

HYDROGEL-BASED WOUND HEALING THERAPEUTICS: TARGETED DRUG DELIVERY AND BIOACTIVE DRESSINGS

Submitted in partial fulfillment of the requirements of the award of

Master of Technology Degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY -1 UNIVERSITY BY UGC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of AAKASH RAJAN. S (42180001) who carried out the project report entitled "**Hydrogel-Based Wound Healing Therapeutics: Targeted Drug Delivery and Bioactive Dressings**" Under my supervision from November 2023 to April 2024.

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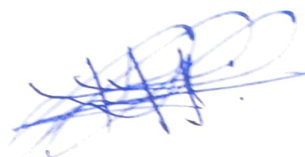
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DECLARATION

I AAKASH RAJAN. S (42810001) hereby declare that the Project Report entitled "**Hydrogel-Based Wound Healing Therapeutics: Targeted Drug Delivery and Bioactive Dressings**" done by me under the guidance **Dr. JAYSHREE NELLORE** at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in Biotechnology.

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ABSTRACT

Hydrogel-based wound healing therapies have attracted significant interest due to their potential in targeted drug delivery and as bioactive dressings. In this study, hydrogels were formulated using extracts from *Kappaphycus* and *Gracilaria*, two types of red seaweeds renowned for their wound healing properties. The effectiveness of these hydrogels in promoting wound healing was assessed through a series of assays. The DPPH assay was employed to evaluate the antioxidant activity of the seaweed extracts, confirming their ability to scavenge free radicals. Encapsulation efficiency studies demonstrated the hydrogels' capability to retain the active compounds present in the extracts. SEM analysis and FTIR were used for the characterization of the hydrogels, revealing insights into their morphology and chemical composition. Viscosity measurements indicated that the hydrogels were suitable for topical application, with the addition of a lipid mixture enhancing their viscosity. Gelation temperature tests revealed that the hydrogels formed at 27°C, suggesting their potential for in situ gel formation at body temperature. MTT assays conducted on cancer cells illustrated the hydrogels' ability to enhance the wound healing cellular pathway, thereby promoting increased wound healing. Additionally, the hydrogels showed promise in removing abnormal or damaged cells by enhancing the natural cell removal process through apoptosis and necrosis. Furthermore, fish embryo toxicity assays confirmed the biocompatibility of the hydrogels, indicating their safety for use in wound healing applications. Overall, the hydrogel-based wound healing therapies developed in this study exhibit significant potential for further advancement as effective and safe treatments for wound healing.

Key words: wound healing, *Kappaphycus* and *Gracilaria*, Hydrogel, Viscosity, Targeted drug delivery, MTT assay.

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LIST OF ABREVIATION

ACRONYM	EXPANSION
EPC	Phosphatidylcholine
FTIR	Fourier Transform Infrared Spectroscopy
G _R	<i>Gracilaria</i>
K _P	<i>Kappaphycus</i>
OA	Oelic Acid
SEM	Scanning Electron Microscopy
UV	Ultra violet visible spectroscopy

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CHAPTER 1

INTRODUCTION

1.1 Preface

Wound healing represents a complex biological process crucial for the restoration of tissue integrity and functionality following injury or trauma. Despite the innate regenerative capabilities of the human body, certain wounds, particularly chronic or severe ones, often necessitate therapeutic interventions to facilitate optimal healing outcomes. In recent years, researchers and clinicians have increasingly turned to innovative approaches to address the challenges associated with wound management, with a particular focus on harnessing the potential of hydrogel-based formulations, liposomes, and natural extracts derived from red algae, such as *Kappaphycus* and *Gracilaria* species.

Hydrogels, owing to their biocompatibility, versatility, and resemblance to the extracellular matrix, have emerged as promising materials for wound healing applications (Liang *et al.*, 2021). These water-swollen networks of polymers offer advantages such as moisture retention, mechanical support, and tunable drug release properties, making them well-suited for the development of advanced wound dressings and delivery systems (Liu *et al.*, 2018).

Liposomes, lipid-based vesicles capable of encapsulating a wide range of therapeutic agents, have garnered significant interest as carriers for targeted drug delivery in wound healing (Doktorovova and Souto, 2008). Their ability to encapsulate both hydrophilic and hydrophobic molecules, along with their biocompatibility and ability to protect encapsulated cargo from degradation, makes them an attractive option for enhancing the stability and efficacy of bioactive compounds (Ali and Ahmed, 2018).

Natural extracts derived from red algae, particularly species belonging to the *Kappaphycus* and *Gracilaria* genera, have long been recognized for their bioactive properties, including antioxidant, anti-inflammatory, and wound healing effects (Beaumont *et al.*, 2021). These extracts contain a rich array of polysaccharides, polyphenols, and peptides that can stimulate various stages of the wound healing

process, including cell proliferation, angiogenesis, and collagen synthesis (Feki *et al.*, 2020).

The integration of liposomes, red algae extracts, and hydrogels, including those derived from *Kappaphycus* and *Gracilaria* species, presents a synergistic approach to wound healing therapeutics. By combining these elements, it is possible to create innovative formulations capable of targeted drug delivery, sustained release of bioactive compounds, and provision of a conducive microenvironment for tissue regeneration.

1.2 Purpose of study:

This study aims to investigate the efficacy of a novel integrated system comprising liposomes, red algae extracts derived from *Kappaphycus* and *Gracilaria* species, and hydrogels in promoting wound healing. Specifically, we seek to evaluate the synergistic effects of these components on key aspects of the wound healing process, including inflammation modulation, cell proliferation, angiogenesis, and extracellular matrix deposition.

1.3 Hypothesis:

We hypothesize that the combined use of liposomes, red algae extract from *Kappaphycus* and *Gracilaria* species, and hydrogels will enhance wound healing outcomes compared to conventional treatment modalities. Specifically, we expect that the integrated system will exhibit:

Improved targeted delivery and sustained release of bioactive compounds to the wound site, leading to enhanced therapeutic efficacy.

Reduction in inflammation and promotion of a favorable wound healing environment, facilitating accelerated tissue regeneration.

Stimulation of cell proliferation, angiogenesis, and extracellular matrix synthesis, resulting in enhanced wound closure and tissue remodeling.

By testing this hypothesis through in vitro and in vivo experiments, we aim to provide valuable insights into the potential clinical applications of this integrated approach and contribute to the advancement of wound healing therapeutics.

CHAPTER 2

LITERATURE SURVEY

2.1 INTRODUCTION TO WOUND HEALING

2.1.1 Overview of the Wound Healing Process

A lot of work has gone into wound care, with a focus on developing new therapeutic strategies and technological advancements for the management of both acute and chronic wounds. Multiple cell types, the extracellular matrix, and the action of soluble mediators including growth factors and cytokines are all involved in wound healing. Despite being an ongoing process, healing can be loosely separated into four stages: (i) hemostasis and coagulation; (ii) inflammation; (iii) proliferation; and (iv) remodelling of the wound with the creation of scar tissue. The clinical result can be effectively influenced by the appropriate approach to wound treatment. This overview covers wound classification, wound treatment techniques, and the physiology of the wound healing process (Velnar *et al.*, 2009).

A number of cutting-edge medical products have been created to address the chronic wound environment and promote skin tissue regeneration, including surgical sutures, wearable wound monitoring, negative pressure wound therapy devices, and wound dressings. The majority of medical devices are a broad category of goods that include bioactive molecules and natural (like chitosan, keratin, casein, collagen, hyaluronic acid, alginate, and silk fibroin) and synthetic (like polyvinyl alcohol, polyethylene glycol, poly[lactic-co-glycolic acid], polycaprolactone, and polylactic acid) polymers. The goal of this study is to provide a crucial theoretical framework for future studies on chronic wound healing by addressing these medical devices with an emphasis on biomaterials and applications (Deng *et al.*, 2022).

2.1.2 Challenges in Conventional Wound Management

wounds with potentially fatal clinical consequences. With co-morbid disorders, chronic wound provide several difficulties. Complementary and alternative

medicine (CAM) is becoming more widely used in the healthcare industry, and its uses in wound care are still similar to "separating the diamonds from the ore." A number of synthetic and natural chemicals have been studied in relation to hallmark genes in an effort to gain a molecular understanding of the wound. We've reviewed the difficulties in healing chronic wounds and the function of complementary and alternative medicine in treating them. The primary focus is on the uses and drawbacks of the current range of treatments for non-healing wounds, as well as the best alternatives to take into account. This data contributes to a greater understanding of the difficulties associated with chronic wound healing, which can be further resolved by combining combination therapies and a multidisciplinary approach (Monika *et al.*,2022)

While standard wound management is routinely used in general practice, scientists have extensively investigated the underlying mechanism of infections. However, the occurrence of biofilm colonization, delayed healing, and drug resistance make strategies for the diagnosis and treatment of wound infections extremely difficult. The common microorganisms associated with both acute and chronic wound infections are outlined in this paper, along with the difficulties in diagnosing patients using non-surgical and surgical approaches (Ding *et al.*,2022)

2.1.3 Rationale for Advanced Therapeutic Strategies

A new and integrated cognitive field has recently emerged as a result of the design and development of a modern health policy in the field of regenerative medicine. This field will require methodical research and study to generate creative solutions and best practices. Even though 285 products have already been registered by the Committee for Advanced Therapies (CAT) as ATMPs, only 10 licenses have been granted, leaving only six (the remaining licenses were for withdrawn products). Academic/research centers and small and medium-sized businesses continue to be the main players in the development and delivery of ATMPs; pharmaceutical companies' involvement is concentrated on the latest advancements in the use of chimeric antigen receptor (CAR)-T cells and in vitro

modified cytotoxic T lymphocytes for the treatment of oncological incidents (Gkioka *et al.*,2020)

Prospective treatments target one or more of these: tau proteins (tau phosphorylation/aggregation and immunotherapy), neuroinflammation, amyloids (secretases, A β 42 generation, amyloid deposition, and immunotherapy), and additional unspecified targets. After 18 years, or since 2003, the FDA has only approved aducanumab for the treatment of AD, despite the significant progress we have made in understanding the underlying pathophysiology of the disease. So, it may be said that in order to find and create therapeutic molecules to combat the century-old AD, new treatment approaches are needed (Athar *et al.*,2021)

2.2 COMPONENTS OF THE MULTIFACETED APPROACH

2.2.1. Liposomes: Precision in Drug Delivery

2.2.1.1 Encapsulation of Therapeutic Agents

Liposomes have attracted significant attention in recent years for their potential to revolutionize drug delivery systems. These lipid-based vesicles provide a versatile platform for encapsulating and delivering various therapeutic agents. Numerous studies have demonstrated their ability to encapsulate a wide range of drugs, including hydrophilic and hydrophobic compounds, peptides, and nucleic acids, with high efficiency and stability (Zhao *et al.*, 2022). The precise drug delivery capability of liposomes is due to their unique structure, which enables controlled release of encapsulated drugs at the desired site of action. By modifying the surface properties of liposomes with ligands or antibodies, researchers have achieved targeted delivery to specific cells or tissues, reducing off-target effects and enhancing therapeutic outcomes (Yang *et al.*, 2022).

2.2.1.1 Targeted Release at the Wound Site

In the realm of wound healing, liposomes show great potential for delivering therapeutic agents directly to the wound site. Chronic wounds, such as diabetic ulcers and pressure sores, pose significant challenges in treatment due to impaired healing processes and microbial colonization. Liposomal formulations offer a promising approach by providing sustained release of antimicrobial agents, growth factors, and anti-inflammatory drugs directly to the wound bed. This promotes tissue regeneration and helps prevent infection (Zamani *et al.*, 2021). Studies (Shakhakarmi *et al.*, 2023) have demonstrated the effectiveness of liposomal delivery systems in improving wound healing outcomes. By encapsulating growth factors such as platelet-derived growth factor (PDGF) or vascular endothelial growth factor (VEGF) within liposomes, researchers have observed accelerated wound closure and enhanced tissue regeneration in animal models.

2.2.1.3 Advantages for Localized Treatment

One of the primary benefits of using liposomal formulations for localized treatment is their capacity to reduce systemic exposure and minimize systemic side effects often linked with conventional drug delivery methods (Bulbake *et al.*, 2017). By delivering therapeutic agents directly to the intended site, liposomes can achieve higher local drug concentrations while avoiding unnecessary exposure of healthy tissues. Additionally, the adaptability of liposomal formulations allows for the integration of multiple therapeutic agents into a single delivery system, facilitating combination therapy for improved effectiveness. This approach has demonstrated potential in treating various localized conditions such as cancer, inflammatory disorders, and infectious diseases (Kulkarni *et al.*, 2018).

2.2.2 Red Algae Extract: Bioactive Healing Agents

2.2.2.1 Rich Sources of Polysaccharides, Polyphenols, and Peptides

According to Vo *et al.* (2020), red algae, also referred to as Rhodophyta, have emerged as a valuable reservoir of bioactive compounds with therapeutic potential in wound healing and tissue regeneration. These marine organisms possess a rich

content of polysaccharides, polyphenols, peptides, and other bioactive molecules that contribute to their healing attributes. Polysaccharides extracted from red algae, such as carrageenans and agarans, have been extensively researched for their wound healing properties. These polysaccharides demonstrate mucoadhesive characteristics, forming a protective layer over the wound surface and fostering cell proliferation and migration (Ismail et al., 2020). Furthermore, polyphenols present in red algae, such as phlorotannins and flavonoids, exhibit antioxidant and anti-inflammatory properties, which play a pivotal role in mitigating oxidative stress and inflammation during the wound healing process (Wan et al., 2019). Additionally, peptides derived from proteins in red algae have exhibited promising bioactivities, including antimicrobial, antioxidant, and immunomodulatory effects, thereby aiding in wound healing and tissue repair (Fleurence and Levine, 2014).

2.2.2.2 Anti-inflammatory and Antimicrobial Properties

The red algae extract offers significant therapeutic advantages, particularly its strong anti-inflammatory and antimicrobial properties. Inflammation and microbial infections often hinder wound healing, causing delays and increasing the risk of complications (Torres et al., 2019). Research has shown that compounds derived from red algae can effectively inhibit the production of pro-inflammatory cytokines and enzymes, such as tumor necrosis factor-alpha (TNF- α) and cyclooxygenase-2 (COX-2), thus reducing the inflammatory response and supporting tissue repair. Red algae extracts demonstrate a wide-ranging antimicrobial effect against diverse pathogens, including bacteria, fungi, and viruses. This antimicrobial effectiveness is linked to the existence of sulfated polysaccharides and bioactive peptides, which disrupt microbial cell membranes and impede microbial growth (Meena et al., 2021).

2.2.2.3 Promotion of Angiogenesis and Tissue Regeneration

Angiogenesis, the process of forming new blood vessels, is vital for wound healing as it supplies oxygen and nutrients to the regenerating tissues. Studies indicate that red algae extract promotes angiogenesis by releasing angiogenic factors and modulating endothelial cell function (El-Sheekh et al., 2016). As stated by Ale et al. (2011), the bioactive elements of red algae, such as polysaccharides

and polyphenols, stimulate cell proliferation, extracellular matrix synthesis, and collagen deposition to support tissue regeneration.

2.2.3 Hydrogel: Creating a Supportive Environment

Hydrogels are a new class of biomaterials that can be used in many different biomedical applications, such as wound healing, drug delivery, and tissue engineering, to create a supportive environment. According to Li and Mooney (2018), they can be used to mimic the natural extracellular matrix and create a favorable microenvironment for cell proliferation and tissue regeneration because of their high water content and adjustable mechanical properties. The goal of recent developments in hydrogel design has been to add bioactive molecules, such as growth factors, peptides, and extracellular matrix components, to the gels to improve both their structural integrity and bioactivity. the study by Yue et al. (2015), these biofunctionalized hydrogels facilitate tissue repair and regeneration by encouraging cell adhesion, migration, and differentiation.

2.2.3.1 Moisture Retention and Wound Bed Protection

Hydrogels are essential in wound healing applications because they keep the wound bed moist and shield it from outside impurities. Their capacity to take in and hold a lot of water aids in wound hydration, encourages autolytic debridement, and quickens the body's natural healing process (meena *et al.*, 2019). Studies by Mao *et al.* (2019) have investigated the application of sophisticated hydrogel formulations for improved moisture retention and wound bed protection, such as self-healing hydrogels and smart hydrogels. These next-generation hydrogels offer enhanced functionality and efficacy in wound management due to their stimuli-responsive behavior, self-repairing qualities, and controlled release of therapeutic agents.

2.2.3.2 Sustained Release of Encapsulated Compounds

The ability of hydrogels to encapsulate and release medicinal compounds in a controlled and sustained manner is one of their main advantages. Longer release kinetics can be achieved by researchers by adding medications, growth factors, or other bioactive agents into the hydrogel matrix. This reduces the need for frequent dosing and increases patient compliance (Hoffman, 2012). The goal of recent

advancements in hydrogel-based drug delivery systems has been to enhance biocompatibility, release kinetics, and drug loading capacity. The performance and adaptability of hydrogel drug carriers have been improved by the use of tactics like molecular imprinting, nanoparticle-hydrogel composites, and microfluidic fabrication methods (Luo *et al.*, 2023).

2.2.3.3 Biocompatibility and Biodegradability

The clinical translation of hydrogel-based therapies necessitates the careful consideration of biocompatibility and biodegradability. The creation of biocompatible and biodegradable hydrogel formulations that reduce adverse reactions and promote tissue integration and remodeling has received significant attention in recent research (Nunes *et al.*, 2022). Alginate, chitosan, and hyaluronic acid are examples of novel hydrogel materials that have demonstrated exceptional biocompatibility and degradation profiles. Furthermore, the development of hydrogels with customized mechanical characteristics and degradation rates has been made possible by advancements in crosslinking chemistries and fabrication techniques, providing exact control over tissue regeneration processes (Chen *et al.*, 2019).

2.2.4 Gellan Gum Hydrogels: Scaffold for Enhanced Healing

Hydrogels made of gellan gum have drawn interest as potentially effective scaffolds for improving healing in a range of biomedical applications, such as tissue engineering and regenerative medicine. According to Famielec and Flieger (2024), these hydrogels provide a three-dimensional network structure that resembles the extracellular matrix, fostering cell attachment, proliferation, and differentiation. Gellan gum hydrogels have been shown in recent research to be useful in fostering tissue regeneration in a variety of anatomical locations, such as bone, cartilage, and skin. Their adjustable characteristics, like porosity, stiffness, and degradation kinetics, enable the creation of scaffolds that are specifically suited to the needs of different tissues (Yang *et al.*, 2023).

2.2.4.1 Structural Integrity and Mechanical Support

Gellan gum hydrogels' superior mechanical and structural integrity is one of their main benefits. These hydrogels are appropriate for load-bearing applications in tissue engineering because they have a high water content and swelling capacity while retaining mechanical stability (das and giri, 2020). Improved mechanical properties and durability have resulted from recent developments in gellan gum hydrogel fabrication techniques, including crosslinking techniques and composite formulations. Gellan gum scaffolds have been made stronger and more stable through the use of techniques like covalent crosslinking, physical reinforcement, and hybridization with reinforcing materials (Claudia *et al.*, 2016).

2.2.4.2 Synergistic Integration with Hydrogel Matrices

To achieve improved functionality and performance, gellan gum hydrogels can be synergistically integrated with other hydrogel matrices. Researchers can modify the characteristics of the resultant hydrogel composite to fulfill particular biomedical needs by mixing gellan gum with other natural or synthetic polymers (Gupta *et al.*, 2014). Gellan gum's synergistic effects with polymers like hyaluronic acid, chitosan, and alginate have been investigated recently in a variety of tissue engineering applications. According to Singh *et al.* (2024) these hybrid hydrogel systems have better mechanical strength, bioactivity, and biocompatibility, which promotes better cell adhesion, proliferation, and tissue regeneration.

2.2.4.3 Facilitation of Tissue Regeneration Processes

Gellan gum hydrogels are essential for supporting the processes involved in tissue regeneration because they create an environment that is favorable for cellular activity. According to Oliveira *et al.* (2010), their bioactive characteristics, such as growth factor binding sites and cell adhesion ligands, stimulate cell-matrix interactions and signaling pathways involved in tissue regeneration and repair. In order to increase the bioactivity and therapeutic efficacy of gellan gum hydrogels, recent research has concentrated on optimizing their composition and structure. To alter cellular responses and hasten tissue healing, techniques like surface

modification, the addition of bioactive molecules, and the regulated release of growth factors have all been investigated (salas *et al.*, 2022).

2.3 SYNERGISTIC EFFECTS AND MECHANISMS OF ACTION

The combination of liposomes, red algae extract, hydrogel, and gellan gum hydrogels offers a unique opportunity to harness the synergistic effects of these versatile biomaterials for improved therapeutic outcomes in wound healing and tissue regeneration. Each component contributes distinct bioactive properties and mechanisms of action that complement and enhance one another, resulting in enhanced efficacy and efficiency of the combined system (rasa *et al.*, 2018). Recent studies have revealed the synergistic interactions between these components at the molecular and cellular levels. These interactions involve intricate signaling pathways, cellular responses, and biochemical processes that regulate inflammation, stimulate angiogenesis, and expedite wound closure. Understanding these mechanisms is essential for optimizing the design and formulation of multifunctional biomaterial-based therapies (Ahmad *et al.*, 2022).

2.3.1 Combined Benefits of Liposomes, Red Algae Extract, Hydrogel, and Gellan Gum Hydrogels

The combined use of liposomes, red algae extract, hydrogel, and gellan gum hydrogels provides numerous advantages for wound healing and tissue regeneration. Liposomes offer a versatile platform for the targeted delivery and sustained release of bioactive compounds. Red algae extract contributes anti-inflammatory, antimicrobial, and pro-angiogenic properties. Hydrogel and gellan gum hydrogels act as scaffolds, creating a supportive environment that facilitates cellular activities and promotes tissue regeneration (Ren *et al.*, 2021). Recent research has highlighted the collective benefits of these biomaterials in speeding up wound closure, reducing inflammation, and supporting tissue repair in both preclinical and clinical studies. The synergistic integration of liposomes, red algae extract, hydrogel, and gellan gum hydrogels presents a holistic approach to wound management, addressing multiple aspects of the wound healing process concurrently (Zhao *et al.*, 2018).

2.3.2 Targeted Delivery and Sustained Release of Bioactive Compounds

The ability of the combined system to deliver bioactive compounds to the wound site with targeted delivery and sustained release is one of its main advantages. Therapeutic agents can be encapsulated in liposomes and directed towards particular cells or tissues; on the other hand, hydrogel and gellan gum hydrogels offer a matrix for the controlled release and extended retention of encapsulated substances (Bercea, 2022). Recent developments in the fields of biomaterial science and nanotechnology have made it possible to precisely control the kinetics of bioactive compound loading, encapsulation, and release within hydrogel, gellan gum hydrogels, liposomes, and red algae extract. Methods to maximize therapeutic efficacy and reduce side effects have been investigated, including surface modification, stimuli-responsive release, and co-delivery of multiple agents (Yang *et al.*, 2023).

2.3.3 Modulation of Inflammation, Promotion of Angiogenesis, and Accelerated Wound Closure

By doing synergistic mechanisms of action, the combined system has a multifaceted effect on the wound healing process by modulating inflammation, promoting angiogenesis, and speeding up wound closure. Red algae extract contains bioactive compounds that promote angiogenesis and improve wound healing, while liposomes deliver growth factors and anti-inflammatory agents to reduce inflammation and stimulate tissue regeneration (Chen *et al.*, 2023). Recent research has shown that gellan gum hydrogels and hydrogels can support cell migration, proliferation, and differentiation by forming a favorable microenvironment, which helps promote tissue regeneration and repair. According to Xu *et al.* (2023) the combined effects of these biomaterials lead to improved functional outcomes and patient satisfaction by accelerating wound closure, improving tissue quality, and reducing scarring.

2.4 APPLICATIONS IN WOUND HEALING AND TARGETED DELIVERY

The integrated approach involving liposomes, red algae extract, hydrogel, and gellan gum hydrogels shows great potential for use in wound healing and targeted delivery of bioactive substances. By harnessing the combined effects and mechanisms of these biomaterials, healthcare providers and researchers can tackle various challenges in wound management, including modulating inflammation, promoting new blood vessel formation, and speeding up wound closure (Yao *et al.*, 2021).

Recent research has proven the effectiveness of this integrated system in stimulating tissue regeneration and reducing healing time in animal models of both acute and chronic wounds. Liposomes allow for the targeted delivery and sustained release of therapeutic substances, while red algae extract offers bioactive compounds known for their anti-inflammatory, antimicrobial, and pro-angiogenic properties. Hydrogel and gellan gum hydrogels act as supportive structures, creating a favorable microenvironment that enhances cellular functions and facilitates tissue repair (Wang *et al.*, 2022).

2.4.1 Potential Clinical Settings and Patient Populations

The combined system's adaptability and customizability make it appropriate for a variety of clinical environments and patient demographics. When it comes to acute wound care—such as those from surgical incisions and traumatic injuries—the combined system can encourage quick healing and lower the chance of problems. The system can promote tissue regeneration and increase wound closure rates in the treatment of chronic wounds, such as pressure sores and diabetic ulcers (Xu *et al.*, 2018). Moreover, this integrated system holds promise for use in specialized wound care settings such as burn units, dermatology clinics, and plastic surgery centers. Its ability to target specific cellular pathways and deliver bioactive substances in a controlled manner makes it particularly well-suited for personalized medicine approaches tailored to the unique needs of each patient (Sun *et al.*, 2023).

2.4.2 Tailoring Formulations for Specific Wound Types

Optimizing the combined system's formulations for particular wound types is crucial to achieving the best possible clinical results and therapeutic effectiveness. Different types of wounds have different needs and challenges, including different levels of tissue loss, infection, and inflammation. To effectively address these challenges, therefore, customized formulations incorporating particular combinations of hydrogel, red algae extract, liposomes, and gellan gum hydrogels may be required (liu et al., 2022). The development of biomaterial-based treatments for particular wound types, such as diabetic foot ulcers, venous leg ulcers, and pressure injuries, has been the focus of recent research. The combined system's suitability for various wound environments and patient populations can be improved by employing strategies like modifying the composition, concentration, and release kinetics of bioactive compounds (Zhao et al., 2023).

2.4.3 Considerations for Translational Research and Future Clinical Trials

There are a number of factors that need to be carefully taken into account when translating the combined system from preclinical research to clinical practice, such as safety, efficacy, regulatory requirements, and commercialization strategies. Subsequent clinical trials ought to concentrate on assessing the combined system's safety profile, dose-response relationship, and long-term results in a range of patient populations (Chen et al., 2022).

Furthermore, in order to advance translational research and remove obstacles to clinical adoption, cooperation between regulatory bodies, industry, and academia is crucial. To demonstrate the clinical utility and commercial viability of the combined system, Fan et al. (2020) emphasize the importance of robust preclinical data, standardized manufacturing processes, and rigorous clinical trial designs.

2.5 CONCLUSION AND FUTURE DIRECTIONS

In summary, the combined system of liposomes, red algae extract, hydrogel, and gellan gum hydrogels represents a promising approach for wound healing and targeted delivery of bioactive compounds. Through synergistic interactions and

mechanisms of action, this multifunctional system offers enhanced therapeutic outcomes in terms of inflammation modulation, angiogenesis promotion, and accelerated wound closure.

2.5.1 Implications for Clinical Practice:

The combined system holds significant implications for clinical practice, offering a versatile and customizable approach to wound management across diverse clinical settings and patient populations. Its potential applications in acute and chronic wound care, specialized wound clinics, and personalized medicine approaches underscore its clinical utility and relevance.

2.5.2 Areas for Further Research and Development:

Despite significant progress, several areas warrant further research and development to optimize the clinical translation and commercialization of the combined system. Future studies should focus on refining formulations tailored to specific wound types, conducting rigorous clinical trials to evaluate safety and efficacy, and addressing regulatory and commercialization challenges.

Overall, the combined system of liposomes, red algae extract, hydrogel, and gellan gum hydrogels holds great promise for advancing the field of wound healing and targeted drug delivery. Continued research efforts and collaborative initiatives are essential for realizing its full potential in clinical practice and improving patient outcomes in wound care.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To investigate the efficacy and synergistic effects of integrating liposomes, red algae extract, hydrogel, and Gellan gum hydrogels for targeted delivery in wound healing therapeutics.

3.2 OBJECTIVE

- Obtain Kappaphycus and Gracilaria red algae species from cultivated sources.
- Characterize the bioactive compounds present in the algae extracts.
- Extract bioactive compounds from Kappaphycus and Gracilaria red algae using methanol as the solvent.
- Evaluation of Antioxidant Activity (DPPH Assay)
- Preparation of Lipid Mixture and Liposomes:
- Characterization of Lipid Mixture and Liposomes:
- Assessment of Encapsulation Efficiency:
- Formulation of Liposomal Hydrogel using Gellan Gum Powder
- Evaluation of Hydrogel Viscosity and Gelation Properties
- Assessment of Cytotoxicity and Biocompatibility

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS:

4.1. COLLETION OF BIOACTIVE COMPOUNDS:

For this experiment two bioactive compound is taken from rhodopyta species which is known as red algae they are *Kappaphycus* and *Gracilaria*. *Kappaphycus* is cultivated from southeast part of India and *Gracilaria* seaweed is cultivated from Kovalam backwaters at Marine Culture Centre Tamilnadu. *Kappaphycus* species are multicellular sea algae distinguished by their red or reddish-brown color.

They usually have cylindrical or flattened thalli (bodies) made of branching or un branched filaments. The size, texture, and appearance of the thalli might vary depending on the species and the habitat. *Kappaphycus* species are commercially cultivated for a variety of applications, including food, agar production, and biotechnology. Carrageenan, a hydrocolloid dutilized as ageing agent, stabilizer, and thickening in a variety of culinary and industrial uses, is abundant in them. *Kappaphycus* species have bioactive substances with potential pharmacological and nutraceutical use. Extracts from these algae have been investigated for antioxidant, anti-inflammatory, and antiviral properties, among other health advantages. *Gracilaria* species are distinguished by their crimson or purple coloring and delicate, branching thalli. *Gracilaria* thalli are normally made up of cylindrical or flattened fronds that vary insize and form depending on the species and the climate.

The placement of reproductive organs along the branches of some *Gracilaria* species gives them a distinctive "beaded" look. *Gracilaria* is commercially grown for a variety of applications, including agar manufacture, food, and biotechnology. Agar, agelatinousmaterial derived from *Gracilaria*, is commonly used in the culinary industry as a gelling agent, thickener, and stabilizer in desserts, soups, and confectionary.

Gracilaria is often used in aquaculture to feed fish and shellfish. *Gracilaria* species include bioactive substances that have potential medicinal, nutraceutical, and cosmetic uses. Extracts from these algae have been investigated for

their antioxidant, anti-inflammatory, antibacterial, and anticancer effects. Gracilaria-derived products find application in cosmetics, dietary supplements, and traditional medicine formulations.

4.1.2. compound required:

Phosphatidylcholine (EPC)

Oleic acid

Cholesterol

Chloroform

Phosphate buffer

Methanol

Distilled water

Gellan gum powder

Doubly distilled water

4.2. METHODOLOGY

4.2.1. Extraction of plant materials:

Take 500g of *Kappaphycus* and *Gracilaria* separately and wash them with water. After cleaning is done through mortar and pestle apparatus grind the plants and add 100ml of methanol for preparing 100ml of plant extract while grinding. After grinding the plants take the methanolic plant extract solution and filter them with Whatmann paper which removes dirt and impurities. And centrifuge for 15 mins and collect supernatant.

4.2.2 DPPH assay antioxidant activity:

Prepare sample extract stock solution serial dilution with 99% methanol of 1, 2, 3, 4, 5 & 6 ml solution in 6 test tubes. Then 4ml of DPPH solution is added to each of the test tube and mix it well. Make up all volume to 10ml with 99% methanol and mix it well. And, Incubate at room temperature for 30 minutes in dark place to complete the reaction.

Observe the color change from dark violet to different degree of discoloration it is depending on the concentration of antioxidant. Then absorbance of the solution is measured absorbance 517nm against blank.

The capability to scavenge the DPPH radical was calculated by.

$$\text{DPPH scavenging effect (\%)} = [(A_c - A_s)/A_c] \times 100$$

A_c = The absorbance of control

A_s = The absorbance of sample

4.2.3. Preparation of lipid mixture:

Preparation of liposomes is done by thin film hydration method. Add EPC: cholesterol: oleic acid at ratio of 7.5: 1.5: 2.8 were dissolved with 5ml of chloroform in 250ml of round bottom flask and spread gently over the wall of flask for to achieve thin film. The dried lipid film was rehydrated with 5ml of phosphate buffer saline (PBS) and then add 1mg/ml of plant extracts to lipid mixture. The resulting solution was vortexed for 1min and the multi lamellar vesicles was obtained by sonication the lipid suspension for 3min. After Tiporbe sonicator was used to sonicate the MLVs for 30s at 20w to produce Luvs-large unilamellar vesicles. And then vesicles was filtered by polycarbonate membrane with 100nm.

4.3. CHARACTERIZATION:

4.3.1. *Scanning electron microscopy:*

Scanning Electron Microscopy (SEM) may be used to examine the lipid mixture, revealing surface shape, particle size, and aggregation behavior of lipid components. SEM analysis is important in the investigation of liposomal lipid mixtures because it can produce high-resolution pictures of liposome surface shape. Liposomes' size and shape have a direct influence on their stability, drug encapsulation efficiency, and interaction with biological systems. SEM offers extensive information on the surface topography, size distribution, and shape features of liposomes, helping researchers to better understand the structural aspects of liposomal lipid combination. Furthermore, SEM analysis may be utilized to assess how formulation parameters such as lipid content, preparation procedures, and encapsulated components affect the structural integrity and physical properties of liposomes. This data is critical for refining the design and performance of liposomal drug delivery systems, as well as quality control in.

4.3.2. *FTIR:*

FTIR analysis, also known as Fourier-transform infrared spectroscopy, is a commonly used technique in chemistry and biochemistry for determining and studying the chemical composition of diverse materials. In recent years, FTIR analysis has been used to examine seaweed and algae, notably kappaphycus and gracilaria species, because to their potential utility in a variety of applications including food, medicines, and biofuel generation. When doing FTIR analysis on kappaphycus and gracilaria, researchers often dry and ground the materials to a fine powder. The powdered samples are then combined with an appropriate infrared- transparent substance, such as potassium bromide, and pressed into a thin, homogenous pellet. The pellets are then put in an FTIR spectrometer, which measures the absorption of infrared light over a variety of frequencies. The obtained spectrum is then examined to determine whether functional groups exist in the samples. The FTIR spectra of kappaphycus and gracilaria often display distinct

peaks corresponding to the numerous functional groups found in seaweeds. Polysaccharides, such as agar and carrageenan, may be recognized by distinct peaks in the carbohydrate portion of the spectrum.

4.3.3. ENCAPSULATION EFFICIENCY:

The encapsulation efficiency (EE%) is defined as the ratio of the concentration of the integrated material (active substances, medicines, scents, proteins, insecticides, antimicrobial agents, and so on) identified in the formulation to the original concentration utilized to create the formulation.

The encapsulation efficiency (EE%) was determined using the formula below:

$$EE\% = (W_T/W_i) \times 100\%$$

W_T = Total amount of integrated material

W_i = Total amount of incorporated material added at the start of the preparation

4.4. LIPOSOMAL HYDROGEL FORMULATION USING GELLAN GUM POWER:

Gellan gum is a natural polysaccharide extracted from a specific type of bacteria that forms a three-dimensional network which is used for drug delivery. Add gellan gum powder 0.2% w/v in 100 ml of double distilled water at 30 mins by continuous stirring cooled to 50°C add (1 mg/g) plant extract powder keep it for 20 min. Gently mix lipid suspension with gellan gum solution.

4.5. VISCOSITY OF HYDROGEL:

The high acyl gellan gum powder is used to make gel. For this viscosity test sodium chloride, potassium chloride, and calcium chloride is used for testing viscosity of gellan gum gel. The first step was to prepare gellan gum gel by magnetic stirring in 85°C for 30 mins. After stirring, gellan gum powder dissolved in distilled water and formed thick and milky fluid.

When the solution cools, it thickens and forms a soft gel that breaks down with agitation and mixing. Secondly, the effect of GLG concentration on viscosity was examined by adding GLG to distilled water at different doses. Thirdly, high temperatures were applied to GLG solutions in order to evaluate the impact of temperature on viscosity. Fourthly, the effects of monovalent and divalent ions on viscosity were measured by adding NaCl, KCl, and CaCl₂ to GLG solutions. Thus the viscosity of GLG is measured by this method.

4.6. GELATION TEMPERATURE AND TIME:

Gellan gum hydrogels still face a significant challenge when it comes to encapsulating live cells in a tissue engineering setting while preserving their injectability. For injectable encapsulation, the T_{gelation} is commercially available, that intact gellan gum is too high (> 42 °C) under physiological cationic circumstances to suspend cells. There are just a few appropriate chemistries and formulations since cells are present during the gelation process. Several studies on gellan gum hydrogels show that the gelation temperature may be adjusted to be medically appropriate by: (i) Adjusting gellan's molecular weight and (ii) functionalization of gellan with carboxymethyl and thiol groups.

4.7. MTT ASSAY:

Cells were seeded into 96-well plates at a density of 5×10^4 cell/mL per well and allowed to attach overnight in 200 μ L medium incubated at 37°C in 5% CO₂ in the air. The seaweed extracts in 21.7 mM ethanol were sterile-filtered prior to addition to plated cells. Seaweed extracts were added at a final concentration of 0.125, 0.25,

0.5, 1 and 2 mg/mL of medium, and the cells left to incubate in the seaweed extract containing medium for 24 h at 37°C and 5% CO₂ [21]. A set of menadione (6.25, 12.5, 25, 50, 100 µM) as positive control and medium without compound as negative control were included in each microtitre plate. After incubation, traces of seaweed extract were removed by washing the cells with 200 µL PBS and applying 200 µL of fresh medium plus 20 µL of 5mg/mLMTT salt to determine the effects of the algal extracts on cell proliferation [25]. Cells were then incubated for 4 h at 37°C, 5% CO₂. After 4 h, the medium was removed carefully and 200 µL of DMSO were added to each well in order to solubilise the purple crystal formazan that were formed in the well bottom. The plate was read using an ELISA microplate reader (I Mark, Bio-Rad, USA) and the absorbance measured at 570 nm. Each concentration of the respective algal extracts was assayed in triplicate.

4.8. FISH EMBRYO TOXICITY ASSAY

4.8.1. Embryo procurement/preparation and maintenance:

The zebrafish (*Danio rerio*) embryos were procured from a lab animal provider in bulk as fertilized eggs; they were then transferred in a large holding vessel for maturation. The embryo formation is visible in 24hrs.

To maintain PH, temperature, and prevent contamination, we use HEPES E3 media which is prepared by adding 16.50ml per liter of distilled water and a few drops of methylene blue for indication purpose. Once prepared, the media is filter-sterilized stored under refrigeration and away from direct sunlight.

For observing and maintaining the growth of embryos, A Sterile segmented petri-dish is taken and is filled with 10-15ml of E3 media, and 10 eggs are added per segment. These Petri dishes are then stored in a cool region with no direct sunlight and disturbance. The embryo is observed every 24 hours under a microscope. Once the observations are finished, the media is replaced by removing the fish from the old petri dish and moving it to new petri-dish filled with media.

4.8.2. Sample loading and observation:

We begin by preparing 5 petri-dish (1X Control, 2X Half strength, 2 X Full strength) and each data such as alive, dead, hatched, hatching, misread of each

petri-dish is logged once every 24 hours on MS Excel sheet after observing under a microscope. Parameters such as Heart rate, Mobility, Physical deformity is also logged

About 500 µl per strength of the sample is loaded onto each segment which has been observed and logged every 24 hours, post media replacement via petri-dish transfer. The observation period is 96 hours to study acute toxicity. Once the 96-hour period is over, the embryos are transferred to a sterile tarson tube filled with E3 media and are stored in a deep freezer until analytical studies can be carried out.

4.9. STATISTICAL ANALYSIS

Statistical tests, including t-tests and ANOVA, were conducted to analyze the data and assess the significance of differences observed between groups. Results were considered significant at $p \leq 0.05$.

CHAPTER 5

RESULT AND DISCUSSION

5.1. EXTRACTED PLANT SAMPLE:

Plant extracts after centrifugation for 15mins.



Figure:5.1.1: plant extracts

In this fig 5.1.1 show the two type of methonolic extract shown one is kappaphycus and another one is *gracilaria* red seaweed which play important role in synthesis of liposome by enacapsulation method. This two type red algae has rich nutrient content which make base for hydrogel formulation with gellan gum powder. After extraction of this two extract lyophilisation is done which convert liquid solution into powder form which very useful to synthesis of liposomes by encapsulation method. Previously Jaiswal et al.2019; reported where the extraction of carrageenan which useful for hydrogel formulation.

5.2. DPPH ASSAY:



Figure: 5.2.1: DPPH assay

In this figure 5.2.1 shows the antioxidant activity of two plant extract one is *kappaphycus* and another is *gracilaria* and the standard is ascorbic acid which mainly used in cosmetic industries and pharmaceutical industries. After 30mins of incubation the absorbance of concentrated samples is measured UV-visible spectroscopy and calculation, tabulation is done as Li et al.,2020; Mussel inspired antioxidant activity.

Table 5.2.1: Standard value of ascorbic acid and scavenging activity

S.no	Concentration (ug/ml)	Absorbance	Blank	%RSA
(1)	250	0.096	0.52	33.53
(2)	300	0.072	0.52	38.15
(3)	350	0.049	0.52	42.19
(4)	400	0.035	0.52	45.26
(5)	450	0.012	0.52	49.69
(6)	500	0.0011	0.52	51.78

Table 5.2.2: Standard value of *Kappaphycus* and scavenging activity

S.no	Concentration (ug/ml)	Absorbance	Blank	%RSA
(1)	250	0.105	0.52	31.8
(2)	300	0.087	0.52	35.26
(3)	350	0.066	0.52	39.3
(4)	400	0.046	0.52	43.15
(5)	450	0.027	0.52	46.8
(6)	500	0.016	0.52	48.92

Table 5.2.3: Standard value of *gracilaria* and scavenging activity

S.no	Concentration (ug/ml)	Absorbance	Blank	%RSA
(1)	250	0.102	0.52	32.57
(2)	300	0.086	0.52	35.46
(3)	350	0.063	0.52	39.88
(4)	400	0.049	0.52	42.57
(5)	450	0.031	0.52	46.02
(6)	500	0.021	0.52	47.96

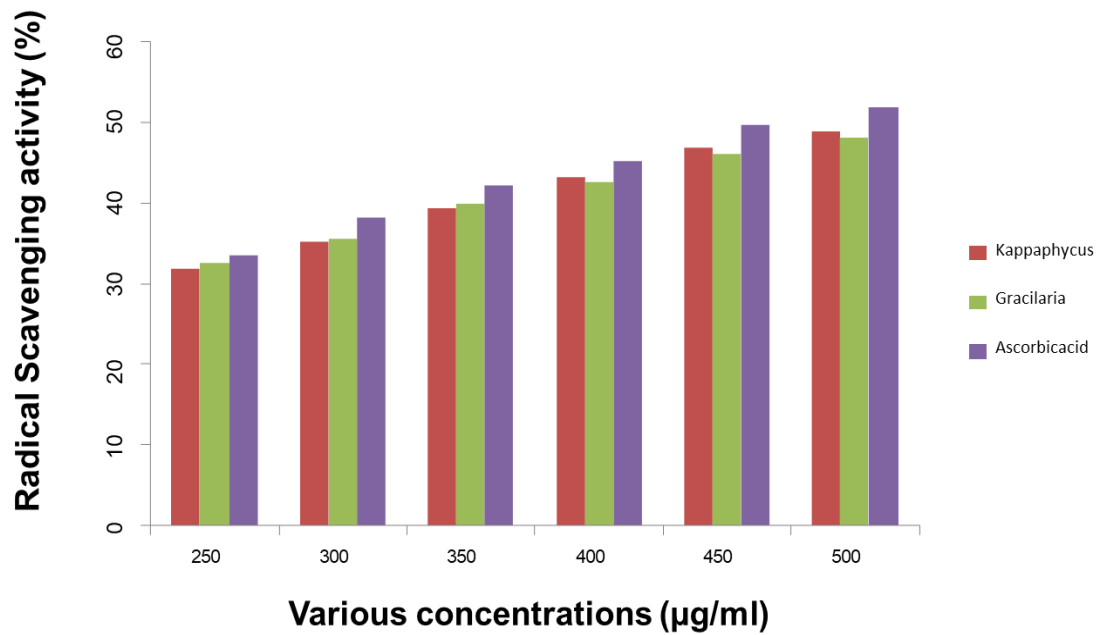


Figure:5.2.2: Comparison Graph of Ascorbic Acid and Red Algae Extracts

In this fig 5.2.2 the graph explain about the standard graph of ascorbic acid concentration is higher than rest of the compound which results the *kappaphycus* and *gracilaria* are more effective than ascorbic acid the red one is *kappaphyucs* and the green one is *gracilaria* and the blue is standard solution ascorbic acid. The standard RSA value of ascorbic acid is start 33.53 which end to 51.78 but *kappahycus* start from 31.8 to 48.92 and then in *gracilaria* start from 32.57 to 47.96. Overall, in graph the concentration of *gracilaria* is lower than comparing other two solution but standard ascorbic has high concentration than other two by Li et al.,2020; Mussel inspired antioxidant activity.

5.3. ENCAPSULATION EFFICINCY:

$$\text{Encapsulation Efficiency}\% = (W_t/W_i) \times 100$$

Where,

W_t is the total amount of integrated material

W_i is the total amount of incorporated material added at the start of the preparation.

Result:

- The encapsulation efficiency of *kappaphycus* extract in liposomes is **$80 \pm 0.2\%$**
- The encapsulation efficiency of *Gracilaria* extract in liposomes is **$80 \pm 0.3\%$**

5.4. CHARATERCORIZATION:

5.4.1. SEM analysis:

SEM analysis is done to lipid mixture to identify the size of liposomes in lipid mixture and their structure.

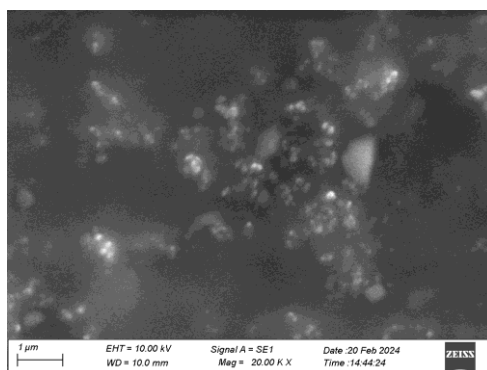


Figure:5.4.1.1:Lipid mixture

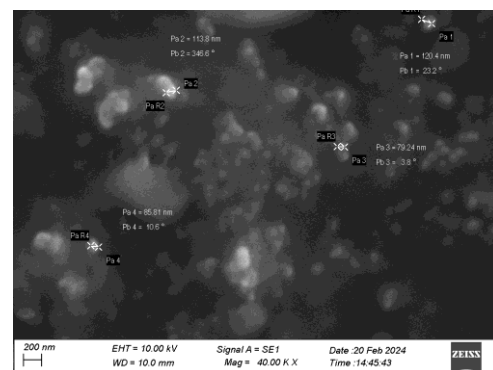


Figure:5.4.1.2: Lipid mixture in size

In this fig 5.4.1.1 and 5.4.1.2 explains the Scanning electron microscopy image which take from 1um and 200nm meter with width of 10.0nm the fig 5.4.1.2 SEM image shows the size of liposomes in lipid mixture which is show in nano meter and the fig 5.4.1.1 shows the presence of liposomes in lipid mixture which helpful for encapsulation. Previously by Fahimirad et al.2021; liposomal SEM analysis for hydrogel formulation.

5.4.2. FTIR analysis:

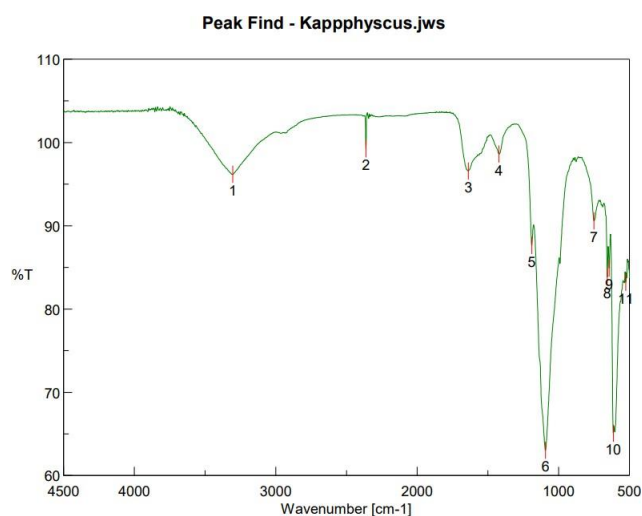


Figure:5.4.2.1 FTIR Analysis Of Kappaphycus

In this figure 5.4.2.1 *Kappaphycus* ftir analysis explains the peak present in powder compound. The image represents only a fraction of the spectrum, especially the wave number range of 4500 to 500 cm⁻¹. For a thorough study, the whole spectrum (usually 4000-400 cm⁻¹) is required. The occurrence of wide peaks across the spectrum shows that the sample is a complex combination of molecules, rather than a single pure material. While exact determination is tricky several common functional groups found in seaweed, such as hydroxyl (-OH), carbonyl (C=O), and amine (N-H), may be present based on typical absorption ranges. The peaks start from 399.193 cm⁻¹ and ends at 4500.08cm⁻¹ as shown as peak in report of Souza et al.2018; in *kappaphycus*.

TABLE 5.4.2.1: *Kappaphycus* position in FTIR graph

NO	POSITIONS	INTENSITY
1	3303.46	96.1658
2	2362.37	99.2398
3	1638.23	96.5737
4	1422.24	98.6134
5	1189.86	87.6357
6	1093.44	63.0373
7	750.174	90.5772
8	656.643	83.8422
9	643.144	84.909
10	614.217	65.0644
11	525.507	83.1919

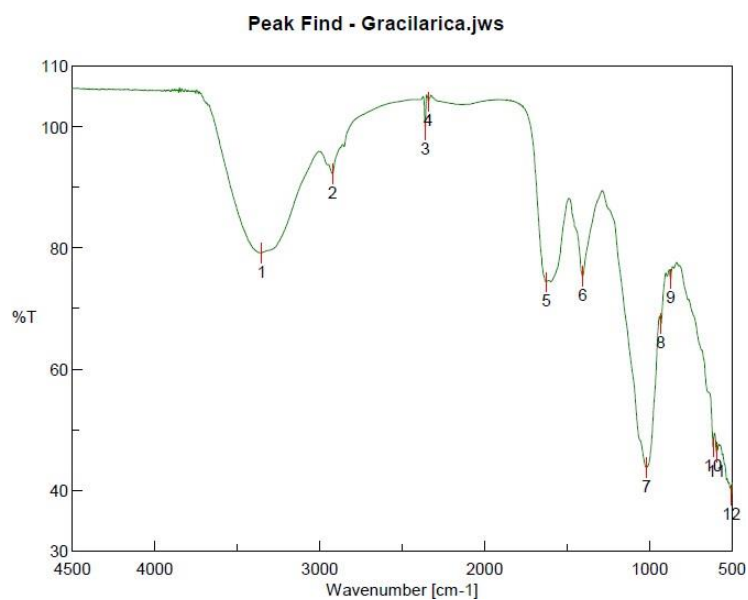


Figure: 5.4.2.2: FTIR analysis of *Gracilaria*

In this figure 5.4.2.2 *Gracilaria* ftir analysis explains the peak and wave number of powdered *gracilaria* compound. The picture only displays a fraction of the spectrum, especially the wave number range of 4500 to 500 cm⁻¹. For a thorough examination, the full spectrum spanning a larger range (usually 4000-400 cm⁻¹) is required for definite identification of functional groupings.

The appearance of wide peaks throughout the spectrum indicates that the *gracilaria* sample might represent a complicated combination of molecules rather than a single pure component. Specific identification is challenging in the absence of a reference spectrum; nevertheless, hydroxyl (-OH), carbonyl (C=O), and amine (N-H), three common functional groups found in seaweed, may be present based on typical absorption ranges as shown as peak in report of Sharaf and El-Naggaer 2018; in *Gracilaria*

The appearance of wide peaks throughout the spectrum indicates that the *gracilaria* sample might represent a complicated combination of molecules rather than a single pure component. Specific identification is challenging in the absence of a reference spectrum; nevertheless, hydroxyl (-OH), carbonyl (C=O), and amine (N-H), three common functional groups found in seaweed, may be present based on typical absorption ranges as shown as peak in report of Sharaf and El-Naggaer 2018; in *Gracilaria*

TABLE 5.4.2.2: *Gracilaria* positions in FTIR graph

NO	POSITIONS	INTENSITY
1	3351.68	79.1461
2	2923.56	92.2173
3	2360.44	99.4645
4	2341.16	104.067
5	1626.66	74.3535
6	1406.82	75.3082
7	1020.16	43.7246
8	931.45	67.452
9	872.631	74.8439
10	616.145	47.1473
11	594.932	46.3307

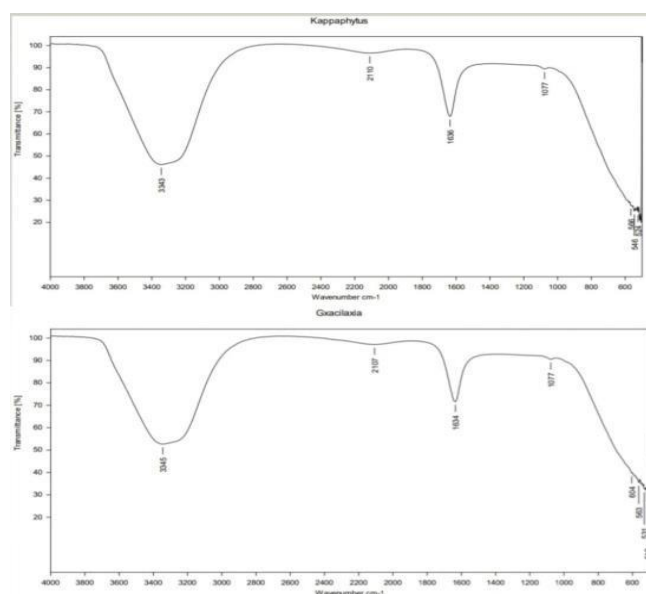


Figure:5.4.2.3: FTIR Analysis of lipid mixture K_p AND G_R

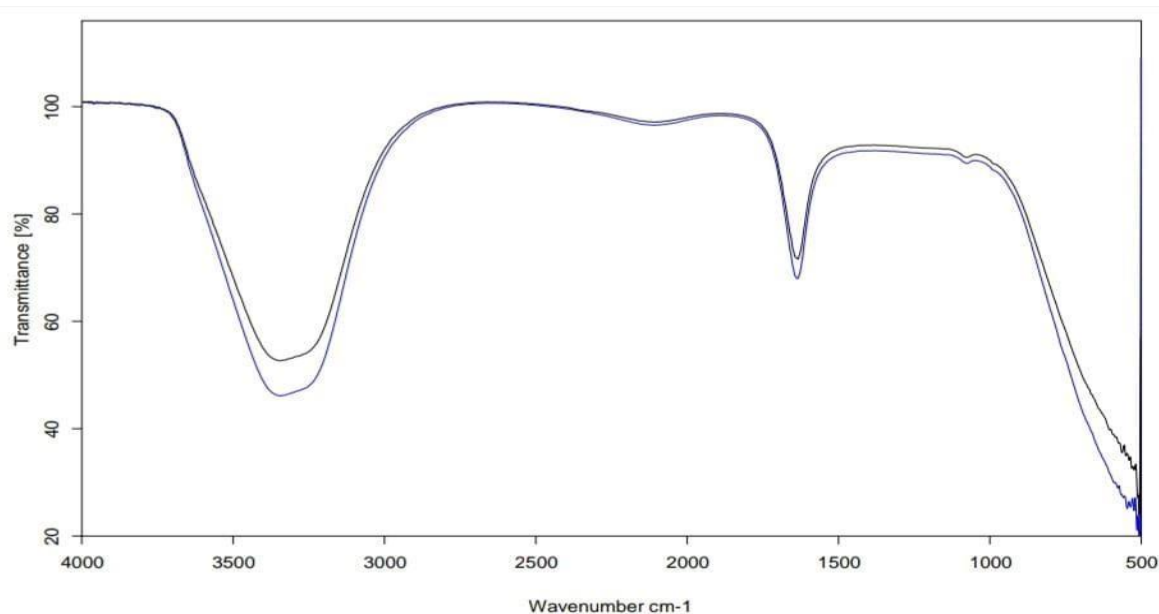


Figure:5.4.2.4: FTIR Analysis Comparing lipid mixture of K_P and G_R

5.5. VISCOSITY RESULTS:

Table 5.5 An overview of the Gellangum gel concentration

S.no	Objectives	Temperature	GLG Concentration (g/ml)	Salt Concentration (g/ml)
(1)	Gellangumgel concentration	25	1-3	Nil
(2)	NaCl	25	2	10
(3)	KCl	25	2	2
(4)	Cacl2	25	2	3

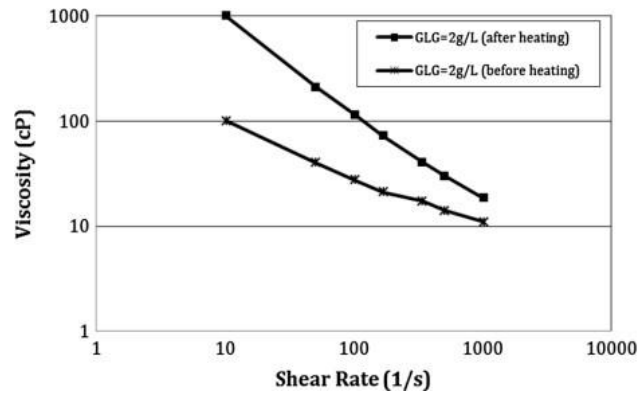


Figure: 5.5.1: Graphical representation of GLG before and after heating

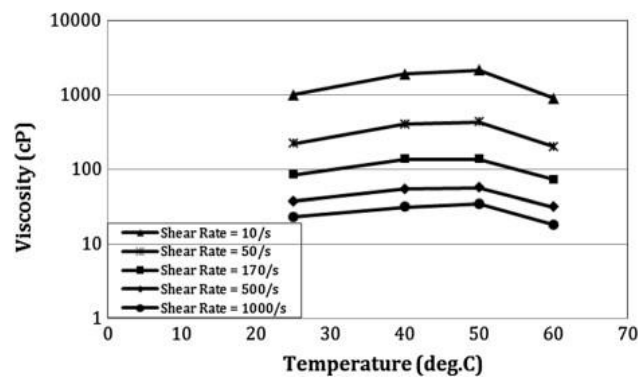


Figure:5.5.2: Graphical representation of GLG solution temperature

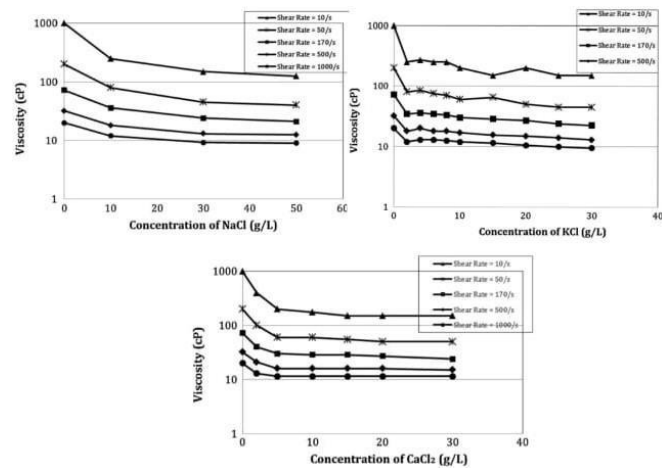
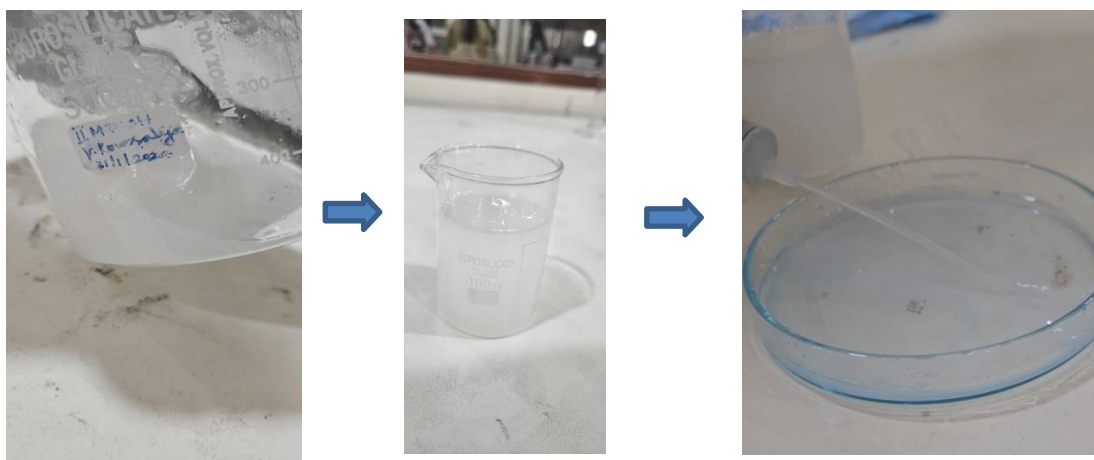


Figure:5.5.3: Graphical representation of concentration of NaCl, KCl, CaCl₂

5.6. GELATION TEMPERATURE:



Gel at 34 °

Gel at 27°C

Fully formulated gel

Figure: 5.6.1: Gelation temperature

In figure 5.6.1 shows the process of gelation time and temperature where after magnetic stirring the GLG solution slightly in liquid at 34⁰c After 20 min the GLG solution become gel form at room temperature 27⁰c. Thus this is the conclusion of gelation temperature as reported done as Xing, Zheng and Duan 2015; Gellan gum gelation temperature.

5.7. MTT assay:

The antioxidant extracts of *Kappaphycus* and *Glacirea* inhibited the viability of hepato carcinoma HepG2 cell in a dose-dependent manner during the 24 h incubation.

Kappaphycus antioxidant extract shows decreased of viability cell at all concentration except at 1.0 mg/mL. The IC₅₀ value for *Kappaphycus* antioxidant extract is 1.8 mg/mL. As for *Gracilaria* antioxidant extract, there were decreased of viability of hepatocarcinoma HepG2 cell at 0.125mg/mL, 1.0mg/mL and 2.0mg/mL only.

The IC₅₀ value for *Gracilaria* antioxidant extract is 0.9 mg/mL. Statistical test showed that there were no significant differences ($p>0.05$) in percentage of viability cell between antioxidant extracts of *Kappaphycus* and *Gracilaria* at all

concentrations except percentage of viability cell at the 2.0 mg/mL treatment level ($p\leq 0.05$). Based on this study, *Gracilaria* could inhibit the proliferation of hepatocarcinoma HepG2 cells and/or cause a cell death of hepatocarcinoma HepG2 cells at lower IC₅₀ concentration than *K. alvarezii* antioxidant extract.

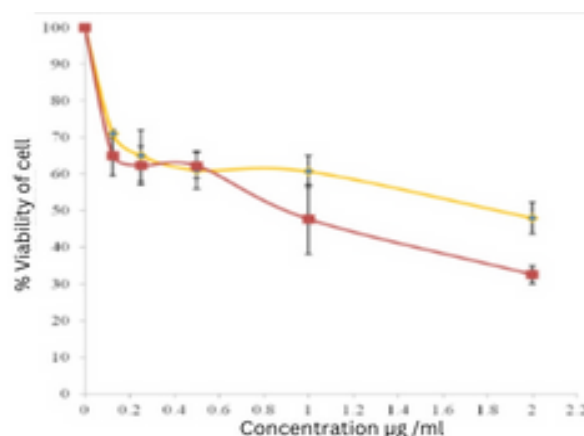


Figure:5.7.1: Graphical repracentation of MTT assay result of Kappaphycus and glaciorea

TABLE 5.7.1: *Kappaphycus* and *glaciorea* encapsulated liposome, negative control and positive control.

SI. NO	Apoptosis(%)±SEM	Necrosis(%)±SEM	Viable cells (%) ±SEM
<i>Kappaphycus</i>	10.2±2.4	2.4 ±1.1	87.4±2.7
<i>Gracilaria</i>	11.3±2.5	2.0±1.3	86.4±1.9
Negative control	9.2±1.7	1.1±0.9	89.7±2.5
Positive control	56.1±1.6	1.5±0.1	42.4±1.7

Relating these results to wound healing, it's important to consider the role of antioxidants and cytotoxicity in the context of tissue repair and regeneration.

Antioxidant Properties: Both *Kappaphycus* and *Gracilaria* extracts are known for their antioxidant properties. Antioxidants play a crucial role in wound healing by neutralizing reactive oxygen species (ROS) and reducing oxidative stress, which can impede the healing process. By inhibiting the viability of cancer cells, these antioxidant extracts may also help mitigate inflammation and promote a favorable environment for wound healing (Angius and Floris, 2015).

Cytotoxicity and Cell Proliferation: The cytotoxic effects observed in cancer cells may also have implications for wound healing. While excessive cytotoxicity can be detrimental to healthy cells, a controlled level of cytotoxicity can be beneficial in eliminating senescent cells, promoting cell turnover, and facilitating tissue remodeling during the wound healing process (Kumar *et al.*, 2018). However, it's essential to balance cytotoxicity with cell proliferation and tissue regeneration to ensure optimal wound healing outcomes (Grela *et al.*, 2018).

Cellular Signaling: The apoptotic and necrotic effects observed in cancer cells indicate potential mechanisms by which these extracts may modulate cellular signaling pathways involved in wound healing (Ishiki *et al.*, 2018). Apoptosis, or programmed cell death, is a natural process that helps remove damaged or abnormal cells, while necrosis may occur in response to severe cellular injury. By influencing these cellular processes, the antioxidant extracts may contribute to tissue repair and regeneration in wounds (Zhao *et al.*, 2021).

5.8. FISH EMBRYO TOXICITY ASSAY:

5.8.1 *Acute Toxicity assay*

5.8.1.1 *0th hour:*

The acute toxicity assay helps determine the toxicity of the compound.

About 30 unhatched embryos were loaded per plate and 500 µl of the sample (***glacire*a encapsulated liposomal hydrogel**) was loaded on to each segment

5.8.1.2 48 hours:

TABLE 5.8.1.2: 48 hours period

<i>Plate</i>	<i>total</i>	<i>hatched</i>	<i>hatching</i>	<i>unhatched</i>	<i>Alive</i>	<i>dead</i>	<i>misreading</i>
<i>Control</i>	30	6	11	7	26	0	4
<i>Full strength</i>	30	8	5	11	24	0	6
<i>Half strength</i>	30	7	6	10	23	0	7

At 48 hrs., the embryos begin to hatch and leave the sack. Few still might not have hatched. There were no deaths reported in any dosage. The accidental added empty or voided embryos are reported as a misreading.

The observed heart rate is at 147-156 BPM

5.8.1.3 72 hours:

TABLE 5.8.1.3 72 hours period

<i>Plate</i>	<i>total</i>	<i>hatched</i>	<i>Hatching</i>	<i>unhatched</i>	<i>Alive</i>	<i>dead</i>
<i>Control</i>	26	23	3	0	26	0
<i>Full strength</i>	24	20	4	0	24	0
<i>Half strength</i>	23	20	3	0	23	0

At 72 hrs., all the embryos have hatched and are mobile, no death was observed and all the embryos were still alive.

The observed heart rate was between 136 – 152 BPM

5.8.1.4 96 hours:

TABLE 5.8.1.4. 96 hours period

Plate	Total	hatched	Hatching	unhatched	Alive	dead
Control	26	26	0	0	26	0
Full strength	24	24	0	0	24	0
Half strength	23	23	0	0	23	0

At 96 hrs., no significant change was observed and no death was reported.

The observed heart is between 148-162 BPM

5.8.2. Morphology imaging:

5.8.2.1 48 hours:



Figure :5.8.1: Image of the embryo at 48 hrs.

At 48 hours we observe that a lot of the embryos begin hatching and a few have completely hatched or are still inside their embryo sac. The complete hatching will take another 24hrs No physical deformity was observed

5.8.2.2 72 hours/96 hours:

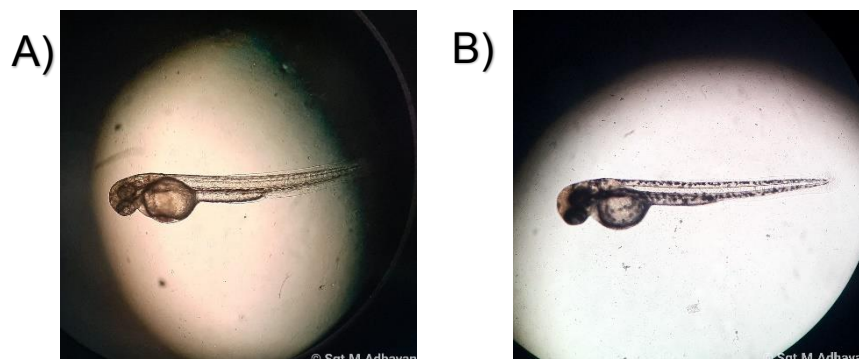


Figure :5.8.2.2: Image of the embryos at A)72 hrs B) 96 hrs.

At 72 hours and 96 hours, all the embryo has hatched and is very mobile, responding to stimulus. No physical deformity was observed and mobility was normal.

The results of the fish embryo toxicity assay indicate that the glaciarea encapsulated liposomal hydrogel, even at full strength, did not exhibit acute toxicity to the fish embryos. Throughout the 96-hour observation period, there were no deaths reported in any dosage group, and the observed heart rates remained within normal ranges. Additionally, morphological imaging revealed no physical deformities in the embryos, and they exhibited normal mobility and responsiveness to stimuli.

Relating these results to wound healing, it's essential to consider the safety profile of any potential therapeutic agent. In this case, the absence of acute toxicity in the fish embryo model suggests that the glaciarea encapsulated liposomal hydrogel is unlikely to cause harm to living tissues. This safety profile is a crucial consideration for wound healing applications, where the goal is to promote tissue repair without inducing adverse effects.

Furthermore, the normal development and mobility observed in the fish embryos treated with the hydrogel indicate that it does not interfere with physiological processes critical for wound healing. This suggests that the hydrogel formulation is biocompatible and unlikely to impede the natural healing cascade.

Overall, the results of the fish embryo toxicity assay support the potential use of the glucirea encapsulated liposomal hydrogel in wound healing applications. Its demonstrated safety and lack of adverse effects in a relevant model system are promising indicators of its suitability for further evaluation and potential clinical use in promoting wound healing and tissue regeneration.

CHAPTER 6

SUMARRY AND CONCLUSION

6.1. SUMMARY

The study investigated the potential of *Kappaphycus* and *Gracilaria* red seaweed extracts, encapsulated in liposomal hydrogel formulations, for wound healing applications. Various assays and analyses were conducted to assess the antioxidant properties, encapsulation efficiency, characterization of the formulations, and acute toxicity using a fish embryo model.

Antioxidant Activity: Both *Kappaphycus* and *Gracilaria* extracts demonstrated significant antioxidant activity, as evidenced by their scavenging abilities against DPPH radicals. The extracts exhibited comparable or even superior antioxidant effects compared to the standard ascorbic acid, highlighting their potential for mitigating oxidative stress during wound healing.

Encapsulation Efficiency: The encapsulation efficiency of both *Kappaphycus* and *Gracilaria* extracts in liposomes was found to be high, indicating successful incorporation into the hydrogel matrix. This efficient encapsulation ensures the sustained release of bioactive compounds, potentially prolonging their therapeutic effects in wound healing.

Characterization: SEM analysis confirmed the presence and size distribution of liposomes in the lipid mixture, providing insights into the structure of the encapsulated formulations. FTIR analysis revealed the complex composition of the seaweed extracts, indicating the presence of various functional groups that contribute to their bioactivity.

Viscosity and Gelation Temperature: The viscosity measurements and gelation temperature determination of the hydrogel formulations provide valuable information for their application and handling. The gelation temperature ensures the stability and consistency of the gel, which is crucial for localized wound treatment.

MTT Assay: The cytotoxicity evaluation using the MTT assay demonstrated the potential of both *Kappaphycus* and *Gracilaria* extracts to inhibit the viability of hepatocarcinoma HepG2 cells in a dose-dependent manner. This cytotoxic effect may

contribute to controlling abnormal cell proliferation and promoting tissue regeneration in wounds.

Fish Embryo Toxicity Assay: The fish embryo toxicity assay revealed that the encapsulated liposomal hydrogel formulations did not exhibit acute toxicity to developing fish embryos, indicating their safety profile for potential biomedical applications.

6.2. CONCLUSION

In conclusion, the study provides comprehensive insights into the antioxidant properties, encapsulation efficiency, characterization, cytotoxicity, and safety profile of *Kappaphycus* and *Gracilaria* red seaweed extracts encapsulated in liposomal hydrogel formulations. These findings support their potential utility as promising therapeutic agents for wound healing applications. Further preclinical and clinical studies are warranted to validate their efficacy, safety, and feasibility for clinical translation in wound management.

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