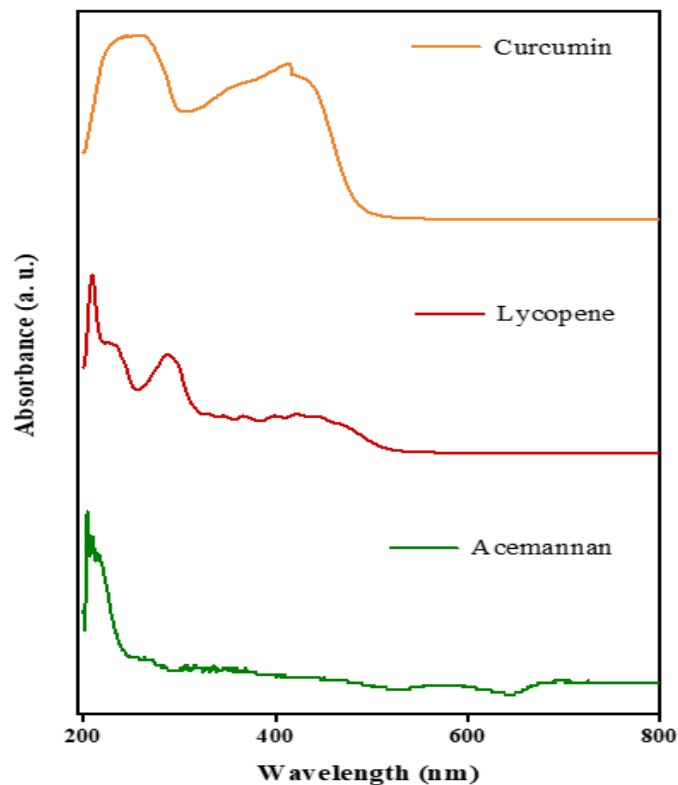


Figure 4: UV Spectra of Natural Bioactive Compounds

4.3 XRD Analysis of Fabricated Bioactive Hydrogel

The surface structure of the fabricated Bioactive Hydrogel was analyzed by X-ray diffraction. Nepal Academy of Science and Technology (NAST) had done this XRD procedure to determine whether the developed hydrogel is crystalline or amorphous in nature. As the peaks are not so sharp, they are curve in observation which indicated that the fabricated biomaterials are amorphous structure. This happens due to presence of bioactive compound that are integrated with the PVA-GE hydrogels which are vital for the burn wound treatment and tissue generation by enhancing the biodegradability and biocompatibility. Some slight shifts are also observed in the diffraction peak position means that PVA and GE are properly interacted during the hydrogel formation. Thus, the

amorphous structure will support for the enhancement of biodegradability, drug release and tissue regeneration during the burn wound healing procedure.

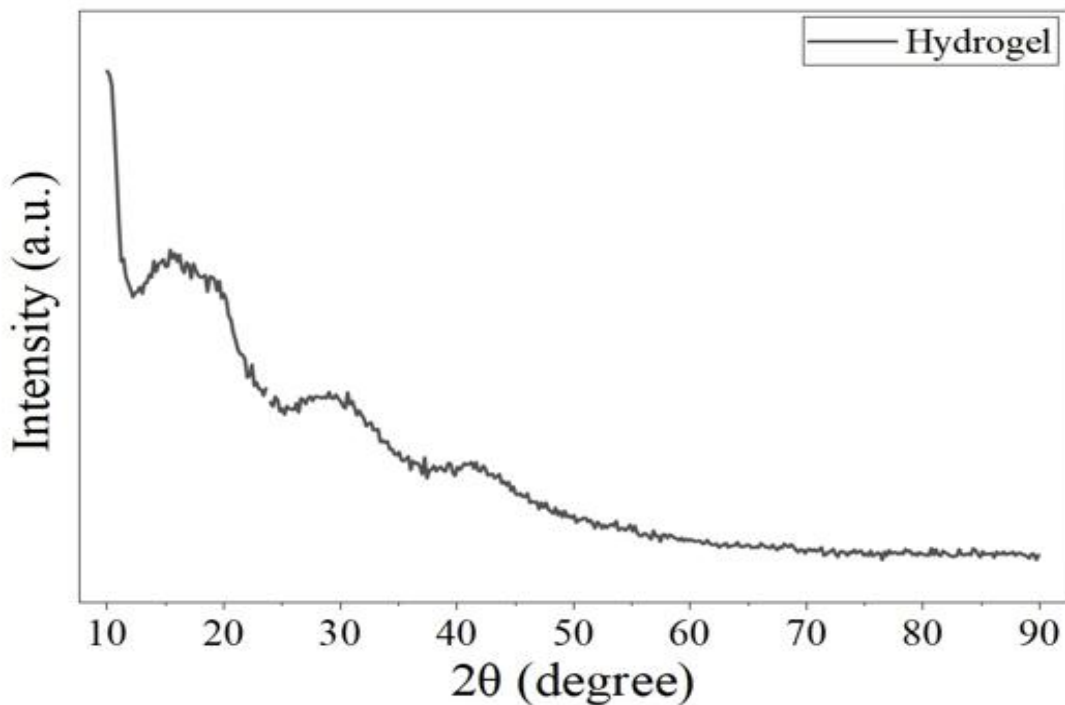


Figure 5: XRD Spectra of Fabricated Bioactive Hydrogels

4.4 SEM Analysis of Fabricated Bioactive Hydrogel

The main purpose of doing SEM is to know about the porosity and distribution of fabricated hydrogels. In both 5 m and 10 m, there are number of pores inside the fabricated bioactive hydrogel which are not uniform in shape. All most all pores have their own diameter and depth at the microscale that can be useful for the nutrient's transports and permeability of oxygen. For burn wound healing application, it can be vital for the release of drugs in balanced way.

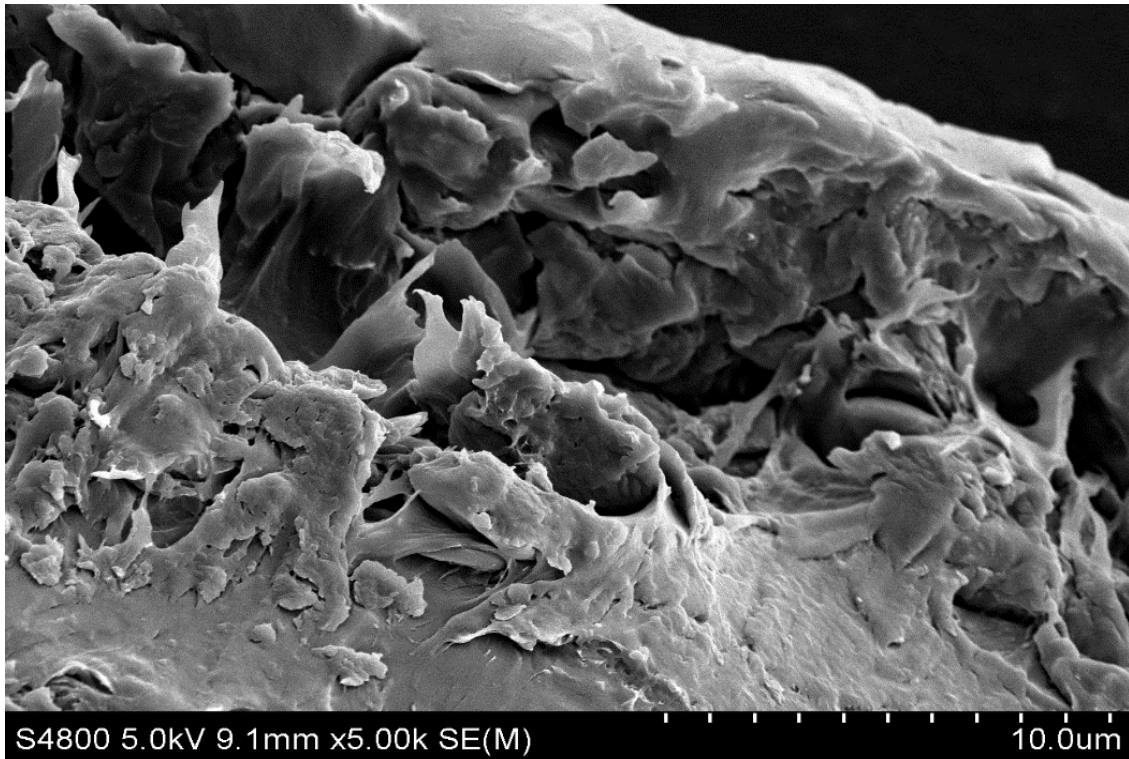


Figure 6: SEM image of the fabricated bioactive hydrogel at 10 μm

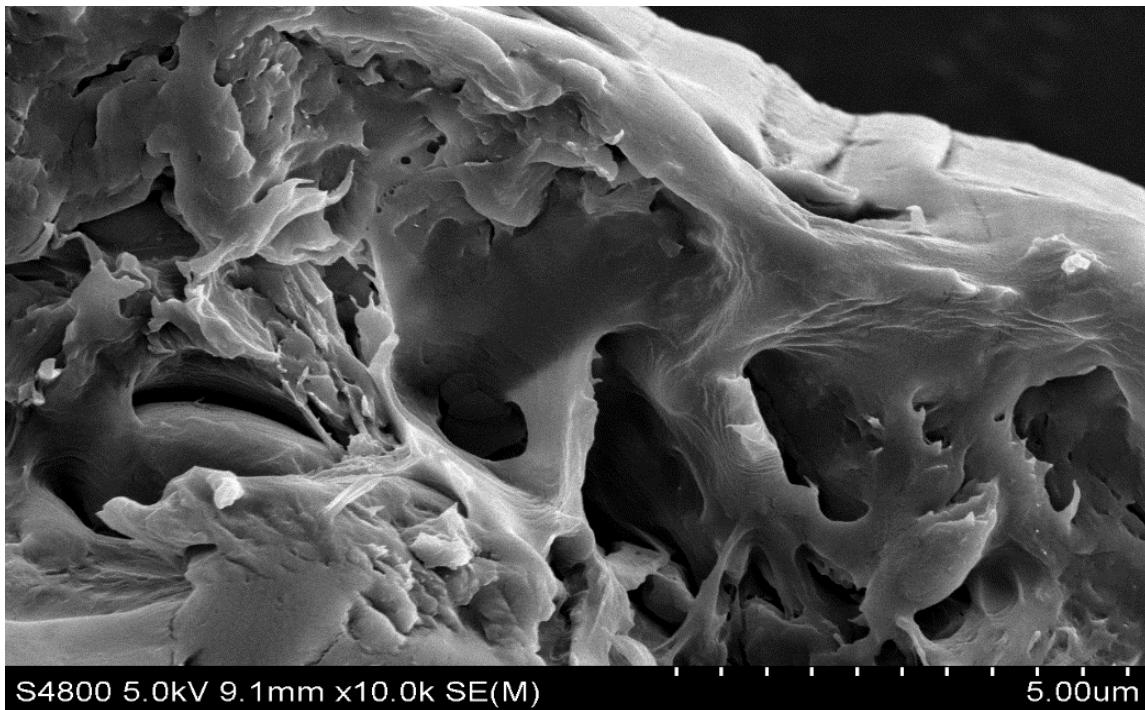


Figure 7: SEM image of the fabricated bioactive hydrogel at 5 μm

4.5 Evidence of Bioactive Hydrogel Formations

The initial dry weight of the Bioactive Hydrogel was 3846 mg determined using analytic balance. The swollen weights of the Bioactive Hydrogel after 1, 2, 4, 12 and 24 hours were recorded as 22610 mg, 22313 mg, 24213 mg, 32215 and 35214 mg respectively. The swelling ratio of the biomaterial at different time intervals (1,2,4,12 and 24 hours) was calculated to be 4, 4, 5, 7 and 8 respectively. It indicates the rapid absorption of the fluid in first and second hours and then reached to equilibrium after 12 to 24 hours maintaining their structural integrity.

4.6 Antimicrobial Activity of Fabricated Bioactive Hydrogel

All 4 sample of fabricate bioactive hydrogel exhibited the notable antimicrobial activities against both pathogens namely *S. aureus* and *E. Coli*. The zone of inhibition (ZOI) observed in the agar diffusion assay confirms that the fabricated bioactive hydrogel effectively suppressed bacterial growth whereas there was no zone of the inhibitions in control PVA-GE Hydrogels that lack the natural bioactive compounds. The inhibition zone was slightly large in size for *S. aureus* comparison to *E. Coli* demonstrating the Gram-positive bacteria with higher susceptibility. The bioactive hydrogel produced a significantly larger ZOI against *S. aureus* confirming enhanced interaction between the bioactive components of bioactive hydrogels and the exposed peptidoglycan layer of gram-positive bacteria. The control samples confirmed the reliability of the assay as no ZOI seen in its surrounding. As the fabricated bioactive hydrogel possess antimicrobial activities which can be attributed to the presence of natural extracts integrating with matrix of polymer. These natural extracts exert their effects through the bacterial cell membranes disruption, reactive oxygen species generation and interference with intracellular metabolic pathways. Gram negative bacteria *E. Coli* possess an additional outer lipid membrane which acts as a permeability barrier making them penetration of antimicrobial agents. Whereas, Gram positive bacteria *S. aureus* lacks outer lipid membrane making them more vulnerable to antimicrobial substances. In addition, the matrix of bioactive hydrogels facilitates the controlled release of the antimicrobial agents which enhanced the prolong interaction bacterial cells & improving overall efficacy. As

burn wound treatment require the continuous antimicrobial action which can done by controlled release behavior of bioactive hydrogels.

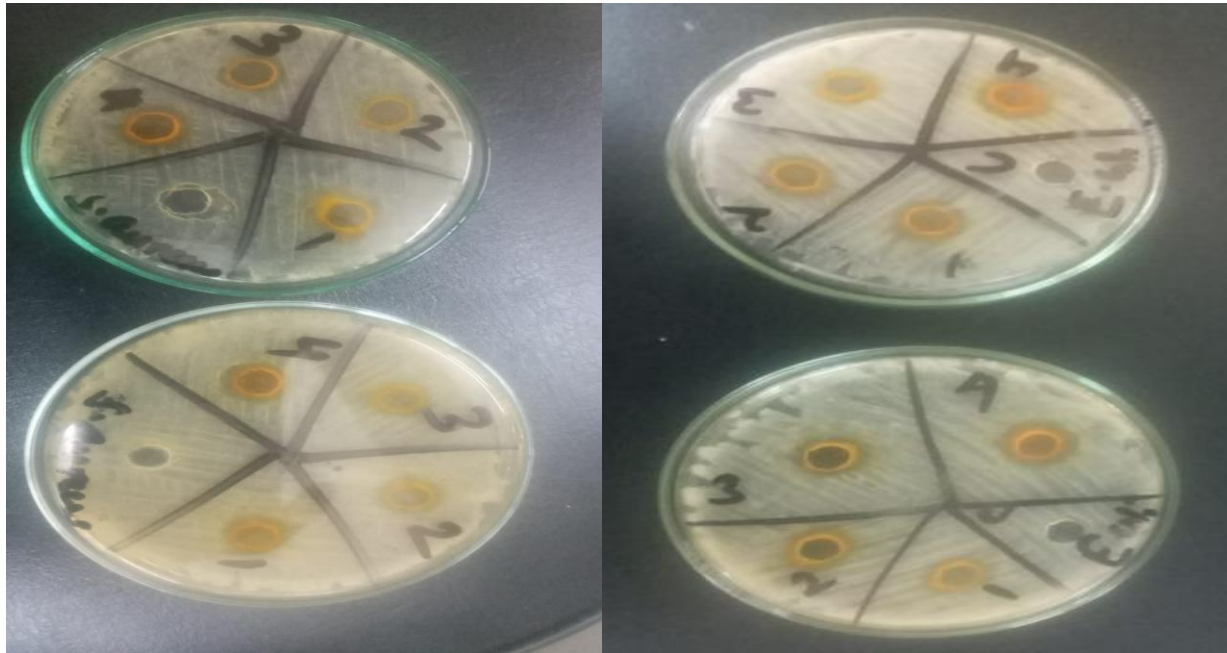


Figure 8: Inhibition zones reflecting the activity of the prepared bioactive hydrogels against pathogens *S.aureus* and *E. Coli*

Table 2: ZOI of Fabricated Hydrogel Samples Against *S. aureus*

Samples	PVA-GE Hydrogel	Curcumin	Lycopene	Acemanan	ZOI
BAH4 (4)	9250mg	700mg	25mg	25mg	15.1 mm
BAH3 (3)	9250mg	600mg	75mg	75mg	14.2 mm
BAH2 (2)	9250mg	500mg	125mg	125mg	13.8 mm
BAH1 (1)	9250mg	400mg	175mg	175mg	13.5 mm

From the demonstration of antibacterial activity of fabricated hydrogel samples against *S. aureus* through ADM, all samples exhibited noticeable inhibitory effects against *S. aureus* confirming the successful incorporation and diffusion of extracts from the hydrogel matrix into the surrounding of agar medium. The yellowish appearance of clear ZOI around the fabricated hydrogel samples indicates the proper suppression of the bacterial growth and demonstrated the proper antibacterial potential of the fabricated bioactive hydrogel for the burn wound healing managements.

The concentration of PVA-GE hydrogel matrix was maintained constant at 9250 mg in all developed bioactive hydrogel samples for the uniformity in the structure of bioactive hydrogel, swelling capacity, porosity and physicochemical properties. Thus, the antibacterial activity of the hydrogel can be accurately evaluated by keeping the PVA-GE matrix in constant and varying the volume of curcumin, lycopene and acemannan. Thus, this synergetic ratio in hydrogels minimized the experimental variation associated with the composition of the hydrogels and enable direct comparison of antibacterial efficiency among the samples.

Among all samples including control, BAH4 possess the highest antibacterial activity with ZOI 15.1mm. The enhanced antibacterial performance of BAH4 due to the higher concentration of curcumin (700mg) combined with lower concentrations of lycopene and acemannan. BAH3 possess ZOI 14.2 mm with 600 mg curcumin and moderate concentration of lycopene and acemannan. In additional, ZOI of BAH2 and BAH1 are 13.8 mm and 13.5 mm respectively.

Table 3: ZOI of Fabricated Hydrogel Samples Against E. coli

Samples	PVA-GE Hydrogel	Curcumin	lycopene	Acemannan	Inhibition Zone Size
BAH4 (4)	9250mg	700mg	25mg	25mg	14.9 mm
BAH3 (3)	9250mg	600mg	75mg	75mg	14.1 mm
BAH2 (2)	9250mg	500mg	125mg	125mg	13.7 mm
BAH1 (1)	9250mg	400mg	175mg	175mg	13.3 mm

From the demonstration of antibacterial activity of fabricated hydrogel samples against E. coli through ADM, all samples exhibited noticeable inhibitory effects against E. coli confirming the successful incorporation and diffusion of extracts from the hydrogel matrix into the surrounding of agar medium. The yellowish appearance of clear ZOI around the fabricated hydrogel samples indicates the proper suppression of the bacterial growth and demonstrated the proper antibacterial potential of the fabricated bioactive hydrogel for the burn wound healing managements.

The concentration of PVA-GE hydrogel matrix was maintained constant at 9250 mg in all developed bioactive hydrogel samples for the uniformity in the structure of bioactive hydrogel, swelling capacity, porosity and physicochemical properties. Thus, the antibacterial activity of the hydrogel can be accurately evaluated by keeping the PVA-GE matrix in constant and varying the volume of curcumin, lycopene and acemannan. Thus, this synergetic ratio in hydrogels minimized the experimental variation associated with the composition of the hydrogels and enable direct comparison of antibacterial efficiency among the samples.

Among all samples including control, BAH4 possess the highest antibacterial activity with ZOI 14.9 mm. The enhanced antibacterial performance of BAH4 due to the higher concentration of curcumin (700mg) combined with lower concentrations of lycopene and acemannan. BAH3

possess ZOI 14.1 mm with 600 mg curcumin and moderate concentration of lycopene and acemannan. In addition, ZOI of BAH2 and BAH1 are 13.7 mm and 13.3 mm respectively.

The gradual increase in ZOI from BAH1 to BAH4 confirms that curcumin plays vital role in the antibacterial activity of fabricated bioactive hydrogel system against both gram positive and gram-negative bacteria. Although lycopene and acemannan also possess antibacterial and antioxidant properties, their antimicrobial activity may be comparatively lower than that of curcumin. Lycopene as antioxidant activity and acemannan as anti-inflammatory activity for the treatment of burn wound healing applications. Higher the concentration lycopene and acemannan plays vital role in antioxidant and anti-inflammatory activity rather than antimicrobial activity comparison to that concentration of curcumin. The antibacterial activity observed in the developed hydrogel samples may also be associated with the diffusion and swelling behavior of PVA-GE hydrogels. The PVA-GE are hydrophilic in nature for the absorption of the moisture and facilitates the balance release of extracts into agar medium. In addition, Due to the porosity that are found in fabricated hydrogel enhances the antimicrobial activity.

The results of through ZOI indicates the promising antibacterial activity of all sample that are loaded with bioactive compounds suitable for the burn wound healing applications. Among the four samples including control, BAH4 possess most effective antibacterial performance due to the bioactive extracts. Through these finding, the optimization of samples can be done where the BAH4 inhibits stronger than other samples in terms of antimicrobial activity. Therefore, this study is helpful to choose the particular concentration of bioactive hydrogels for the particular burn wound healing applications.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The PVA-GE bioactive hydrogel has been successfully developed by integrating acemannan, curcumin and lycopene into PVA-GE hydrogel matrix through physical crosslinking mechanism to enhance the porosity, non-toxicity, biodegradability, structural integrity and biocompatibility. FTIR spectroscopy of individual natural bioactive compounds and the fabricated bioactive hydrogel confirmed the presence of functional groups and the interaction between PVA and GE, as evidenced by characteristic absorption peaks. The absence of new peaks in the fabricated bioactive hydrogel indicated the hydrogel formation occurred via physical crosslinking rather than chemical modification. UV-Visible spectroscopy confirmed the presence, purity and concentration of the individual bioactive extracts. X-ray diffraction (XRD) analysis revealed that the fabricated hydrogel exhibits a predominantly amorphous structure indicating the favorable swelling behavior and diffusion properties. Scanning electron microscopy (SEM) analysis showed a highly non-uniform porous microstructure with numerous non uniform cavities and a rough surface morphology which is suitable for burn wound healing applications. Crosslinking the PVA-GE hydrogels with natural bioactive compounds improved the natural antimicrobial activities that can be used to design for the therapeutic treatment of the burn healing system. ZOI were observed through ADM in fabricated bioactive hydrogel indicating the potential antibacterial activity in the burn healing part. The fabricated bioactive hydrogel is able to maintain the hydrophilicity and crystallinity based on its physical, behavioral and biological characterization. Overall, the combined results confirm the successful fabrication of bioactive PVA/GE based hydrogel incorporating with natural bioactive compounds exhibiting the desirable properties such as stability, biocompatibility, mechanical integrity, biodegradability, hydrophilicity, crystallinity and antimicrobial activities for burn wound care system.

5.2 LIMITATION

The hydrogel integrated with curcumin and lycopene has limited capacity without affecting stability. As curcumin and lycopene are hydrophobic in nature with poor solubility in water may reduce the uniform dispersion. The mechanical strength of natural polymer bioactive hydrogels often has lower comparison to synthetic hydrogels. The study is limited to in-vitro analysis of fabricated bioactive hydrogel. In-vivo test and detailed drug release were not assessed. Bioactive extracts like curcumin and lycopene may degrade due to exposure to environment and may degrade over time affecting stability. Despite the successful fabrication and characterization of bioactive hydrogel, XRD and FTIR although effective in identifying the structure and functional group provide the limited quantitative information. The exact degree of the crosslinking and network density was yet not identified. There was no direct measurement during the physical crosslinking resulting the precise contribution of each interaction type to overall bioactive hydrogel stability remains partially qualitative. The swelling behavior was calculated under the controlled laboratory condition but may not fully represent the complex environments for the real-world engineering. Mechanical stress, pH variation, temperature fluctuation and ionic strength were not systematically investigated which can influence the performance of fabricated bioactive hydrogel. The evaluation of tensile strength, comprehensive modulus and viscoelastic behavior that are essential for the assessment of bioactive hydrogels especially in load bearing and burn wound healing were not done. In addition, the long-term stability, reusability and degradation behavior of the hydrogel were not assessed restricting conclusion on its durability and lifecycle performance.

5.3 RECOMMENDATION

The fabricate bioactive hydrogels need to conduct the in-vivo test after the succession of in-vitro test. NMR spectroscopy can be done to determine the network homogeneity, crosslinking density and viscoelastic behavior with greater precision. The swelling behavior can be conducted under pH levels, temperatures and ionic strength for the better simulation in practical condition that are important for the stability and responsiveness of the bioactive hydrogel in various environment. The elasticity, material strength and deformation behavior can be done that are important for the application specific design. Investigation of multiple hydrogel formulation with various polymer ratios or crosslinking techniques will allow the systematic optimization of crystallinity, swelling behavior and molecular interactions. Overall, biocompatibility assessment or controlled release experiments can be explored to validate the bioactive hydrogel's functional potential beyond fundamental characterization that will lead to fabrication of more robust, tunable and engineering ready bioactive hydrogel systems in the treatment of burn wound.

REFERENCES

- Abou-Okeil, A., Refaei, R., Moustafa, S. E., & Ibrahim, H. M. (2024). Magnetic hydrogel scaffold based on hyaluronic acid/chitosan and gelatin natural polymers. *Scientific Reports*, 14(1). <https://doi.org/10.1038/s41598-024-78696-6>
- Alberts, A., Moldoveanu, E. T., Niculescu, A. G., & Grumezescu, A. M. (2025). Hydrogels for Wound Dressings: Applications in Burn Treatment and Chronic Wound Care. In *Journal of Composites Science* (Vol. 9, Number 3). <https://doi.org/10.3390/jcs9030133>
- Annas, B., Intifada, A., Evatri, C., & Pramusita, P. (2025). Wound healing activity of cream formulation containing tomato juice in guinea-pig: Development of measurement analysis and its application. *Journal of Phytomedicine and Therapeutics*, 24(1), 2020–2040. <https://doi.org/10.4314/jopat.v24i1.20>
- Ashtaputrey, P., & Ashtaputrey, S. (2018). STUDY OF SWELLING BEHAVIOR AND DETERMINATION OF SWELLING PARAMETERS OF SPHERICAL HYDROGELS IN WATER. *Journal of Drug Delivery and Therapeutics*, 8(4). <https://doi.org/10.22270/jddt.v8i4.1775>
- Brumberg, V., Astrelina, T., Malivanova, T., & Samoilov, A. (2021). Modern wound dressings: Hydrogel dressings. In *Biomedicines* (Vol. 9, Number 9). <https://doi.org/10.3390/biomedicines9091235>
- Burgess, M., Valdera, F., Varon, D., Kankuri, E., & Nuutila, K. (2022). The Immune and Regenerative Response to Burn Injury. In *Cells* (Vol. 11, Number 19). <https://doi.org/10.3390/cells11193073>
- Çevik, Ö., Oba, R., MacIt, Ç., Çetinel, Ş., Çilingir Kaya, Ö. T., Şener, E., & Şener, G. (2012). Lycopene inhibits caspase-3 activity and reduces oxidative organ damage in a rat model of thermal injury. *Burns*, 38(6). <https://doi.org/10.1016/j.burns.2012.01.006>

- Datye, A., & DeLaRiva, A. (2023). Scanning Electron Microscopy (SEM). In Springer Handbooks. https://doi.org/10.1007/978-3-031-07125-6_18
- de Szalay, S., & Wertz, P. W. (2023). Protective Barriers Provided by the Epidermis. In International Journal of Molecular Sciences (Vol. 24, Number 4). <https://doi.org/10.3390/ijms24043145>
- Dhanaji, K., Mukade, S. G., Phadtare, R. A., Khilari, A. N., & Korade, V. N. (2023). Formulation and evaluation of emollient cream from marigold. International Journal of Advanced Research and Development Www.Multidisciplinaryjournal.Net, 8(3).
- Dodda, J. M., Deshmukh, K., Bezuidenhout, D., & Yeh, Y.-C. (2023). Hydrogels: Definition, History, Classifications, Formation, Constitutive Characteristics, and Applications. In Multicomponent Hydrogels. <https://doi.org/10.1039/bk9781837670055-00001>
- Dolgachev, V. A., Ciotti, S. M., Eisma, R., Gracon, S., Wilkinson, J. E., Baker, J. R., & Hemmila, M. R. (2016). Nanoemulsion therapy for burn wounds is effective as a topical antimicrobial against Gram-negative and Gram-positive bacteria. Journal of Burn Care and Research, 37(2). <https://doi.org/10.1097/BCR.0000000000000217>
- Elsayed, A. E., Attia, S. K., Mahmoud, G. A., Mostafa, Y. M., R.Taman, A., & Osman, D. I. (2023). Environmentally friendly radiation EDTA modified hydrogel based on gelatin for adsorptive removal of cationic and anionic dye from synthetic wastewater. Egyptian Journal of Petroleum, 32(4). <https://doi.org/10.1016/j.ejpe.2023.10.002>
- Fang, Y., Nie, T., Li, G., Wang, L., Du, J., & Wu, J. (2024). Multifunctional antibiotic hydrogel doped with antioxidative lycopene-based liposome for accelerative diabetic wound healing. Chemical Engineering Journal, 480. <https://doi.org/10.1016/j.cej.2023.147930>
- Fathi, M., Ahmadi, N., Forouhar, A., & Hamzeh Atani, S. (2022). Natural Hydrogels, the Interesting Carriers for Herbal Extracts. In Food Reviews International (Vol. 38, Number S1). <https://doi.org/10.1080/87559129.2021.1885436>
- George, B., Bhatia, N., Kumar, A., Gnanamani, A., Thilagam, R., Shanuja, S. K., Vadakkadath Meethal, K., Shiji, T. M., & T. V, S. (2022). Bioinspired gelatin based sticky hydrogel for

- diverse surfaces in burn wound care. *Scientific Reports*, 12(1).
<https://doi.org/10.1038/s41598-022-17054-w>
- Ghosh, S., & Nandi, S. (2024). A Comprehensive Review on UV-Visible Spectroscopy and Its application. In *International Journal of All Research Education and Scientific Methods (IJARESM)* (Vol. 12, Number 1).
- Gusarova, A. Yu., & Diomidova, V. N. (2025). Structure and morphological features of human skin (literature review). *Acta Medica Eurasica*, (3). <https://doi.org/10.47026/2413-4864-2025-3-44-54>
- Hussain, M. B., Ahmad, R. S., Waheed, M., Rehman, T. U., Majeed, M., Khan, M. U., Shariati, M. A., Plygun, S. A., & Glinushkin, A. P. (2017). EXTRACTION AND CHARACTERIZATION OF LYCOPENE FROM TOMATO AND TOMATO PRODUCTS. *Russian Journal of Agricultural and Socio-Economic Sciences*, 63(3), 195–202.
<https://doi.org/10.18551/rjoas.2017-03.24>
- Intiaz, N., Niazi, M. B. K., Fasim, F., Khan, B. A., Bano, S. A., Shah, G. M., Badshah, M., Mena, F., & Uzair, B. (2019). Fabrication of an original transparent PVA/gelatin hydrogel: In vitro antimicrobial activity against skin pathogens. *International Journal of Polymer Science*, 2019.
<https://doi.org/10.1155/2019/7651810>
- Iosageanu, A., Mihai, E., Seciu-Grama, A. M., Utoiu, E., Gaspar-Pintiliescu, A., Gatea, F., Cimpean, A., & Craciunescu, O. (2024). In Vitro Wound-Healing Potential of Phenolic and Polysaccharide Extracts of Aloe vera Gel. *Journal of Functional Biomaterials*, 15(9).
<https://doi.org/10.3390/jfb15090266>
- Jaipan, P., Nguyen, A., & Narayan, R. J. (2017). Gelatin-based hydrogels for biomedical applications. *MRS Communications*, 7(3). <https://doi.org/10.1557/mrc.2017.92>
- Jiang, S., Liu, S., & Feng, W. (2011). PVA hydrogel properties for biomedical application. *Journal of the Mechanical Behavior of Biomedical Materials*, 4(7).
<https://doi.org/10.1016/j.jmbbm.2011.04.005>

- Kim, H., Yang, G. H., Choi, C. H., Cho, Y. S., & Kim, G. H. (2018). Gelatin/PVA scaffolds fabricated using a 3D-printing process employed with a low-temperature plate for hard tissue regeneration: Fabrication and characterizations. *International Journal of Biological Macromolecules*, 120. <https://doi.org/10.1016/j.ijbiomac.2018.07.159>
- Kondej, K., Zawrzykraj, M., Czerwiec, K., Deptuła, M., Tymieńska, A., & Piękuła, M. (2024). Bioengineering Skin Substitutes for Wound Management—Perspectives and Challenges. In *International Journal of Molecular Sciences* (Vol. 25, Number 7). <https://doi.org/10.3390/ijms25073702>
- Kusjuriansah, K., Rodhiyah, M., Syifa, N. A., Luthfianti, H. R., Waresindo, W. X., Hapidin, D. A., Suciati, T., Edikresnha, D., & Khairurrijal, K. (2023). Composite Hydrogel of Poly(vinyl alcohol) Loaded by Citrus hystrix Leaf Extract, Chitosan, and Sodium Alginate with In Vitro Antibacterial and Release Test. *ACS Omega*. <https://doi.org/10.1021/acsomega.3c10143>
- M., A., L., K., I., A. M., & M., E. (2022). Moroccan medicinal plants traditionally used for the treatment of skin diseases: From ethnobotany to clinical trials. *Journal of Ethnopharmacology*, 297.
- Mansur, H. S., Sadahira, C. M., Souza, A. N., & Mansur, A. A. P. (2008). FTIR spectroscopy characterization of poly (vinyl alcohol) hydrogel with different hydrolysis degree and chemically crosslinked with glutaraldehyde. *Materials Science and Engineering C*, 28(4). <https://doi.org/10.1016/j.msec.2007.10.088>
- Mohamed, S. A., & Hargest, R. (2022). Surgical anatomy of the skin. In *Surgery* (United Kingdom) (Vol. 40, Number 1). <https://doi.org/10.1016/j.mpsur.2021.11.021>
- Muhandri, T., Adawiyah, D. R., Listyawati, S., & Fadhilatunnur, H. (2024). Effectiveness of sugar palm fruit (*Arenga pinnata* (Wurmb) Merr.) and chitosan as edible coating on cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*). *Food Research*, 8(1). [https://doi.org/10.26656/fr.2017.8\(1\).134](https://doi.org/10.26656/fr.2017.8(1).134)

- Mujahid, M., Zubair, M., Yaqoob, A., Shahzad, S., & Ullah, A. (2025). Formulation and Evaluation of Licorice-Extract-Enhanced Chitosan, PVA, and Gelatin-Derived Hydrogels for Wound Dressing. *Bioengineering*, 12(5). <https://doi.org/10.3390/bioengineering12050439>
- Nguyen, T.-H., Ventura, R., Min, Y.-K., & Lee, B.-T. (2016). Genipin Cross-Linked Polyvinyl Alcohol-Gelatin Hydrogel for Bone Regeneration. *Journal of Biomedical Science and Engineering*, 09(09), 419–429. <https://doi.org/10.4236/jbise.2016.99037>
- Nur, A., Hajrah, H., Ilyas, A., Aisyah, A., & Escalante, M. C. L. (2022). Determination and Characterization of Lycopene Compounds from N-Hexane Fraction of Tomato Fruit (*Solanum Lycopersocum Grandyfolium*) by using Fourier Transform Infrared and UV-Vis Spectrophotometry. *Walisongo Journal of Chemistry*, 5(1). <https://doi.org/10.21580/wjc.v5i1.10885>
- Osmokrovic, A., Stojkovska, J., Krunic, T., Petrovic, P., Lazic, V., & Zvicer, J. (2025). Current State and Advances in Antimicrobial Strategies for Burn Wound Dressings: From Metal-Based Antimicrobials and Natural Bioactive Agents to Future Perspectives. In *International Journal of Molecular Sciences* (Vol. 26, Number 9). <https://doi.org/10.3390/ijms26094381>
- Pal, S., Raj, M., Singh, M., Saurav, K., Paliwal, C., Saha, S., Sharma, A. K., & Singh, M. (2024). The Effect of Aloe vera on Skin and Its Commensals: Contribution of Acemannan in Curing Acne Caused by *Propionibacterium acnes*. In *Microorganisms* (Vol. 12, Number 10). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/microorganisms12102070>
- Palshetkar, A. D., - Adurkar, A. T., & Kshitija, R. (2024). Formulation and Evaluation of a Film-Forming Gel for Enhanced Burn Wound Healing. In *Pharma Vision : Research And Reviews* (Number 2).
- Pangesty, A. I., Dwinovandi, C. S., Sunarso, Tarigan, S. J. A. P., Rahman, S. F., Katili, P. A., Azwani, W., Whulanza, Y., & Abdullah, A. H. (2024). PVA/gelatin hydrogel loaded with propolis for the treatment of myocardial infarction. *Journal of Science: Advanced Materials and Devices*, 9(3). <https://doi.org/10.1016/j.jsamd.2024.100732>

- Pappas-Taffer, L. (2024). Burn Classifications. In Ferri's Clinical Advisor 2025: 5 Books in 1. <https://doi.org/10.1016/B978-0-443-11724-4.00170-8>
- Radeef, Y. A., & Kadhum Al-Mari, A. M. (2023). A comparative study of the effect of nanoparticles and antibiotics on the antimicrobial susceptibility of antibiotics for some bacteria isolated from wounds and burns infections. *Bionatura*, 8(CSS3). <https://doi.org/10.21931/RB/CSS/2023.08.03.34>
- Rahman Khan, M. M., & Rumon, M. M. H. (2025). Synthesis of PVA-Based Hydrogels for Biomedical Applications: Recent Trends and Advances. In *Gels* (Vol. 11, Number 2). <https://doi.org/10.3390/gels11020088>
- Regina Leong, Z. L., Wan, H. L., & Teo, S. S. (2023). Antimicrobial activity of hydrogel loaded with *Capsicum annum*, *Auricularia auricula judae* and *Kappaphycus alvarezii*. *Research Journal of Chemistry and Environment*, 27(4). <https://doi.org/10.25303/2704rjce01006>
- Seeleamngam, S., Sangvanich, P., Thunyakitpisal, P., & Vongsutilers, V. (n.d.). Characterization of quality attribute and specification for standardization of acemannan extracted from *Aloe vera* for tissue regeneration. Retrieved <http://www.tjps.pharm.chula.ac.th>
- Sepe, F., Valentino, A., Marcolongo, L., Petillo, O., Conte, R., Margarucci, S., Peluso, G., & Calarco, A. (2025). Marine-Derived Polysaccharide Hydrogels as Delivery Platforms for Natural Bioactive Compounds. In *International Journal of Molecular Sciences* (Vol. 26, Number 2). <https://doi.org/10.3390/ijms26020764>
- Shannon, M., Lafeuille, J. L., Frégière-Salomon, A., Lefevre, S., Galvin-King, P., Haughey, S. A., Burns, D. T., Shen, X., Kapil, A., McGrath, T. F., & Elliott, C. T. (2022). The detection and determination of adulterants in turmeric using fourier-transform infrared (FTIR) spectroscopy coupled to chemometric analysis and micro-FTIR imaging. *Food Control*, 139. <https://doi.org/10.1016/j.foodcont.2022.109093>
- Shpichka, A., Butnaru, D., Bezrukov, E. A., Sukhanov, R. B., Atala, A., Burdukovskii, V., Zhang, Y., & Timashev, P. (2019). Skin tissue regeneration for burn injury. In *Stem Cell Research and Therapy* (Vol. 10, Number 1). <https://doi.org/10.1186/s13287-019-1203-3>

- Sikka, M. P., Bargir, J. A., & Garg, S. (2025). Modern developments in burn wound dressing. *Research Journal of Textile and Apparel*, 29(3). <https://doi.org/10.1108/RJTA-08-2023-0084>
- Sordi, M. B., Cruz, A., Fredel, M. C., Magini, R., & Sharpe, P. T. (2021). Three-dimensional bioactive hydrogel-based scaffolds for bone regeneration in implant dentistry. In *Materials Science and Engineering C* (Vol. 124). <https://doi.org/10.1016/j.msec.2021.112055>
- Stoica, A. E., Chircov, C., & Grumezescu, A. M. (2020). Hydrogel dressings for the treatment of burn wounds: An up-to-date overview. In *Materials* (Vol. 13, Number 12). <https://doi.org/10.3390/ma13122853>
- Sun, M., Wang, Y., Yao, L., Li, Y., Weng, Y., & Qiu, D. (2022). Fabrication and Characterization of Gelatin/Polyvinyl Alcohol Composite Scaffold. *Polymers*, 14(7). <https://doi.org/10.3390/polym14071400>
- Sun, S., & Chen, J. (2024). Recent Advances in Hydrogel-Based Biosensors for Cancer Detection. In *ACS Applied Materials and Interfaces* (Vol. 16, Number 36). <https://doi.org/10.1021/acsami.4c02317>
- Surowiecka, A., Strużyna, J., Winiarska, A., & Korzeniowski, T. (2022). Hydrogels in Burn Wound Management—A Review. In *Gels* (Vol. 8, Number 2). <https://doi.org/10.3390/gels8020122>
- Wang, Y., Beekman, J., Hew, J., Jackson, S., Issler-Fisher, A. C., Parungao, R., Lajevardi, S. S., Li, Z., & Maitz, P. K. M. (2018). Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring. In *Advanced Drug Delivery Reviews* (Vol. 123). <https://doi.org/10.1016/j.addr.2017.09.018>
- Wanniarachchi, P. C., Paranagama, I. T., Idangodage, P. A., Nallaperuma, B., Samarasinghe, T. T., & Jayathilake, C. (2025). Natural polymer-based hydrogels: Types, functionality, food applications, environmental significance and future perspectives: An updated review. *Food Biomacromolecules*, 2(1). <https://doi.org/10.1002/fob2.70000>

- Yarahmadi, A., Dousti, B., Karami-Khorramabadi, M., & Afkhami, H. (2024). Materials based on biodegradable polymers chitosan/gelatin: a review of potential applications. In *Frontiers in Bioengineering and Biotechnology* (Vol. 12). <https://doi.org/10.3389/fbioe.2024.1397668>
- Zainuddin, A. N. Z., Razif, R., Nizam, A. A. K., Maarof, M., Fadilah, N. I. M., Kim, Y. H., Mahmoudi, E., & Fauzi, M. B. (2025). Kelulut Honey-Incorporated Hybrid Gelatin-PVA Hydrogel for Wound Healing: Fabrication and In Vitro Characterization. *Polymers*, 17(19). <https://doi.org/10.3390/polym17192618>
- Zemljič, L. F., Volmajer, J., Ristić, T., Bracic, M., Sauperl, O., & Kreže, T. (2014). Antimicrobial and antioxidant functionalization of viscose fabric using chitosan–curcumin formulations. *Textile Research Journal*, 84(8), 819–830. <https://doi.org/10.1177/0040517513512396>
- Zhang, J., Yu, H., Man, M. Q., & Hu, L. (2024). Aging in the dermis: Fibroblast senescence and its significance. In *Aging Cell* (Vol. 23, Number 2). <https://doi.org/10.1111/accel.14054>
- Zhao, L., Zhou, Y., Zhang, J., Liang, H., Chen, X., & Tan, H. (2023). Natural Polymer-Based Hydrogels: From Polymer to Biomedical Applications. In *Pharmaceutics* (Vol. 15, Number 10). <https://doi.org/10.3390/pharmaceutics15102514>
- Żwierzeło, W., Piorun, K., Skórka-Majewicz, M., Maruszewska, A., Antoniewski, J., & Gutowska, I. (2023). Burns: Classification, Pathophysiology, and Treatment: A Review. In *International Journal of Molecular Sciences* (Vol. 24, Number 4). <https://doi.org/10.3390/ijms24043749>

APPENDICES



Accredited by University Grants
Commission (UGC) Nepal 2020

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
इन्जिनियरिङ्ग अध्ययन संस्थान
INSTITUTE OF ENGINEERING

पुल्चोक क्याम्पस
PULCHOWK CAMPUS

5-521260
5-521611
5-522104
5-522809

पुल्चोक, ललितपुर ।
Pulchowk, Lalitpur



Date: May 8, 2026

To Whom It May Concern:

This is to certify that the paper titled "*Physicochemical Development and Multi-Technique Characterization of PVA/Gelatin Based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Applications*" (Submission ID #1146), with **Prabhakar Thapa Magar** as the first author, was accepted through the peer-review process and has been presented at the 18th IOE Graduate Conference, organized at Pulchowk Campus, Lalitpur, Nepal, from May 7 to 9, 2026.

Please note that inclusion of the accepted manuscript in the conference proceedings is contingent upon timely compliance with any further editorial requirements during the publication process.

Prof. Sangeeta Singh
Convener
18th IOE Graduate Conference





IOE Graduate Conference

Certificate of Participation

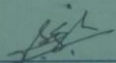



This certificate is awarded to

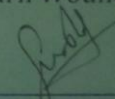
Prabhakar Thapa Magar

in recognition of an invaluable contribution to
the academic discourse and research dissemination
at the 18th IOE Graduate Conference held from May 7 to 9, 2026
organized by Pulchowk Campus, Lalitpur, Nepal
through the **Paper Presentation** entitled

"Physicochemical Development and Multi-Technique Characterization of PVA/Gelatin based Hydrogel Incorporating Natural Bioactive Compounds for Potential Burn Wound Healing Applications."


Prof. Sangeeta Singh
Conference Convener


Prof. Sanjay Uprey
Campus Chief, Pulchowk Campus


Prof. Susmi Bahadur Bajracharya
Dean, Institute of Engineering