EVALUATION OF ANTICANCER ACTIVITY OF GELIDIELLA ACEROSA: A MARINE RHODOPHYTA AGAINST THE HUMAN HEAD AND NECK CANCER CELL LINES

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

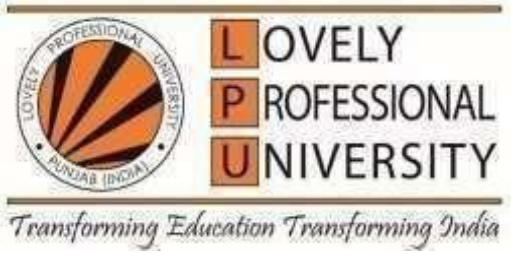
Clinical Biochemistry

By: Shruti Singh

Registration Number: 11919237

Supervised By
Dr.Louis Cojandaraj
Professor
Medical Laboratory and Technology
Lovely Professional University

Co-Supervised by
Dr. Krishnaraj
Industrial scientist
Director of Apex Biotechnology Training and Research Institute, Guindy, Chennai,



LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2026

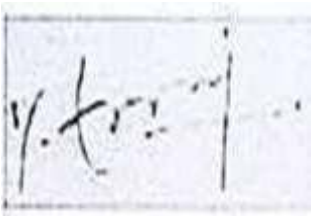
DECLARATION

I, hereby declared that the presented work in the thesis entitled ―Evaluation of anticancer activity of *Gelidiella acerosa*: a marine Rhodophyta against the human head and neck cancer cell lines in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Louis Cojandaraj.A, working as Professor, in the Department Medical Laboratory Sciences, School of Allied Medical Sciences, Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

Shruti Singh
Registration No.: 11919237
Department/school: Medical Laboratory Sciences Lovely Professional University,
Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Evaluation of anticancer activity of *Gelidiella acerosa*: a marine Rhodophyta against the human head and neck cancer cell lines"submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Allied Medical Sciences is a research work carried out by Shruti Singh, Registration No. 11919237, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



Name of Supervisor:
Dr. Louis Cojandaraj
Designation: Professor
School of Medical Laboratory and Technology
Lovely Professional University, Punjab

Name of Co-Supervisor:
Dr. M. Krishnaraj
Designation: Industrial Scientist
Director of Apex Biotechnology Training and Research Institute,
Guindy, Chennai

ACKNOWLEDGMENT

First and foremost, praises and thanks to God, the Almighty, for his blessings throughout my research work and for completing the research successfully.

This thesis is the end of my journey to obtain my Ph.D. degree. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my parents, family, well-wishers, friends, and colleagues.

I acknowledge and deem it a privilege to have Dr. Louis Cojandaraj, Professor, Department of Medical Laboratory Sciences as a guide and express sincere gratitude for his keen interest, patient hearing, untiring effort, skilful instructions, esteemed guidance, and suggestions instilled in me the spirit of confidence to complete the task. I thank him for his unflagging encouragement and for serving as a role model, the enthusiasm and scientific temperament he has for research and her caring ways were contagiously motivating even during tough times in every step of my work.

I thank Dr. Krishnaraj, Director of Apex Biotechnology Training and Research Institute, Guindy, Chennai, for his help and support in completing my research work successfully.

I am always obliged to my father Sh. J.S Randhawa and my mother Smt. Anita Randhawa, who gave me emotional support to complete this work.

I want to thank my better half, Mr. Satvinder Singh who always stood by me in all ups and downs. I wish to place my deep regard and love for his constant care, inspiration, cooperation, and constant support during the research period.

I cannot express my feelings in words towards my little daughter ―ANAYSHA who always acts as my motivating force directly and indirectly.

I appreciate the unending love and affection from my sister and brother. A special thanks to my in-laws Sh. Baljeet Singh and Smt. Sushma Devi.

I sincerely thank Prof. (Dr.) Ashwani Kumar, Head, Department of Biochemistry, Shri Lal Bahadur Shastri Government Medical College, Nerchowk, Mandi, who has been a friend, teacher, and guiding force. His constant motivation and encouragement inspired me to pursue my higher studies and achieve this milestone.

I would like to sincerely thank my friend, Avinash Joseph and Akanksha Pangotra for their generous support and assistance in sample collection and cleaning, which significantly contributed to this research.

Thank you to everyone who has been part of this journey, your support has been valuable. I want to thank all the near and dear ones whose name is not mentioned.

Shruti Singh

DEDICATED TO MY BELOVED PARENTS, DAUGHTER AND TO MY FAMILY

TABLE OF CONTENT

ABBREVIATIONS

	ABBREVIATIONS
1)	Abs - Absorbance
2)	ABTS – 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
3)	Akt – Protein Kinase B
4)	APTT – Activated Partial Thromboplastin Time
5)	ATP – Adenosine Triphosphate
6)	Bcl-2 – B-cell lymphoma 2
7)	BSA – Bovine Serum Albumin
8)	$CaCO_3$ – Calcium Carbonate
9)	CDK – Cyclin-Dependent Kinase
10)	CHO – Chinese Hamster Ovary(cells)
11)	COX-2 – Cyclooxygenase-2
12)	DMSO – Dimethyl Sulfoxide
13)	DNA – Deoxyribonucleic Acid
14)	IP- Inhibitory percentage
15)	EGF – Epidermal Growth Factor
16)	EGFR – Epidermal Growth Factor Receptor
17)	ELISA – Enzyme-Linked Immunosorbent Assay
18)	ERK – Extracellular Signal-Regulated Kinase
19)	FCR – Folin-Ciocalteu Reagent
20)	FTIR – Fourier Transform Infrared Spectroscopy
21)	GAE – Gallic Acid Equivalence
22)	GPx – Glutathione Peroxidase
23)	H_2O_2 – Hydrogen Peroxide
24)	HepG2 – Human Liver Cancer Cells
25)	HNC – Head and Neck Cancer
26)	HNSCC – Head and Neck Squamous Cell Carcinoma
27)	HPV – Human Papillomavirus
28)	HRAS – Harvey Rat Sarcoma Viral Oncogene
29)	HPLC – High-Performance Liquid Chromatography
30)	IC_{50} – Inhibitory Concentration 50%

31)	IMRT – Intensity-Modulated Radiation Therapy
32)	LC-MS – Liquid Chromatography-Mass Spectrometry
33)	MCF-7 – Michigan Cancer Foundation-7 (breast cancer cells)
34)	MAE – Microwave-Assisted Extraction
35)	MAPK – Mitogen-Activated Protein Kinase
36)	MDA – Malondialdehyde
37)	MTT – Methyl thiazolyl Diphenyl-tetrazolium Bromide
38)	NMR – Nuclear Magnetic Resonance
39)	NO – Nitric Oxide
40)	p53 – Tumour Protein 53
41)	PBS – Phosphate-Buffered Saline
42)	PI3K – Phosphatidylinositol 3-Kinase
43)	PD-1 – Programmed Death-1
44)	PD-L1 – Programmed Death Ligand-1
45)	Rb – Retinoblastoma Protein
46)	ROS – Reactive Oxygen Species
47)	SFE – Supercritical Fluid Extraction
48)	SOD – Superoxide Dismutase
49)	TPC – Total Phenol Content
50)	TORS – Transoral Robotic Surgery

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study investigates the bioactive potential of the marine red seaweed *Gelidiella acerosa*, with a specific focus on its anticancer properties. Seaweeds, rich in various bioactive compounds, have gained attention in pharmaceutical and nutraceutical research due to their potent therapeutic properties. The study involved extracting key metabolites from *Gelidiella acerosa* using Soxhlet extraction with solvents such as acetone, chloroform, ethyl acetate, and methanol. The phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins, phenols, terpenoids, and polysaccharides. These extracts were subjected to several in vitro assays to assess their antioxidant and anticancer activities.

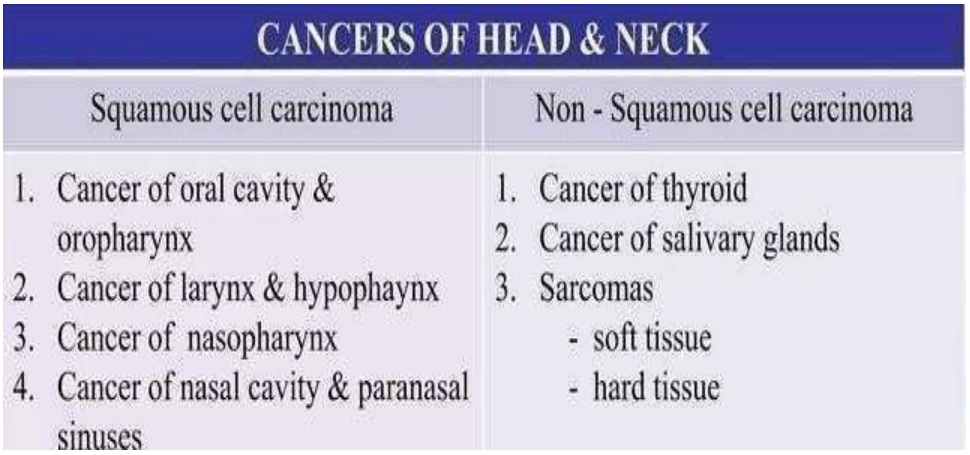
Cytotoxicity assays, including MTT tests, were conducted on human cell lines such as Hep2 (Human larynx carcinoma),KB (Human oral epidermal carcinoma) and HaCaT (Human keratinocyte cell), demonstrating that the acetone extract displayed significant cytotoxic activity. Further, fractionation and purification of the acetone extract led to the identification of key bioactive compounds, which were characterized using analytical techniques like Thin Layer Chromatography (TLC), High- Performance Liquid Chromatography (HPLC), and Fourier Transform Infrared Spectroscopy (FTIR). One of the key compounds, 3-methylidenecyclopentene was found to exhibit strong anticancer activity.

In addition to its anticancer potential, *Gelidiella acerosa* exhibited strong antioxidant properties, as demonstrated through assays such as DPPH radical scavenging and ABTS assays. The combination of antioxidant and anticancer activities makes *Gelidiella acerosa* a promising candidate for pharmaceutical development. This study contributes to the growing body of research on marine biota as a source of novel therapeutic agents, highlighting the potential for *Gelidiella acerosa* in drug discovery, particularly in cancer therapy.

Keywords: Seaweed- *Gelidiella acerosa*, photochemicals, antioxidants, cytotoxicity, 3-methylidenecyclopentene , cancer therapy

CHAPTER 1
INTRODUCTION

Head and neck cancers (HNCs) are a diverse group of malignancies originating in the epithelial tissues of the oral cavity, pharynx, larynx, and associated structures (Figure1). They represent a significant public health concern due to their complex pathophysiology, diverse etiological factors, and evolving treatment strategies (Johnson et al., 2020). Understanding the nuances of HNC is essential for advancing preventive measures, diagnostic techniques, and therapeutic interventions (Argiris, Karamouzis, Raben, & Ferris, 2008). This introduction provides a comprehensive overview of the pathophysiology, risk factors, and treatment strategies for HNC, based on recent research and clinical advancements.



CANCERS OF HEAD & NECK	
Squamous cell carcinoma	Non - Squamous cell carcinoma
1. Cancer of oral cavity & oropharynx 2. Cancer of larynx & hypophaynx 3. Cancer of nasopharynx 4. Cancer of nasal cavity & paranasal sinuses	1. Cancer of thyroid 2. Cancer of salivary glands 3. Sarcomas - soft tissue - hard tissue

Figure 1: Classification of Head and Neck Cancer

1.1 Pathophysiology of Head and Neck Cancer

The development of head and neck cancer involves a complex interplay of genetic, epigenetic, and environmental factors that drive the transformation of normal epithelial cells into malignant tumors (Leemans, Snijders, & Brakenhoff, 2018). Head and neck squamous cell carcinoma (HNSCC) is the most prevalent form of HNC, and its pathogenesis is characterized by a series of molecular events that disrupt cellular homeostasis and promote tumor progression (Seiwert & Cohen, 2005).

1.1.1 Molecular Alterations in HNC

Recent studies have highlighted several key molecular pathways involved in the pathogenesis of HNSCC. Genetic mutations and chromosomal alterations play a crucial role in the

development and progression of these cancers. Notable among these are mutations in the NOTCH1, PIK3CA, and HRAS genes. NOTCH1 Mutations*: Mutations in the NOTCH1 gene are among the most frequently observed genetic alterations in HNSCC. The NOTCH signaling pathway is critical for regulating cell fate, differentiation, and proliferation. Loss-of-function mutations in NOTCH1 disrupt these processes and contribute to tumorigenesis (Leemans, Braakhuis, & Brakenhoff, 2011). PIK3CA and HRAS Mutations**: PIK3CA encodes a component of the PI3K-AKT signaling pathway, and mutations in this gene lead to aberrant activation of the pathway, promoting cellular proliferation and survival. Similarly, HRAS mutations are involved in the activation of the MAPK signaling pathway, which drives tumor growth and progression (Agrawal et al., 2011; van Houten et al., 2006).

1.1.2 HPV and Viral Oncogenesis

Human papillomavirus (HPV) infection is a significant factor in the development of oropharyngeal cancers. HPV-positive tumors are characterized by distinct molecular profiles and clinical features compared to HPV-negative tumors. HPV oncoproteins E6 and E7 promote carcinogenesis by degrading tumor suppressor proteins such as p53 and Rb, leading to uncontrolled cellular proliferation (Gillison, Chaturvedi, Anderson, & Fakhry, 2015).

1.1.3 Genetic and Epigenetic Modifications

In addition to genetic mutations, epigenetic alterations contribute to HNC pathogenesis. DNA methylation, histone modification, and non-coding RNAs affect gene expression and cellular transformation. For example, promoter hypermethylation of tumor suppressor genes can silence their expression, facilitating tumor development (Stransky et al., 2011). These epigenetic changes provide insights into the mechanisms of carcinogenesis and potential therapeutic targets.

1.2 Risk Factors and Epidemiology

Identifying and understanding the risk factors for head and neck cancer are crucial for developing effective prevention and early detection strategies. The risk factors for HNC include both environmental and lifestyle factors, as well as viral infections.

1.2.1 Tobacco and Alcohol Use

Tobacco smoking and alcohol consumption are major risk factors for HNC. Tobacco carcinogens, such as polycyclic aromatic hydrocarbons and nitrosamines, cause direct DNA

damage and promote tumor formation. Alcohol enhances the carcinogenic effects of tobacco and introduces acetaldehyde, which can induce DNA adducts and mutations (Sturgis & Cinciripini, 2007). The synergistic effect of tobacco and alcohol significantly increases the risk of developing HNC.

1.2.2 Human Papillomavirus (HPV) Infection

HPV infection, particularly with high-risk types such as HPV-16, is a major risk factor for oropharyngeal cancers. HPV-positive HNCs are associated with a younger age of onset, distinct clinical features, and a better prognosis compared to HPV-negative cancers (Ang et al., 2010). The increasing incidence of HPV-associated HNC highlights the need for effective vaccination and screening programs to mitigate its impact on public health (Gillison et al., 2015).

1.2.3 Other Risk Factors

Additional risk factors for HNC include exposure to occupational carcinogens, such as asbestos and formaldehyde, and the presence of predisposing conditions like oral leukoplakia and dysplasia. Genetic predispositions and inherited syndromes, such as Fanconi anemia and LiFraumeni syndrome, also increase susceptibility to HNC (Argiris, Karamouzis, Raben, & Ferris, 2008; Economopoulou et al., 2016). Understanding these risk factors is crucial for identifying individuals at high risk and implementing targeted prevention strategies.

1.3 Clinical Treatment Strategies

The management of head and neck cancer requires a multidisciplinary approach that includes surgery, radiotherapy, chemotherapy, and emerging targeted and immunotherapies. Each treatment modality has specific indications, benefits, and limitations, and treatment plans are often personalized based on individual patient and tumor characteristics.

1.3.1 Surgery

Surgical resection is a cornerstone of treatment for localized HNC. The objective is to achieve complete tumor removal while preserving functional and aesthetic aspects. Advances in surgical techniques, such as transoral robotic surgery (TORS) and minimally invasive approaches, have improved surgical precision and outcomes (Bonner et al., 2010). However, surgery alone is often insufficient for advanced or high-risk tumors, necessitating the integration of other treatment modalities.

1.3.2 Radiotherapy

Radiotherapy is essential for treating both localized and advanced HNC. Advances in radiotherapy techniques, including intensity-modulated radiation therapy (IMRT) and proton therapy, have enhanced the precision and efficacy of treatment while minimizing damage to surrounding healthy tissues (Nutting et al., 2011). IMRT allows for targeted delivery of radiation, reducing side effects and improving treatment outcomes. Radiotherapy is also used as an adjunct to surgical resection or as a standalone treatment when surgery is not feasible.

1.3.3 Chemotherapy

Chemotherapy is commonly employed in the treatment of advanced HNC, either as neoadjuvant, adjuvant, or palliative therapy. Agents such as cisplatin, fluorouracil, and taxanes have shown efficacy in managing HNC, often in combination with radiotherapy (Vermorken et al., 2007). Recent research focuses on optimizing chemotherapy regimens to enhance efficacy and reduce toxicity, as well as exploring novel agents and combinations for better treatment outcomes.

1.3.4 Targeted Therapies

Targeted therapies offer a promising approach to treating HNC by specifically targeting molecular alterations associated with tumor growth. Cetuximab, an anti-EGFR monoclonal antibody, has shown efficacy in combination with radiotherapy and chemotherapy (Ferris et al., 2017). Other targeted agents are being investigated, including inhibitors of the PI3K-AKT and MAPK pathways, which aim to disrupt specific signaling pathways critical for tumor survival and growth (Burtness et al., 2017).

1.3.5 Immunotherapy

Immunotherapy represents a significant advancement in the treatment of HNC, particularly for recurrent and metastatic disease. Immune checkpoint inhibitors, such as pembrolizumab and nivolumab, have demonstrated promising results in clinical trials, providing durable responses and improved survival rates for patients with advanced HNC (Seiwert et al., 2016). These agents work by inhibiting immune checkpoints such as PD-1 and PD-L1, enhancing the immune system's ability to recognize and attack tumor cells. Ongoing research aims to identify biomarkers that predict response to immunotherapy and explore combination strategies with other treatments (Burtness et al., 2019).

1.4 Need for Alternative Therapies

1.4.1 Limitations of Current Treatments

The conventional approaches to treating HNC, while effective, are associated with a range of adverse effects. Surgical treatments, although potentially curative, can lead to significant functional and aesthetic issues (Bonner et al., 2010). Radiotherapy, while crucial for managing localized disease, often causes damage to surrounding healthy tissues, leading to complications such as mucositis and xerostomia (Nutting et al., 2011). Chemotherapy, while targeting rapidly dividing cancer cells, can result in systemic toxicity affecting various organs (Vermorken et al., 2007). These limitations highlight the need for alternative or adjunct therapies that can mitigate side effects and enhance overall treatment efficacy.

1.4.2 Role of Complementary Therapies

Complementary therapies, including dietary supplements and natural products, are increasingly recognized for their potential to support cancer treatment and improve quality of life. Among these, marine biota, particularly seaweeds, have shown promise due to their diverse bioactive compounds and potential anti-cancer properties (Cheng et al., 2016). Seaweeds are rich in polysaccharides, polyphenols, and other phytochemicals that may offer therapeutic benefits, including anti-inflammatory, antioxidant, and cytotoxic effects against cancer cells (Jiang et al., 2021).

1.5 Seaweed

Seaweeds, or macroalgae, are marine plants that thrive in a diverse range of coastal environments, playing essential roles in aquatic ecosystems. Classified within three primary groups—red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta)—seaweeds exhibit a remarkable variety of forms, sizes, and colors, contributing to the richness of marine biodiversity (Critchley & Ohno, 1998). These organisms are found in both intertidal and subtidal zones, often attached to rocky substrates or floating freely in the water column.

Seaweeds are vital for maintaining the health of marine ecosystems. They serve as primary producers, converting sunlight into energy through photosynthesis and providing food and habitat for a wide range of marine organisms, including fish, invertebrates, and other aquatic life (Huisman et al., 2021). Furthermore, they contribute to coastal protection by reducing wave energy and preventing erosion, thereby maintaining the integrity of marine environments (Barbier et al., 2011).

Beyond their ecological importance, seaweeds have garnered significant attention in recent years for their potential economic and nutritional benefits. They are rich in essential nutrients, including vitamins, minerals, and dietary fiber, making them valuable in food production and health supplements. In addition, seaweeds are increasingly recognized for their bioactive compounds, which exhibit various pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities (Wang et al., 2018). This biotechnological potential has led to the exploration of seaweeds as sources of novel drugs and functional foods.

Despite their many benefits, the sustainable harvesting and cultivation of seaweeds are critical for ensuring their availability for future generations. With the rising demand for seaweed-based products, there is a pressing need for research focused on the cultivation, conservation, and sustainable management of seaweed resources (Chopin et al., 2020). Understanding the ecological, nutritional, and economic aspects of seaweeds will contribute to their sustainable utilization and the advancement of marine biotechnology.

1.6 Seaweed as a Potential Therapeutic Agent

1.6.1 Bioactive Compounds in Seaweed

Seaweeds, encompassing a wide range of marine algae, have garnered interest for their therapeutic potential due to their rich composition of bioactive compounds. Among the most studied seaweeds are *Laminaria*, *Sargassum*, and *Porphyra*. These seaweeds contain diverse polysaccharides, polyphenols, and other bioactive substances that exhibit potential anti-cancer properties.

Fucoidans are sulfated polysaccharides predominantly found in brown seaweeds, such as *Laminaria* and *Sargassum*. These compounds have shown promising anti- tumor activity. Fucoidans induce apoptosis in cancer cells by triggering various molecular pathways. Studies have reported that fucoidans can activate caspases, a group of enzymes crucial for apoptosis, and inhibit the proliferation of ―cancer cells‖ by disrupting the cell cycle (Kang et al., 2018). Additionally, fucoidans have demonstrated potential in inhibiting angiogenesis, a crucial process in tumor development and spread is metastasis (Kang et al., 2018).

Carrageenans, derived from red seaweeds like *Porphyra*, are another significant group of seaweed polysaccharides with therapeutic potential. Carrageenans have been recognized for their ability to modulate immune responses and exhibit anti- cancer properties. Research

indicates that carrageenans can enhance the immune response by activating macrophages and promoting the release of cytokines that target cancer cells (Roh et al., 2014). Moreover, carrageenans have been shown to limit the proliferation of cancer cells and induce apoptosis, making them potential candidates for ―cancer‖ therapy (Roh et al., 2014).

Phlorotannins, another class of polyphenolic compounds found in brown seaweeds, have also attracted attention due to their antioxidant and anti-cancer properties. These compounds are believed to scavenge free radicals, reduce oxidative stress, and inhibit cancer cell growth (Cheng et al., 2016). The diverse array of bioactive compounds in seaweeds presents a promising opportunity for developing novel cancer treatments.

1.7 Mechanisms of Action

The anti-cancer effects of seaweed extracts can be attributed to several underlying mechanisms. One prominent mechanism involves the modulation of key cellular signaling pathways that are frequently dysregulated in cancer, including the PI3K-AKT and MAPK pathways.

The PI3K-AKT pathway is critical in regulating cell growth, survival, and metabolism. Dysregulation of this pathway is commonly observed in HNSCC, leading to increased cell proliferation and resistance to apoptosis. Seaweed-derived polysaccharides, such as fucoidans, have been shown to inhibit the activation of the PI3K-AKT pathway, thereby inducing apoptosis and reducing tumor growth (Cheng et al., 2016). This effect suggests that seaweed compounds can counteract one of the key mechanisms driving cancer progression.

The MAPK pathway, which includes proteins such as ERK, JNK, and p38 MAPK, plays a crucial role in regulating cellular responses to stress, proliferation, and differentiation. Seaweed extracts have been reported to modulate the MAPK pathway, affecting cell cycle progression and apoptosis in cancer cells (Jiang et al., 2021). By influencing these pathways, seaweed compounds can potentially reduce cancer cell proliferation and enhance the efficacy of conventional treatments.

Additionally, seaweed extracts have shown potential in enhancing the efficacy of conventional therapies and mitigating side effects. Some studies suggest that seaweed compounds can enhance the sensitivity of cancer cells to chemotherapeutic agents, thereby improving treatment outcomes (Jiang et al., 2021). Moreover, the anti-inflammatory and antioxidant properties of seaweed extracts can help alleviate treatment-related side effects, such as mucositis and oxidative stress, which are common in cancer therapy.

1.8 Clinical and Preclinical Evidence

Preclinical studies have provided valuable insights into the potential of seaweed extracts in cancer treatment. In vitro experiments have demonstrated that seaweed polysaccharides can inhibit the proliferation of HNSCC cells and induce apoptosis. Studies have shown that fucoidans from brown seaweeds can reduce the viability of HNSCC cells and enhance their sensitivity to chemotherapeutic agents (Kang et al., 2018). These findings highlight the potential of seaweed-derived compounds to serve as adjuncts to traditional cancer therapies.

In vivo studies using animal models have further supported the anti-cancer potential of seaweed extracts. Research has indicated that seaweed polysaccharides can inhibit tumor growth and metastasis in animal models of cancer (Cheng et al., 2016). However, while these preclinical results are promising, clinical trials are necessary to confirm the efficacy and safety of seaweed based therapies in human patients.

Clinical studies exploring the use of seaweed extracts in cancer treatment are still limited. Nonetheless, existing studies have provided some evidence of the potential benefits of incorporating seaweed-based interventions into cancer care. A few case reports and pilot studies have suggested that seaweed supplements may improve quality of life and reduce treatment-related side effects in cancer patients (Jiang et al., 2021). Further research is needed to establish the clinical efficacy of seaweed-based therapies and determine their optimal use in combination with existing cancer treatments.

1.9 Integrating Seaweed into HNC Management

1.9.1 Potential Benefits

Integrating seaweed-based therapies into the management of head and neck cancer (HNC) presents several compelling advantages, offering both direct and supportive benefits to patients undergoing treatment. Seaweeds, rich in bioactive compounds, can play a multifaceted role in improving patient outcomes through their therapeutic properties and nutritional benefits.

1.9.2 Reduction of Treatment-Related Side Effects

One of the significant challenges in managing HNC is the management of treatment-related side effects, particularly those arising from radiotherapy and chemotherapy. Mucositis, a common complication of radiotherapy, is characterized by inflammation and ulceration of the mucous membranes lining the oral cavity and throat. This condition can severely affect a

patient's quality of life, causing pain, difficulty swallowing, and increased risk of infection (Roh et al., 2014). Seaweed extracts, particularly those from brown and red seaweeds, have demonstrated potential in mitigating such side effects. The anti-inflammatory properties of seaweed polysaccharides, such as fucoidans and carrageenans, are instrumental in reducing inflammation and promoting mucosal healing. For example, fucoidans derived from brown seaweeds have been shown to significantly reduce inflammation and oxidative stress, thus alleviating symptoms of mucositis (Kang et al., 2018). Similarly, carrageenans have been reported to possess soothing effects on mucosal tissues, helping to prevent and manage mucositis (Roh et al., 2014).

The antioxidant properties of seaweed compounds further contribute to their efficacy in managing side effects. Seaweeds are rich in antioxidants, such as polyphenols and phlorotannins, which neutralize free radicals and reduce oxidative damage to healthy tissues (Cheng et al., 2016). By minimizing oxidative stress, seaweed extracts can help mitigate the adverse effects of radiation and chemotherapy, improving overall patient comfort and potentially accelerating recovery.

1.9.3 Enhancement of Conventional Treatments

Seaweeds may also be utilized in terms of their therapies to enhance the effectiveness of the traditional treatments against cancer. Indeed, one of the most significant mechanisms by which seaweeds exert their therapeutic effects is through the modulation of cellular signaling pathways which are often dysregulated in cancer. Polysaccharides extracted from the seaweed may affect PI3K-AKT and MAPK pathways that regulate cell proliferation, survival, and drug resistance (Jiang et al., 2021).

The PI3K-AKT pathway is frequently aberrant in ―HNSCC‖, leading to increased cancer cell proliferation and resistance to apoptosis. Seaweed compounds, such as fucoidans, have been shown to inhibit this pathway, thereby promoting cancer cell death and enhancing the effectiveness of chemotherapeutic agents (Cheng et al., 2016). This effect is particularly valuable in overcoming drug resistance, a common challenge in cancer therapy.

Similarly, seaweed extracts can modulate the MAPK pathway, which includes proteins such as ERK, JNK, and p38 MAPK. These proteins play a role in cellular responses to stress and are often dysregulated in cancer cells. Seaweed-derived compounds have been reported to affect the MAPK pathway, leading to reduced cell proliferation and enhanced apoptosis (Jiang et al., 2021). Bytargeting these key pathways, seaweed extracts can potentially improve the efficacy

of conventional therapies, such as chemotherapy and radiotherapy.

In addition, compounds in seaweed have been shown to be capable of reducing the side-effects that entail conventional treatments. The antioxidant and anti-inflammatory properties of extracts of sea-weed may eradicate or reduce symptoms such as nausea, fatigue, and mucositis that cancer patients develop during their treatments (Roh et al., 2014). Seaweed-based therapies can improve compliance and overall treatment outcomes by reducing these side effects.

1.9.4 Nutritional Support and Overall Well-Being

In addition to their therapeutic effects, seaweeds offer substantial nutritional benefits that can support overall health and well-being during cancer treatment. Seaweeds are rich in essential vitamins, minerals, and dietary fiber, which play a crucial role in maintaining immune function and supporting general health.

Seaweeds contain a variety of vitamins, including vitamin A, vitamin C, and vitamin K, which are essential for immune system function and overall health. Vitamin A, is crucial for maintaining mucosal integrity and supporting immune responses (Cheng et al., 2016). Vitamin C, an antioxidant, helps to protect cells from oxidative damage and supports wound healing. Vitamin K plays a role in blood clotting and bone health, which can be particularly beneficial for cancer patients experiencing treatment-related bleeding or bone issues.

Minerals such as iodine, calcium, and magnesium are also present in seaweeds and contribute to various physiological functions. Iodine is essential for thyroid function, which can influence overall metabolic health. Calcium and magnesium support bone health and muscle function, which is important for patients who may experience bone loss or muscle weakness due to cancer treatment (Cheng et al., 2016).

Dietary fiber in seaweeds can aid in maintaining digestive health, which is crucial for cancer patients who may experience gastrointestinal issues as a result of treatment. Fiber supports regular bowel movements and can help prevent constipation, a common side effect of chemotherapy (Jiang et al., 2021).

Overall, the integration of seaweed-based therapies into HNC management offers a holistic approach to improving patient outcomes. By reducing treatment-related side effects, enhancing the efficacy of conventional treatments, and providing nutritional support, seaweeds have the potential to contribute significantly to the management of HNC and improve the quality of life for patients undergoing cancer treatment.

1.10 *Gelidiella acerosa*

1.10.1 Introduction

This species of red seaweed belonging to the family Gelidiellaceae, is widely distributed along the coasts of the Indian Ocean and the Pacific Ocean. This seaweed has garnered attention due to its diverse range of bioactive compounds, which are of significant interest for various industrial and pharmaceutical applications. Its potential therapeutic benefits have been explored extensively, highlighting its role in marine biotechnology and natural product research.

1.10.2 .Importance of Gelidiella acerosa

Ecological Role: *Gelidiella acerosa* is a red alga found in Tamil Nadu's coastal waters, particularly in the Gulf of Mannar and Palk Bay. It forms part of the macroalgal community that supports marine biodiversity by providing habitat and food resources for various marine organisms (Krishnan & Narayanakumar, 2013). The species contributes to the structure and function of coastal ecosystems, influencing the dynamics of marine communities.

Economic Importance: *Gelidiella acerosa* is economically valuable due to its use in agar production. Agar, a gel-like substance derived from this seaweed, is used in food products, pharmaceuticals, and microbiological research (Rao & Mantri, 2010). The agar from *Gelidiella acerosa* is noted for its high gel strength and clarity, making it a preferred choice for various applications. This economic value highlights the importance of sustainable management practices to ensure the continued availability of this resource .

Conservation Efforts: Conservation initiatives in Tamil Nadu include the establishment of marine protected areas, such as the Gulf of Mannar Biosphere Reserve, which aims to protect coral reefs, seagrass meadows, and mangrove forests (Vel et al., 2014). Community- based management strategies, including sustainable seaweed harvesting and mangrove restoration, are also important for preserving marine biodiversity (Krishnan & Narayanakumar, 2013).

1.10.3 Industrial and Pharmaceutical Applications

Gelidiella acerosa has several applications in industries ranging from food to pharmaceuticals due to its unique chemical composition and biological activities.

Food Industry: Agar extracted from *Gelidiella acerosa* is widely used as a gelling agent in food products. Its ability to form gels at low concentrations makes it an essential ingredient in various culinary applications (Dawes & Mathieson, 1993).

Pharmaceutical Industry: The bioactive compounds of *Gelidiella acerosa* have potential applications in drug development. Its antioxidant and anti-cancer properties make it a candidate for developing novel therapeutic agents. Additionally, its antiinflammatory properties could be harnessed for designing treatments for inflammatory conditions (Senthilkumar et al., 2019).

Cosmetics: The moisturizing and antioxidant properties of *Gelidiella acerosa* make it a valuable ingredient in cosmetics and skincare products. Its extracts are used in formulations aimed at improving skin hydration and reducing oxidative stress (López-Hortas et al., 2021).

CHAPTER 2
REVIEW OF LITERATURE

2.1 Marine Biota in India

2.1.1 Introduction

The marine biota refers to the wide array of living organisms, including plants, animals, and microorganisms, that inhabit marine ecosystems. Globally, marine biota play a critical role in maintaining ecological balance, supporting fisheries, and contributing to global biodiversity (Gattuso et al., 2015). India, with its extensive coastline stretching over 7,500 kilometres, is endowed with diverse marine ecosystems that include coral reefs, mangroves, seagrass beds, and estuaries, all of which support a myriad of species (Rao & Mantri, 2010).

India's Exclusive Economic Zone (EEZ), which covers over 2 million square kilometres, is home to more than 2,000 species of fish, 1,200 species of mollusks, and a wide variety of other marine organisms (Singh et al., 2012). However, in recent years, there has been increasing concern about the impact of human activities on these ecosystems, including overfishing, coastal pollution, and climate change (Pandolfi et al., 2011).

2.1.2 Marine Biodiversity in Indian Waters

Marine biodiversity in Indian waters is both diverse and unique, with some of the richest ecosystems found in the coral reefs of the Andaman and Nicobar Islands, the Gulf of Mannar, and Lakshadweep. These ecosystems are home to many endemic species and serve as critical habitats for endangered species such as dugongs, sea turtles, and various species of cetaceans (Wilkinson, 2013).

The mangrove forests along the eastern coast, particularly in the Sundarbans, are among the largest in the world and serve as important breeding grounds for many marine species, while also providing ecosystem services such as coastal protection and carbon sequestration (Kathiresan & Bingham, 2011). Mangrove ecosystems are also crucial for their role in supporting fisheries, providing habitat and nursery grounds for a wide range of commercially important species.

Coral reefs in India have been under increasing stress from climate change, particularly due to coral bleaching caused by rising sea temperatures. Studies indicate that repeated bleaching

events have led to a decline in coral cover, affecting the overall biodiversity of these reefs (Pratchett et al., 2013). This has direct consequences for the fish populations and other marine organisms that rely on coral reefs for shelter and food.

2.1.3 Seaweed Biodiversity in India

Seaweed, often referred to as macroalgae, is an essential component of marine biodiversity. India is home to a vast variety of seaweed species, with over 841 species identified across its coastal waters (Rao & Mantri, 2010). These species are found predominantly along the western coast (Gujarat), southern coast (Tamil Nadu), and the Lakshadweep and Andaman & Nicobar Islands.

The diversity of seaweed species includes ―*red algae*‖ (*Rhodophyceae*), ―*green algae" (Chlorophyceae*), and ―*brown algae"* (Phaeophyceae), each playing a crucial role in marine ecosystems (Mishra et al., 2012). Brown algae such as *Sargassum* and *Turbinaria* are known to form dense beds that provide habitat for fish and invertebrates (Yuan et al., 2013). Additionally, seaweed plays a vital role in primary productivity and nutrient cycling in coastal waters.

The commercial importance of seaweed has increased significantly in India, with certain species such as *Gracilaria* and *Kappaphycus* being cultivated for use in the food, pharmaceutical, and biofuel industries (Thirumaran et al., 2014). Moreover, seaweed farming is being promoted as a sustainable livelihood option for coastal communities, particularly in Tamil Nadu and Gujarat, where seaweed cultivation has been successfully integrated into local economies (Krishnan & Narayanakumar, 2013).

Seaweed ecosystems are not only important for biodiversity but also act as natural biofilters, removing excess nutrients such as nitrogen and phosphorous from the water. This helps to mitigate the effects of eutrophication, which is a growing problem in many coastal areas due to agricultural runoff and untreated sewage (Kim et al., 2015). By absorbing these excess nutrients, seaweed helps maintain water quality and promote the health of other marine organisms.

2.1.4 Marine Biodiversity Overview

Tamil Nadu, a state on the southeastern coast of India, is known for its diverse marine ecosystems, including coral reefs, seagrass meadows, mangroves, estuaries, and coastal waters. These ecosystems are crucial for maintaining ecological balance and supporting a rich array of marine species. This literature review focuses on the marine biodiversity of Tamil Nadu, with

a particular emphasis on *Gelidiella acerosa*, a significant seaweed species in the region.

2.2 Gelidiella acerosa :Algae of Interest

2.2.1 Research and Conservation: Recent research on *Gelidiella acerosa* has focused on its resilience to environmental changes and its role in marine ecosystems. ((Kumar et al. 2021) explored the species' ability to tolerate varying environmental conditions.

2.2.2 Phytochemical Composition

Gelidiella acerosa is known for its rich content of bioactive compounds that contribute to its potential therapeutic properties. The primary components include polysaccharides, polyphenols, and essential fatty acids.

Gelidiella acerosa belongs to the kingdom *Plantae* and is a species of red algae classified under the phylum *Rhodophyta*. It is a member of the class *Florideophyceae* and the order *Gelidiales*, within the family *Gelidiellaceae*. The genus *Gelidiella* includes this species, which is commonly found in tropical and subtropical marine environments. *G. acerosa* is of particular interest due to its economic and ecological significance, especially in relation to its potential bioactive properties (Guiry et.al 2010).

Polysaccharides: Seaweeds are rich in carbohydrates, with their cell membranes containing distinct polysaccharides specific to different algae types. For instance, brown algae contain alginate and fucoidan, green algae have Ulvan, and red algae possess agar and carrageenan. These polysaccharides have gained attention due to their unique physicochemical properties (Shanura et.al 2018). As biopolymers derived from natural sources, polysaccharides serve as eco-friendly alternatives to conventional plastics and synthetic polymers. They also function as structural components and energy reserves in various organisms, including plants and marine species. In seaweed, polysaccharides are the predominant macromolecules, making up more than 80% of their total weight. Based on their role, they are categorized as either cell-membrane polysaccharides or storage polysaccharides. Most seaweed polysaccharides belong to the cell-membrane category, except for those stored within cell plastids. Additionally, they can be classified as food-grade or non-food-grade, depending on their application (Hii et.al 2016).

Polyphenols: Polyphenols are another crucial group of bioactive compounds found in Gelidiella acerosa. These compounds are known for their antioxidant properties, which can help in neutralizing free radicals and reducing oxidative stress. Studies have identified several

polyphenolic compounds in Gelidiella acerosa, including flavonoids and tannins, which contribute to its potential health benefits (Kumar et al., 2019).

Essential Fatty Acids: *Gelidiella acerosa* contains essential fatty acids, such as omega- 3 and omega-6 fatty acids, which are vital for maintaining cardiovascular health and reducing inflammation. These fatty acids presence adds to the seaweed's nutritional value (Giménez et al., 2019).

2.3 Types of Phytochemicals

2.3.1 Polysaccharides

Gelidiella acerosa is renowned for its high content of polysaccharides, including agar and carrageenan. These polysaccharides have significant industrial applications due to their gelling, thickening, and stabilizing properties.

Agar: Agar is a key polysaccharide extracted from *Gelidiella acerosa*. It is used extensively as a gelling agent in food products, such as desserts and jellies, and as a medium for microbial culture. Agar's ability to form gels at low concentrations and its stability under a range of conditions make it a valuable ingredient (Yuan et al., 2016).

Carrageenan: Carrageenan, another polysaccharide obtained from *Gelidiella acerosa*, is used for its thickening and stabilizing properties in the food industry. It is widely used in dairy products and meat processing (Khan et al., 2018).

2.3.2 Phenolic Compounds

Phenolic compounds in *Gelidiella acerosa* include flavonoids and phenolic acids, which are renowned for their antioxidants and anti-inflammatory characterstics.

2.3.3 Flavonoids: Flavonoids such as quercetin and catechins are present in *Gelidiella acerosa*. These compounds exhibit strong antioxidant activity, which contributes to algae's potential health benefits. (Elshikh et al. 2019) reported that ethanol extracts of *Gelidiella acerosa* are rich in flavonoids, which help in neutralizing free radicals and reducing oxidative stress.

2.3.4 Phenolic Acids: Phenolic acids like caffeic acid are also found in *Gelidiella acerosa*. These compounds have antioxidant properties and may contribute to anti-inflammatory and anticancer activities (Garg et al., 2016).

2.3.5 Terpenoids: Terpenoids are a diverse group of phytochemicals found in *Gelidiella acerosa*. They have various biological activities, including antimicrobial and ―anti-inflammatory‖ effects.

Terpenoids are often extracted using organic solvents or supercritical fluid extraction methods. (Yang et al. 2017) reported that terpenoids from marine algae, including *Gelidiella acerosa*, have potential applications in medicine due to their bioactivity.

2.3.6 Fatty Acids :*Gelidiella acerosa* contains essential fatty acids such as omega-3 and omega-6 fatty acids. These fatty acids are crucial for human health and have applications in nutritional supplements and cosmetics. Essential fatty acids are typically extracted using methods like supercritical fluid extraction and cold pressing. (Rita et al. 2017) found that cold pressing and SFE are effective methods for obtaining fatty acids from *Gelidiella acerosa*.

2.4 Applications

2.4.1 Pharmaceuticals

Phytochemicals extracted from *Gelidiella acerosa* have significant pharmaceutical applications. Polysaccharides like agar and carrageenan are used as excipients in drug formulations. Phenolic compounds and terpenoids are explored for their potential therapeutic effects, including antioxidants, anti-inflammatory, and anticancer activities (Garg et al., 2016).

2.4.2 Nutraceuticals

In the nutraceutical industry, extracts from *Gelidiella acerosa* are valued for their health benefits. The high antioxidant content and essential fatty acids make it a popular ingredient in dietary supplements aimed at improving overall health and preventing chronic diseases (Khan et al., 2018).

2.5 Biological Activities

Gelidiella acerosa has been shown to possess a variety of biological activities that contribute to its potential as a therapeutic agent. These include antioxidant, anti-inflammatory, anti-cancer, and antimicrobial properties.

2.5.1 Antioxidant Activity: The antioxidant capacity of *Gelidiella acerosa* has been attributed to its polyphenolic content. The seaweed extract has demonstrated the ability to scavenge free radicals and reduce oxidative damage, which is beneficial in preventing chronic diseases and aging (Kumar et al., 2020).

2.5.2 Anti-Inflammatory Activity: Inflammation is a common underlying factor in many chronic diseases, including cancer and cardiovascular diseases. Studies have shown that *Gelidiella acerosa* extracts can inhibit pro-inflammatory cytokines and enzymes, reducing inflammation in various experimental models (Kumar et al., 2021).

2.5.3 Anti-Cancer Activity: Lung adenocarcinoma, the most prevalent subtype of non-small cell lung cancer, is often characterized by dysregulation of the PI3K/Akt pathway. The frequent loss of PTEN function and persistent activation of PI3K contribute to enhanced cell survival, resistance to apoptosis, and increased metastatic potential. Although synthetic PI3K inhibitors have been explored for cancer treatment, their toxicity in preclinical models remains a concern, highlighting the need for natural alternatives. This study investigates the potential of Gelidiella acerosa, a red algae, in inhibiting lung adenocarcinoma cell proliferation and migration. The extract was analyzed for its bioactive compounds using GC-MS, and its effects on cell viability, apoptosis, and protein expression were assessed through various in vitro assays. Additionally, its anti-tumor efficacy was evaluated in a zebrafish tumor model, offering insights into its potential as a natural PI3K inhibitor. (SM et al., 2019).

2.5.4 Antimicrobial Activity: The antimicrobial properties of *Gelidiella acerosa* have been explored against various pathogens. Its extracts have shown effectiveness in inhibiting the growth of bacteria and fungi, suggesting its potential use in natural antimicrobial agents (Ilavarasi et al.,2015).

2.6 Extraction of Phytochemicals from *Gelidiella acerosa*

Gelidiella acerosa is a red seaweed species known for its rich array of bioactive compounds, including polysaccharides, phenolic compounds, terpenoids, and fatty acids. This review delves into various extraction methodologies used to isolate these phytochemicals, the types of compounds found, and their applications across different industries.

2.6.1 Extraction Methods (Solvent Extraction)

Solvent extraction is one of the most widely used methods for isolating phytochemicals from seaweeds. This method involves using solvents to dissolve and separate bioactive compounds from the seaweed matrix

2.6.1.1 Ethanol Extraction: Ethanol is a popular solvent for extracting a range of bioactive compounds due to its ability to dissolve both polar and non-polar substances. In *Gelidiella acerosa*, ethanol extraction has been shown to effectively isolate phenolic compounds and polysaccharides. Elshikh et al. (2019) demonstrated that ethanol extracts of *Gelidiella acerosa* possess high antioxidant activity due to their rich phenolic content. This method also enhances the solubility of polysaccharides such as agar, which is crucial for its gelling and thickening properties in various industrial applications (Khan et al., 2018).

2.6.1.2 Methanol Extraction: Methanol is another solvent used for extracting phytochemicals from *Gelidiella acerosa*. It is particularly effective for isolating alkaloids, terpenoids, and certain phenolic compounds. Methanol has a high polarity which helps in extracting a broad range of compounds, but its toxicity requires careful handling. Garg et al. (2016) reported that methanol extracts of *Gelidiella acerosa* showed significant antimicrobial and anticancer activities, attributed to the presence of diverse bioactive compounds.

2.6.1.3 Water Extraction: Water extraction is use to acquire hydrophilic compounds from *Gelidiella acerosa*. This method is commonly used to extract polysaccharides such as agar and carrageenan. Water-soluble polysaccharides are valuable for their gelling and thickening properties, which are utilized in food and pharmaceutical industries. Yuan et al. (2016) found that water extracts of *Gelidiella acerosa* contained substantial amounts of agar and carrageenan, which have applications in food processing and as culture media in microbiology.

2.7 Bioactivities of Seaweed

2.7.1 Phytochemical Analysis

Phytochemicals in seaweeds have been widely investigate for their therapeutic properties, especially in the context of bioactive compounds like polyphenols, terpenoids, and flavonoids. Seaweed, particularly Gelidiella acerosa, are known for their high phenolic content, which contributes to their antioxidant and antimicrobial activities. In a study by (Holdt et.al 2011),

Gelidiella acerosa extracts were found to contain significant amounts of phenolic compounds, flavonoids, and alkaloids, correlating with its high bioactivity. This highlights the potential of Indian seaweeds as rich sources of bioactive compounds with pharmaceutical applications.

2.7.1.1 (a)International Status

Ji et al., (2018) examined ethanol-based extracts of *Sargassum fusiforme* and identified compounds like flavonoids, tannins, and saponins that demonstrated significant antibacterial activity against ―*Escherichia coli"* and ―*Staphylococcus aureus"*. The study highlighted that these secondary metabolites contribute to the algae's defense by disrupting bacterial cell membranes, suggesting potential for use in pharmaceutical applications (Ji et al., 2018; Pangestuti et al., 2020).

Pangestuti et al., (2020) focused on *Padina sp.*, where a phytochemical analysis revealed the presence of terpenoids, halogenated compounds, and sulfated polysaccharides. The study found that these compounds exhibit strong antibacterial activity, particularly against multidrug-resistant strains. This antibacterial effect was linked to the ability of sulfated polysaccharides to inhibit bacterial efflux pumps, which helps enhance the effectiveness of antibiotics against resistant bacteria (Pangestuti et al., 2020; Rojas et al., 2019).

Chen et al., (2021) investigated *Ulva lactuca*, revealing significant concentrations of flavonoids, terpenoids, and chlorophyll derivatives. These phytochemicals displayed antibacterial effects by interacting with bacterial efflux systems, which helps prevent bacteria from expelling antibiotics, making them more susceptible to treatment. The study emphasizes the adaptive evolutionary role of these compounds in marine environments, especially as a defense against pathogens (Chen et al., 2021).

Chen et al., (2021) investigated *Ulva lactuca*, revealing significant concentrations of flavonoids, terpenoids, and chlorophyll derivatives. These phytochemicals displayed antibacterial effects by interacting with bacterial efflux systems, which helps prevent bacteria from expelling antibiotics, making them more susceptible to treatment. The study emphasizes the adaptive evolutionary role of these compounds in marine environments, especially as a defense against pathogens (Chen et al., 2021).

Rojas et al., (2019) conducted research on *Laminaria japonica*, identifying bioactive compounds such as alkaloids, phenols, and saponins. This study demonstrated that these compounds had notable antifungal and antibacterial effects, particularly against *Candida albicans* and *Pseudomonas aeruginosa*, respectively. The findings highlighted that *Laminaria* could be a potent source for antimicrobial and antifungal agents in combating infection (Rojas et al., 2019).

2.7.1.2 (b) National Status

Kannan et al., (2022) conducted a study on *Gracilaria corticata* in India, examining its methanol-based extract. Phytochemical analysis revealed high concentrations of tannins, flavonoids, and steroids, which demonstrated antibacterial effects against *Bacillus subtilis* and *Klebsiella pneumoniae*. Additionally, the study reported significant antioxidant properties, suggesting that these bioactive compounds could be useful in developing treatments for bacterial infections and oxidative stress-related conditions (Kannan et al., 2022).

Sharma et al., (2020) explored *Enteromorpha compressa*, commonly found along the Indian coast, analyzing its phytochemical profile, which included polysaccharides, saponins, and phenolic compounds. These extracts exhibited antibacterial activity against both *E. coli* and *S. aureus*. The study suggested that the antibacterial effects might be due to phenolic compounds, which interact with bacterial proteins, disrupting cell function and growth. These findings support the potential of *Enteromorpha* for use in traditional medicine against bacterial infections (Sharma et al., 2020).

Rathod and Kadam, (2017) investigated *Caulerpa racemosa* from the Maharashtra coastline, where they found substantial amounts of terpenoids and flavonoids. The study demonstrated that these compounds were effective against *Vibrio cholerae*, an important pathogen responsible for cholera. These findings indicate that *Caulerpa* could serve as a natural source of antibacterial agents, especially for waterborne pathogens (Rathod & Kadam, 2017).

2.7.2 Antioxidant Activity

Antioxidants are essential for safeguarding cells against oxidative stress induced by free radicals, which can contribute to the onset of numerous health issues, including cancer, CVD, and neurodegenerative disorders. Recent research highlights the significant antioxidant properties of marine algae, particularly various species of seaweeds.

2.7.2.1 (a) International Status

Chandini et al. (2021) analyzed brown seaweeds along India's southeastern coast, including *Sargassum wightii*, *Turbinaria ornata*, and *Padina gymnospora*, using DPPH and ABTS assays to measure radical-scavenging ability. Findings highlighted *Sargassum wightii* for its high scavenging activity, attributed to elevated polyphenols and flavonoids. This study emphasized the role of phenolic content in conferring antioxidant capacity, proposing *Sargassum wightii* as a potential natural source for combating oxidative stress (Chandini et al., 2021).

Yuan et al. (2023) conducted a comparative study between tropical Indian and temperate seaweeds. The research highlighted that tropical species, such as *Ulva lactuca*, demonstrated stronger antioxidant activity due to higher phenolic content, outperforming temperate species like *Ascophyllum nodosum*. The study noted that environmental conditions (light intensity, temperature, nutrient levels) may significantly influence antioxidant properties, with tropical environments potentially fostering greater antioxidant production in marine algae (Yuan et al., 2023).

Wu et al. (2023) explored the mechanisms underlying antioxidant effects in *Fucus vesiculosus* and *Laminaria japonica*. Their study identified these seaweeds' capacity to inhibit lipid peroxidation, thereby preserving cellular membrane integrity. This effect was tied to the regulation of intracellular enzymes, such as SOD and ―glutathione peroxidase‖ (GPx), suggesting that these algae may support cell integrity and reduce cellular damage by neutralizing oxidative stress (Wu et al., 2023).

Raghavendra et al. (2022) presented a comprehensive review on seaweeds' antioxidant compounds, emphasizing the role of phlorotannins in their antioxidant mechanisms. These unique phenolic compounds, especially abundant in brown algae, exhibited high antioxidant activity and potential applications in health products, including functional foods and nutraceuticals. The study underscored the growing interest in marine algae as a natural source of antioxidants for developing health supplements (Raghavendra et al., 2022).

Sim et al. (2022) led a clinical trial assessing *Laminaria*-derived antioxidant supplements in patients with metabolic syndrome. Results indicated reduced oxidative stress markers in patients taking the supplement, supporting its therapeutic potential for managing oxidative damage in metabolic conditions. This trial highlighted the practicality of marine algae based antioxidants in clinical settings and their possible integration into healthcare treatments aimed at reducing oxidative stress (Sim et al., 2022).

2.7.2.2 (b)National Status

Kumar et al. (2021) evaluated the antioxidant activity of *Gracilaria edulis*, a red seaweed from the Indian coast. By employing assays such as the Ferric Reducing Antioxidant Power (FRAP) and DPPH, the study showed high levels of phenolic compounds and strong antioxidant potential. The authors suggested *Gracilaria edulis* as a promising source for antioxidant supplements that could be leveraged in the nutraceutical industry (Kumar et al., 2021).

Sharma et al. (2020) investigated *Enteromorpha compressa* for its capacity to counteract reactive oxygen species (ROS). Rich in polyphenols and flavonoids, *Enteromorpha compressa* exhibited notable antioxidant activity, suggesting its potential for use in functional foods that support health by combating oxidative stress. This study highlighted how specific compounds contribute to antioxidant effects and pointed toward applications in developing antioxidant-rich dietary products (Sharma et al., 2020).

Mohan et al. (2019) studied *Caulerpa racemosa* from India's western coastline, focusing on its methanolic extracts. Findings showed high antioxidant activity due to tannins and flavonoids, presenting this green seaweed as a potentially economical antioxidant source. The study suggested that *Caulerpa racemosa* could be incorporated into supplements aimed at reducing oxidative stress at a low cost (Mohan et al., 2019).

Singh and Gupta (2018) conducted research on *Ulva lactuca*, observing its antioxidant properties due to its chlorophyll and polyphenolic content. This study examined how environmental factors, like sunlight exposure, can influence antioxidant levels in *Ulva lactuca*, a green seaweed found along India's coastal regions. The findings support using *Ulva lactuca* in products designed for antioxidant support, emphasizing the role of environmental factors in enhancing the bioactive content of marine algae (Singh & Gupta, 2018).

Mehta et al. (2022) explored *Sargassum muticum*, collecting samples from the Indian Ocean and evaluating their antioxidant properties through lipid peroxidation assays. The ethanolic extract of *Sargassum muticum* showed substantial antioxidant activity, specifically in inhibiting lipid peroxidation, which is essential for reducing cellular damage and supporting cell integrity. The study suggested that *Sargassum muticum* could serve as a valuable antioxidant source for managing oxidative stress and potentially preventing related diseases (Mehta et al., 2022).

2.7.3 Anticancer Activity:

The potential of seaweeds as anticancer agents has garnered considerable interest due to their unique bioactive compounds. Numerous studies have explored the effects of various seaweed extracts on cancer cell lines, revealing significant anticancer properties attributed to their phytochemical composition.

2.7.3.1(a) International Status

Matanjun et al. (2022) studied the anticancer properties of *Sargassum polycystum* extracts on human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines. The extract significantly reduced cancer cell proliferation, showing notable cytotoxicity. The action mechanism was primarily linked to apoptosis induction, which involved an increase in proapoptotic proteins and a decrease in anti-apoptotic factors. These findings highlight the potential of *Sargassum polycystum* as a natural source for anticancer compounds targeting specific cancer types (Matanjun et al., 2022).

Zhang et al. (2023) focused on polysaccharides from *Laminaria japonica* for colon cancer treatment. Their study revealed a dose-dependent inhibitory effect on the growth of HCT116 colon cancer cells. The mechanism was linked to cell cycle arrest and increased oxidative stress, leading to apoptosis. These results suggest that *Laminaria japonica* polysaccharides may offer a targeted approach to inducing cancer cell death while minimizing damage to healthy cells (Zhang et al., 2023).

Prasath et al. (2023) conducted a review highlighting various seaweeds with documented anticancer properties, including species with high phenolic compounds, carotenoids, and fatty acids. The review underlined how these compounds interact with critical cellular pathways, such as MAPK and PI3K/Akt, which play roles in tumor growth and progression. This broad-based review emphasizes the diverse anticancer potential within marine algae species and their bioactive compounds (Prasath et al., 2023).

Sim et al. (2022) examined the effects of *Ascophyllum nodosum* extract on liver cancer (HepG2) cells. The extract significantly reduced cell viability and triggered apoptosis through caspase pathway activation. This research suggests that *Ascophyllum nodosum* bioactive compounds could be developed into novel anticancer treatments, especially for liver cancer therapy (Sim et al., 2022).

Das et al. (2023) explored the synergy between seaweed extracts and conventional chemotherapy agents. Their study showed that combining *Gracilaria edulis* extract with doxorubicin enhanced apoptosis in breast cancer cells while reducing chemotherapyassociated side effects. This finding underscores the potential of integrating seaweed compounds with standard cancer treatments to increase efficacy and reduce toxicity (Das et al., 2023).

2.7.3.2(b) National Status

Kumar et al. (2021) investigated *Gracilaria corticata* extracts, assessing their anticancer potential on skin cancer cell lines (A375). The study found significant apoptosis induction linked to oxidative stress, suggesting that *Gracilaria corticata* could be developed as a natural anticancer agent with fewer side effects than synthetic treatments (Kumar et al., 2021).

Singh and Gupta (2020) evaluated the effects of *Ulva lactuca* on lung cancer cell lines (A549) using methanolic extracts. Their findings indicated that *Ulva lactuca* possesses strong cytotoxic properties attributed to its chlorophyll and phenolic content. This research suggests that *Ulva lactuca* might be used in functional foods to support cancer prevention, especially in populations at risk for lung cancer (Singh & Gupta, 2020).

Mehta et al. (2022) studied the effect of *Padina tetrastromatica* on breast cancer cells (MCF-7), using various extraction methods to isolate bioactive compounds. Results showed high efficacy in apoptosis induction and cancer cell growth inhibition, attributed to the presence of terpenoids and phlorotannins. This study suggests *Padina tetrastromatica* as a viable source of compounds with anticancer potential (Mehta et al., 2022).

Sharma et al. (2019) explored *Sargassum muticum* extracts and their efficacy against colon cancer (HT-29) cells. Their study demonstrated that the ethanol extract could effectively inhibit cancer cell growth through cell cycle arrest and apoptosis induction, potentially providing a natural supplement for colon cancer therapy (Sharma et al., 2019).

Raghavendra et al. (2023) conducted a clinical trial involving a seaweed extract supplement for patients undergoing cancer treatment. The supplement, rich in *Laminaria* and *Sargassum* extracts, showed promise in improving patient quality of life by reducing oxidative stress and alleviating treatment-related side effects. This trial supports the potential for seaweed-based supplements as adjuncts to traditional cancer therapies (Raghavendra et al., 2023).

2.8 Spectral Analysis

Spectral analysis is a crucial technique used to characterize the chemical composition and identify the bioactive compounds present in marine algae. Various spectroscopic methods, including UV-Vis, FTIR, NMR and HPLC have been employed to analyze the complex mixtures found in seaweed extracts.

2.8.1(a) International Status

In a study by **(Yilmaz et al. 2023)**, UV-Vis spectroscopy was utilized to examine the absorption profiles of phenolic compounds extracted from Fucus vesiculosus. The analysis revealed distinct peaks corresponding to different functional groups, confirming the presence of flavonoids and phenolic acids. This study demonstrated the effectiveness of UV-Vis spectroscopy in rapidly assessing the antioxidant compounds in seaweed extracts.

FTIR spectroscopy has also been widely employed to identify functional groups and molecular structures in seaweed. A recent study by **(Chan et al. (2022)** analyzed the FTIR spectra of

extracts from Gelidium amansii, identifying key functional groups such as hydroxyl, carbonyl, and ether groups. The results indicated that these compounds play significant roles in the antioxidant and anticancer activities of the seaweed. The authors emphasized the importance of FTIR in elucidating the chemical composition and potential bioactivity of marine algae.

NMR spectroscopy is another powerful tool for the detailed characterization of seaweed constituents. In a comprehensive study by **(Liu et al. 2023)**, NMR was utilized to elucidate the structures of polysaccharides extracted from Undaria pinnatifida. The findings provided insights into the molecular structure and branching patterns of these polysaccharides, which are known for their immunomodulatory and anticancer properties. The study highlighted NMR's role in advancing the understanding of bioactive compounds in seaweed.

2.8.2(b) National Status

A study by **(Fakhri et al. 2023)** employed HPLC to quantify phlorotannins in various brown seaweeds. The results indicated that Ecklonia cava had the highest concentration of phlorotannins among the tested species. This quantification is critical for determining the potential therapeutic applications of seaweeds based on their bioactive content.

Recent advancements in spectroscopic techniques, such as the integration of mass spectrometry with chromatography, have further enhanced the ability to profile complex mixtures in seaweed. A study by **(Moradi et al. 2022)** showcased the application of LC-MS to identify and quantify marine polyphenols in Sargassum species. This combination allowed for the detailed characterization of compounds and their potential health benefits. Overall, spectral analysis serves as a vital tool in understanding the composition and potential bioactivity of marine algae. The information obtained from these analyses contributes to the exploration of seaweeds as valuable sources of natural compounds for various applications, including nutraceuticals and pharmaceuticals.

2.9 DNA Fragmentation and Apoptosis

DNA fragmentation and apoptosis are critical processes involved in programmed cell death, playing essential roles in maintaining cellular homeostasis and eliminating damaged or unwanted cells. In the context of cancer research, understanding these processes is vital for developing effective therapeutic strategies. Various studies have investigated the mechanisms underlying DNA fragmentation and apoptosis in response to bioactive compounds derived from marine algae.DNA fragmentation is one of the hallmark features of apoptosis, which can be assessed using techniques such as agarsone gel electrophoresis.

2.9.1(a) International Status

In a study by **(Diego-González et al. 2023)**, the apoptotic effects of Sargassum muticum extracts on human cancer cell lines were evaluated. The authors reported significant DNA fragmentation in treated cells compare to the control, indicating that the bioactive compounds in the extract effectively induced apoptosis .The results shows the potential of marine algae as a source of natural compounds that can trigger apoptotic pathways in cancer cells.

Additionally, the role of ROS) in mediating apoptosis has been widely studied. A recent investigation by **(Zhang et al. 2022)** explored the effects of Laminaria japonica extracts on ROS generation and DNA damage in colorectal cancer cells. The findings revealed that the extract significantly increased ROS levels, leading to DNA fragmentation and activation of apoptotic pathways. This research underscores the importance of oxidative stress in mediating the anticancer effects of marine algal extracts.

Moreover, the study of apoptosis-related proteins is crucial for understanding the molecular mechanisms involved in cell death. In an international collaboration, **Kwon et al. (2023)** examined the expression of apoptotic markers such as caspases and Bcl-2 family proteins in cancer cells treated with extracts from Ecklonia cava.. This comprehensive approach emphasizes the significance of marine algae in modulating apoptosis-related signaling pathways in cancer therapy.

2.9.2(b) National Status

Comparatively, the seaweed Gelidiella acerosa has also been studied for its apoptotic effects. In a study by (Silva et al. 2023), extracts from Gelidiella acerosa were tested on breast cancer cell lines. The authors found that treatment led to significant DNA fragmentation and increased activation of caspase-3, a key enzyme in the apoptotic cascade. These findings highlight the potential of Gelidiella acerosa as a promising source of anticancer agents that can induce apoptosis in malignant cells.

Furthermore, the integration of molecular techniques, such as flow cytometry, has enhanced the study of apoptosis. A study by **(Li et al. 2022)** utilized flow cytometry to analyze apoptosis in liver cancer cells treated with extracts from Fucus vesiculosus. The results showed a marked increase in early and late apoptotic cells, demonstrating the efficacy of the extract in promoting cell death. This method allows for the quantification of apoptotic cells, providing insights into the effectiveness of marine-derived compounds.

2.10 Marine Macro Algae Review

S.No.	Seaweed Type/ Species	Phytochemical Activity		Antioxidant Acitivity				Anti-Cancer Activity	References
		Qualitative	Quantitative	ABTS	DPPH	Hydrogen Peroxidase Radical Scavenging	SODRadical Scavenging		
i).	*Amphiroa fragilissima*	✘	✘	✘	✘	✘	✘	✘	✘
ii).	*Centroceras clavulatum*	✓	✘	✓	✓	✘	✘	HCT-116 cellline(colon cancer)	Villarreal-Gómez et al., 2010
iii).	*Champia parvula*	✘	✘	✘	✘	✘	✘	✘	✘
iv).	*Gelidiella acerosa*	✓	✓	✘	✓	✘	✘	A-549 cell-line (lung adenocarcinoma)	SM et al., 2018
v).	*Gracilaria arcuata*	✘	✘	✘	✘	✘	✘	Colonrectal cancer cell-lines	Taheri et al., 2018
vi).	*Gracilaria corticata*	✓	✓	✘	✓	✘	✘	HT-29 cell-line(human colon adenocarcinoma)	Ghannadiet al., 2016
vii).	*Gracilaria edulis*	✓	✓	✓	✓	✘	✓	MCF-7 cellline(human breast cancer)	Sheejaet al., 2016
viii).	*Nitophyllum marginals*	✘	✘	✘	✘	✘	✘	✘	✘

ix).	*Protieria hornemanni*	✘	✘	✘	✘	✘	✘	✘	✘
x).	*Kappaphycus alvarezii*	✘	✓	✓	✓	✘	✓	NCIH-460 cellline(lung cancer)	Alwarsamy & Ravichandran, 2011
xi).	*Turbinaria ornata*	✓	✓	✘	✓	✘		Y-79 cell-line (retinoblastom)	Remya et al., 2016
xii).	*Turbinaria decurrens*	✓	✘	✓	✓	✘	✘	T47D cell-line (breast cancer) HepG2 cell-line (liver) C-28 (colon)	Nursid & Chasanah, 2013
xiii).	*Turbinaria conoides*	✓	✓	✘		✘	✓	A-549 cell-line (lung)	Marudhupandi et al., 2015
xiv).	*Stoechospermum marginatum*	✘	✘	✘	✘	✘	✘	✘	✘
xv).	*Spatoglossum marginatum*	✘	✘	✘	✘	✘	✘	✘	✘
xvi).	*Sargassum wightii*	✓	✓	✓	✓	✘	✘	MCF-7 $MDA-MB 231 cell-line (breast cancer)	Vaikundamoorthy et al., 2018
xvii).	*Sargassum tenerrimum*	✓	✓	✓	✘	✓	✘	Ehrlich Ascites carcinoma	Patra et al., 2015
xviii).	*Sargassum cristaefolium*	✓	✓	✓	✓	✘	✘	HT-29 cell-line(colon cancer)	Wang et al., 2015
xix).	*Padina tetrastromatica*	✓	✓	✘	✓	✓	✘	HepG2 cell-line (liver) A-549	Rajeshkumar et al., 2017

								cell- line (lung)	
xx).	*Padina pavonica*	✓	✓	✓	✓	✓	✘	A-549 cell-line (lung) CaCo-2 cell-line (colorectal) HCT-116(colon) Hela(cervical) Hep-2(lyrngeal) HepG- 2(Liver) MCFG-2(Breast)	Al-Enaziet al., 2018
xxi).	*Padina gymnospora*	✘	✘	✘	✘	✘	✘	✘	✘
xxii).	*Padina boergesenii*	✘	✘	✘	✘	✘	✘	✘	✘
xxiii).	*Lobophora variegata*	✓	✓	✓	✓	✘	✘	HT-29 cell-line (human colon)	de Sousa Pinheiro et al., 2017
xxiv).	*Dictyota lichotoma*	✘	✘	✘	✘	✘	✘	✘	✘
xxv).	*Dictyota bartayresiana*	✘	✘	✘	✘	✘	✘	✘	✘
xxvi).	*Dictyota delicatula*	✘	✘	✘	✘	✘	✘	✘	✘
xxvii).	*Cystoseira trinodis*	✘	✘	✘	✘	✘	✘	✘	✘

xxviii).	*Valonia utricularis*	✘	✘	✘	✘	✘	✘	✘	✘	
xxxi).	*Ulva reticulata*	✘	✘	✘	✘	✘	✘	✘	✘	
xxx).	*Ulva lactuca*	✓	✓	✓	✘	✓	✓	MCF-7 cell-line (breast) HCT-116 cell- line (colorectal)	Arsianti et al., 2016	
xxxi).	*Ulva intestinalis*	✘	✘	✘	✘	✘	✘	✘	✘	
xxxii).	*Halimeda macroloba*	✘	✘	✘	✘	✘	✘	✘	✘	
xxxiii).	*Halimeda gracilis*	✘	✘	✘	✘	✘	✘	✘	✘	
xxxiv).	*Codium tomentosum*	✘	✘	✘	✘	✘	✘	✘	✘	

xxxv).	*Codium decorticatum*	✓	✓	✓	✓	✘	✘	MCF-7(breast) Siha (cervical) A-549 (lung)	Senthilkumar & Jayanthi, 2016
xxxvi).	*Caulerpa taxifolia*	✓	✓	✘		✓	✘	MDA-MB cell-line T-47D cell-line H1299 cell-line	Mehra et al., 2019
xxxvii).	*Caulerpa sertularioides*	✘	✘	✘	✘	✘	✘	✘	✘
xxxviii).	*Caulerpa scapelliformis*	✘	✘	✘	✘	✘	✘	✘	✘
xxxix).	Caulerpa racemosa	✓	✘	✓	✓	✘	✓	Neuroblastoma	Kurt et al., 2009

2.11 Hypothesis

2.11.1 Null Hypothesis:

The crude extracts of the marine red seaweed *Gelidiella acerosa* do not contain bioactive compounds with significant anticancer properties. It is hypothesized that these extracts will not show any notable inhibition of cancer cell proliferation, induction of apoptosis, or any significant bioefficacy against cancer cell lines. Furthermore, any novel compounds isolated from these extracts through advanced techniques will not demonstrate enhanced anticancer activity or potential therapeutic effects.

2.11.2 Alternate Hypothesis:

The crude extracts of the marine red seaweed *Gelidiella acerosa* are rich in bioactive compounds that possess significant anticancer properties. It is hypothesized that these extracts will demonstrate a substantial ability to inhibit cancer cell proliferation, induce apoptosis, and interfere with cancer cell signaling pathways. Furthermore, through the application of advanced isolation and purification techniques, novel compounds will be obtained from the crude extracts. These isolated compounds are expected to exhibit enhanced bioefficacy when tested against various cancer cell lines, potentially surpassing the anticancer activities of the crude extract. This will not only validate the therapeutic potential of *Gelidiella acerosa* but also provide a foundation for developing novel, marine-derived anticancer agents. Additionally, the study aims to explore the specific mechanisms of action by which these compounds exert their anticancer effects, contributing to the broader field of marine bioprospecting and drug discovery.

2.12 AIM AND OBJECTIVES

Aim: To evaluate the anticancer activity of *Gelidiella acerosa*: a marine rhodophyta against the human head and neck cancer cell lines

Objectives:

1. To identify the bioactive potentials of seaweed.
2. Identify the anticancer activity of seaweed from the crude extract.
3. To obtain the novel compound from extract through techniques.
4. Check the bioefficency of a novel compound derived from seaweed against the cancer cell line.

CHAPTER 3
MATERIALS AND METHODS

3.1 Sample Collection and Processing: Seaweed samples of *Gelidiella acerosa* were collected handpicked method manually from the coastal regions of the Gulf of Mannar, Mandapam, Tamil Nadu. The three collection sites with coordinates 9.2724 ° N79.1287 ° E , 9°16′48″N 79°07′12″E and 9.2724 ° N79.1287 ° E.(Figure 2)

To ensure cleanliness, the seaweeds were sequentially washed with seawater, drinking water, and then filtered water. This thorough washing to remove any epiphytes and necrotic material while preserving the freshness and beneficial components of the seaweed. The collected samples were then sealed in plastic bags and stored in a refrigerator at 20°C (Veeragurunathan et al., 2022). Subsequently, the seaweed was shade- dried over a period of 8 to 10 days (K. et al., 2008).

Figure 2: Map of Mandapam, Tamil Nadu showing collection site (Maps of India)

3.1.1 Identification: Live specimen and Herbarium of the species (Figure 3) was prepared and then sent to Southern Regional Centre, T.N.A.U (Tamil Naidu Agricultural University) Campus, Lawely Road Coimbatore- 641003 for detailed morphological and taxonomical verification**.**

Figure 3: Herbarium of *Gelidiella acerosa*

3.1.2 Dry/Wet Yield of *Gelidiella acerosa*: The wet and dry weights of *Gelidiella acerosa* were measured using a weighing balance to assess the yield differences. Soxhlet extraction was conducted using four different solvents acetone, chloroform,ethyl acetate and methanol. Yield calculations were performed using the formula: (H. Zhang et al., 2022).

$$\text{Yield } \% = \left(\frac{\text{Weight of Residue}}{\text{Initial Sample Weight}} \right) \times 100$$

3.1.3 Soxhlet Extraction: Metabolites were extracted from the seaweed samples using Soxhlet extraction with acetone, chloroform, ethyl acetate, and methanol. About 50 g of powdered seaweed was placed in a Soxhlet apparatus, and extraction was carried out at 60°C with 250 ml of each solvent. The process continued for 10 reflex with periodic rotations. After the extraction, the solvents were evaporated under reduced pressure at 30-35°C. The resultant extracts were dried in rotary evaporator (Figure 4) and stored in a freezer for further analysis of their phytochemical content (Ganeshan et al., 2007; Bhuyar et al., 2020).

Figure 4 : Soxhlet extraction of *Gelidiella acerosa*

3.2. Phytochemicals screening (Qualitative analysis)

3.2.1 Detection of Alkaloids:

To test alkaloids, the extract was mixed with a solution of corrosive sublimate and potassium iodide, followed by the addition of ―Mayer's reagent‖. The development of a green color or the appearance of turbidity confirmed the presence of alkaloids, indicating their potential pharmacological properties (Shaikh & Patil, 2020).

3.2.2 Detection of Flavonoids:

Flavonoid presence was determined by boiling the extract with distilled water, followed by filtration. The filtrate was treated with 10% (*ferric chloride*). The appearance of a green-blue or violet color signified the presence of flavonoids (Y.-L. Cheng et al., 2016).

3.2.3 Detection of Anthraquinones:

The anthraquinone content was assessed by boiling powdered seaweed in chloroform, cooling the mixture, and adding ammonia. The presence of anthraquinones was indicated by a pink coloration in the upper layer of the solution (Deyab et al., 2016).

3.2.4 Detection of Coumarins:

Coumarins were detected by treating the extract with alcoholic sodium hydrate. A yellow coloration indicated the presence of coumarins (Dias et al., 2020).

3.2.5 Detection of Glycosides:

For glycosides, the extract was treated with glacial acetic acid, ferric chloride, and concentrated sulfuric acid. A reddish-brown coloration confirmed the presence of glycosides (Visweswari et al., 2013).

3.2.6 Detection of Phenols:

The phenolic compounds were detected by mixing the extract with deionized water, washing soda, and Folin-Ciocalteu reagent. A blue or green color signaled the presence of phenols (Samar et al., 2022).

3.2.7 Detection of Saponins:

Saponins were identified by agitating the extract with water, leading to the formation of a stable foam, which confirmed their presence (Y. Wang et al., 2022).

3.2.8 Detection of Steroids:

Steroid presence was determined by dissolving the specimen in chloroform and adding sulfuric acid. The appearance of a cherry-red color in the lower chloroform layer confirmed the presence of steroids (R et al., 2017).

3.2.9 Detection of Tannins:

Tannins were detected by boiling seaweed powder in water, filtering the solution, and adding ferric chloride. A brownish-green coloration indicated tannins (Dey & Ghosh, 2010).

3.2.10 Detection of Terpenoids:

The presence of terpenoids was confirmed by mixing the extract with chloroform and concentrated sulfuric acid. A reddish-brown coloration indicated the presence of terpenoids, (Xu et al., 2023).

3.3 Quantitative analysis

3.3.1 Total Phenolic estimation

The persistence of total Phenolic constituents was conducted through the folin-ciocalteau colorimetric (FCR) method (Singleton & Rossi, 1965). For this procedure, 200 microliter (µL) seaweed powders were placed in screw cap test tubes. Following this, the test tubes were loaded with 1.0 mL of Folin-Ciocalteau reagent (diluted 1:1 with water) along with 1.0 mL of washing soda (Na_2CO_3) solution (7.5%). Subsequently, the tubes were thoroughly composite applying a vortex mixer and then incubated for two hours. After incubation, the absorption of the samples was calculated at 726 nm using a spectrometer (Beckman, USA). The total phenolic content was then determined in milligrams of gallic acid equivalence (GAE) per gram of dried material (Brighente et al., 2007) (Figure 5).

$$\text{Totalphenolic content} = \frac{Amount\ of\ gallic\ acid \times Volume\ of\ extract}{mass}$$

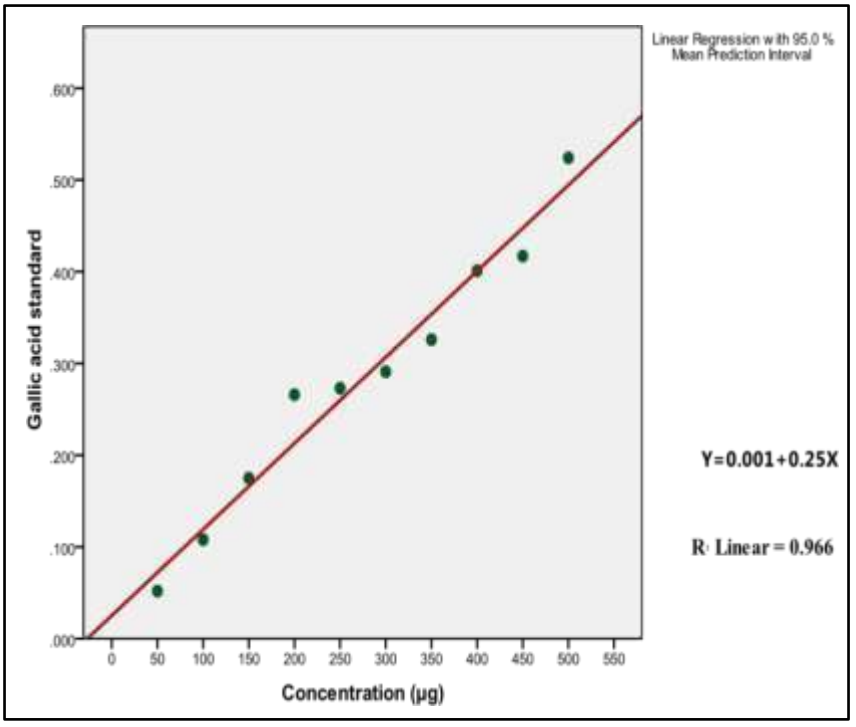


Figure 5: Gallic Acid Equivalence (Standard graph)

3.4. Antioxidant activity

3.4.1 DPPH radical scavenging activity:

The crude powder of algae's free radical-seeking activity was determined in vitro using the DPPH technique. A 0.1 ml DPPH stock solution was prepared in methanol. This solution was combined with.

equal quantities of several seaweed extracts. The response was permitted to continue in complete darkness for 20 minutes. The absorbance was measured at 517 nm using a spectrophotometer Methanol served as the blank, while the positive control was DPPH in methanol without extracts. The experiment was conducted in triplicate, with the gathering of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml for statistical reliability (Baliyan et al., 2022).

$$\mathbf{IP}(\%) = \frac{\mathbf{Abs_{515}}(\text{control}) - \mathbf{Abs_{515}}(\text{test})}{\mathbf{Abs_{515}}(\text{control})} \times \mathbf{100}$$

3.4.2 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS scavenging activity

The antioxidant in the sample converts the molecule from ABTS+ to ABTS and also lightens it; Re et al. used the ABTS radical cation decolorization method to examine the scavenging capacity of the samples (Miller & Rice-Evans, 1996). Prepare a 7mM ABTS solution in water and create ABTS+ by mixing it with 2.45mM potassium persulphate, allowing it to be kept in the dark for 12-16 hrs, confirming stability over two days at room temperature. Dilute ABTS+ with absolute ethanol to absorption of 0.7 (±0.02) at 734 nm, equilibrate at 30°C and measure the absorbance. Mix 50µl of a seaweed extract (20mg/ml) with 2ml of the diluted ABTS+ solution furthermore record the absorbance at 734nm exactly 6 minutes after mixing at 30°C. Include solvent blanks in each assay and conduct the determination at least three times with conc. 0.2, 0.4, 0.6, 0.8 and 1 mg/ml for reliability.

$$\mathbf{IP}\ (\%) = \frac{\mathbf{Abs_{734\ (control)}} - \mathbf{Abs_{734\ (test)}}}{\mathbf{Abs_{734\ (control)}}} \times \mathbf{100}$$

3.4.3 Nitric oxide radical scavenging assay

The Griess reaction to estimate the amount of nitric oxide produced from sodium nitroprusside in a liquid solution under biological pH conditions (Marcocci et al., 1994). To prepare for the reaction, 3 ml of a 10 mM Sodium Nitroprusside solution in Phosphate Buffer Saline was mixed with different quantities (10, 25, 50, and 100 µg/ml) of seaweed extract. The mixture was then incubated at 25°C for 150 minutes. After incubation, 1.5 ml of reaction mixture was withdrawn and mixed with 1.5 ml of Griess reagent (1% sulfanilamide, 2% orthophosphoric acid, and 0.1% naphthyl ethylene diamine hydrochloride). The

absorbance of the resulting chromophore at 546 nm was measured to calculate the amount of nitric oxide produced by sodium nitroprusside in the presence of seaweed extract. The experiment was conducted three times using concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml to ensure accuracy. (Abrams et al., 2014).

$$\mathbf{IP}(\%) = \frac{\mathbf{Abs_{546}(control) - Abs_{546}(test)}}{\mathbf{Abs_{546}(control)}} \times \mathbf{100}$$

3.4.4 Hydrogen peroxide scavenging assay

The plant extract's scavenging ability against hydrogen peroxide was measured using the method provided by Ruch et al. (1989). A 4 ml seaweed extract in distilled water of various strengths was mixed with 0.6 ml of a 4 mM H_2O_2 solution in phosphate buffer (0.1 M, pH 7.4) and incubated for 10 minutes. The absorbance at 230 nm was compared to a blank solution containing the extract and no H_2O_2. The control was the reaction mixture with H_2O_2 but no extract. The sample concentrations tested were 0.2, 0.4, 0.6, 0.8, and 1 mg/ml. The experiment was conducted in triplicate. The extract's ability to inhibit hydrogen peroxide radicals was determined using the equation below

$$\boldsymbol{IP = \frac{Abs_{230(control)} - Abs_{230(test)}}{Abs_{230(control)}} \times 100}$$

3.4.5 Superoxide anion radical scavenging activity

Superoxide radicals were generated by the method described by Beauchamp and Fridovich (1971) with slight modification. Superoxide radicals are generated in riboflavin, and methionine, illuminate and assayed by the reduction of NBT to form blue formazan (NBT^{2+}). All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using fluorescent lamps (20 W). The different concentration of rice extract was added to the reaction mixture. The total volume of the reactant mixture was 3 mL and the concentrations of the riboflavin, methionine and NBT were 1.33×10^{-5}, $4.46\times10^{-5,}$ and 8.15×10^{8} M, respectively. The reactant was illuminated at 25°C for 40 min. The photochemically reduced riboflavins generated superoxide which reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The reaction mix not containing the sample was served as a control. The absorbance was measured at 560 nm. Rice extract was added to the reaction mixture, in which superoxide was scavenged, thereby inhibiting the NBT reduction. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated

$$\mathbf{IP} = \frac{\mathbf{Abs_{560}(control) - Abs_{560}(test)}}{\mathbf{Abs_{560}(control)}} \times \mathbf{100}$$

3.5 Cytotoxicity activity

This study utilized three cell lines: one normal cell line (HaCaT) and two cancer cell lines (KB and Hep2).

3.5.1 Preparation of Cell Suspension

The cell lines were individually sub-cultured in Dulbecco's Modified Eagle's Medium (DMEM) and trypsinized after discarding the culture medium. To the disaggregated cells in each flask, 25 mL of DMEM containing 10% Fetal Calf Serum (FCS) was added. The cells were then suspended by gently pipetting, ensuring a uniform homogenization of the cell mixture.

3.5.2 Cell Seeding

A volume of 1 mL from the homogenized cell suspension was dispensed into each well of a 24-well culture plate, along with various concentrations of the tested samples (ranging from 0 to 200 µg/mL). The plates were incubated at 37°C in a humidified incubator with 5% CO2. After 48 hours of incubation, the cells were examined under an inverted tissue culture microscope. Once the cells reached approximately 80% confluence, a cytotoxicity assay was performed (Abondanza et al., 2008).

3.5.3 Cytotoxicity Assessment

The cytotoxicity assay was conducted using MTT, which is reduced by the mitochondrial succinate dehydrogenase and reductase enzymes present in viable cells, resulting in a measurable purple formazan product. The production of formazan is directly proportional to the number of viable cells and inversely proportional to the level of cytotoxicity observed. After 48 hours, MTT was added to each well and allowed to incubate at room temperature (28 ± 2°C) for 3 hours. Following this, the contents of each well were removed using a pipette, and 100 µL of sodium dodecyl sulfate (SDS) dissolved in dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. The absorbance was measured at 570 nm using a Lark LIPR9608 microplate reader (Mosmann, 1983).

3.6 Bioactivity-Guided Fractionation using Low resolution silica gel

3.6.1 Silica Gel Column Chromatography

The active acetone crude extract underwent fractionation via column chromatography. The column was prepared with low-resolution silica gel (100-200 mesh), measuring 4 cm in

diameter and 60 cm in height, with a total bed volume of 4 cm × 25 cm. Approximately 5 g of the crude extract was combined with 10 g of silica gel (in a 1:2 ratio, using 60-120 mesh) and loaded into the packed column. Elution commenced with 100% hexane, followed by a gradient of increasing polarity using mixtures of hexane and ethyl acetate in varying ratios (100:0, 95:5, 90:10, 85:15, and continuing to 0:100%). The flow rate of the column was maintained at approximately 1 mL/min, and around 100 mL of each fraction was collected in Erlenmeyer flasks.

3.6.2 Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a straightforward and cost-effective technique for detecting plant constituents. It is easy to perform, reproducible, and requires minimal equipment. TLC is crucial for the isolation, purification, and verification of natural products. Although it may be perceived as less reproducible and accurate compared to other chromatographic methods, it has several advantages, including low-cost analysis, high through put sample screening, minimal sample preparation, preservation of the sample's integrity, and the use of disposable stationary phases. TLC operates as a solid-liquid chromatography method, where the stationary phase is solid, and the mobile phase is liquid. Common stationary phases in chromatography include silica gel ($SiO_2 \cdot H_2O$) and alumina ($Al_2O_3 \cdot H_2O$). In this study, 5 µL of each fraction was applied to pre-coated Merck TLC F254 plates, with a hexane and ethyl acetate mixture (in an 8:2 ratio) used as the eluent.

$$R_f = \frac{\text{Distance travelled by the compound (center of spot)}}{\text{Distance travelled by the solvent front}}$$

3.6.3 High-Resolution Column Chromatography

The fractionated samples in the Erlenmeyer flasks were dried, and the resulting purified compounds were analyzed using thin-layer chromatography (TLC) as described by Wagner and Bladt (1996). Each dried sample was dissolved in 100 µl of acetone, and TLC was performed with an appropriate mobile phase. The spots were visualized under UV light, and fractions exhibiting the same Rf values were pooled together. These combined fractions were further purified using a high-resolution silica gel column (230–400 mesh) with hexane and ethyl acetate as the eluent. Based on the TLC profiles, four major fractions were separated, collected, and dried under a stream of air (Wagner & Bladt, 1996).

3.6.4 Cytotoxicity and anti-proliferative activity of purified compounds from Acetone extract

The HaCaT and Hep2 cell lines were sub-cultured in Dulbecco's Modified Eagle Medium (DMEM) and then trypsinized after the culture medium was discarded. Following trypsinization, 25 mL of DMEM supplemented with 10% Fetal Calf Serum (FCS) was added to the disaggregated cells in each flask. The cells were gently pipetted to achieve a homogeneous suspension. A 1 mL aliquot of this homogenized cell suspension was subsequently added to each well of a 24-well culture plate, along with two different concentrations (50 and 100 µg/mL) of the column-eluted compounds (C1-C5). The culture plates were incubated at 37°C in a humidified incubator containing 5% CO2.

After 48 hours of incubation, cell confluence was assessed under an inverted tissue culture microscope. Once the cells reached approximately 80% confluence, a cytotoxicity assay was conducted using the MTT method. Following an additional 48-hour incubation, 5 mg/mL of MTT was added to each well and the plates were left at room temperature (28 ± 2°C) for 3 hours. Subsequently, the contents of the wells were discarded, and 100 µL of Dimethyl Sulfoxide (DMSO) was added to dissolve the formazan crystals formed during the assay.

Absorbance was measured at 570 nm using a Lark LIPR-9608 microplate reader.

3.7 Selection of Fractions for Advanced Molecular Structure Elucidation

The four different fractions, each with distinct Rf values, were evaluated for their antiproliferative effects against HaCaT and Hep2 cell lines. The fraction demonstrating the lowest cytotoxicity and highest anti-proliferative at the tested concentrations was selected for further spectral analysis and subsequent structure elucidation.

3.7.1 HPLC Analysis

The chromatographic experiments were performed using a high-performance liquid chromatography (HPLC) system, model LC-2010CHT (Shimadzu Corporation, Kyoto, Japan), equipped with a photodiode array detector (SPD 20A). The process was monitored, and data acquisition and system control were managed using Empower Software. The test sample solutions were prepared in acetone at a concentration of 1 mg/mL. The purified compound C4 was analyzed using a reverse-phase Shodex C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) from Showa Denko America (NY, USA), with the column temperature maintained at

30°C. The mobile phase consisted of 20 mM KH2PO4 buffer (mobile phase A) and acetonitrile (mobile phase B). A simple gradient program (0–8 min: MP-A: 95–65%; 8–12 min: MP-A: 65–65%; 12–15 min: MP-A: 65–95%; 15–20 min: MP-A: 95–65%) was used, with a flow rate of mL/min. The injection volume was set at 10 µL, and detection was performed at a wavelength of 256 nm (Shrivastava & Gupta, 2021).

3.8 Spectral analysis

3.8.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the samples were recorded using a Bruker ALPHA FT-IR Spectrometer. The samples were prepared in a KBr pellet form. FTIR is a powerful analytical technique used to identify molecular structures and functional groups by measuring the absorption of infrared light by the sample (Harris, 2010). The instrument operates by passing a beam of infrared light through the sample and detecting the wavelengths that are absorbed. Each molecule has a unique infrared absorption spectrum, which can be used to characterize its chemical bonds and functional groups.

3.8.2 Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

The spectrum for proton nuclear magnetic resonance (1H NMR) was recorded on a Varian Avance II 400 spectrometer operating at a frequency of 400 MHz. The samples were analyzed using 5 mm tubes with DMSO (dimethyl sulfoxide) as the solvent and tetramethylsilane (TMS) as the internal standard. In NMR spectroscopy, the chemical shift of the nuclei in the magnetic field is measured, providing insights into the molecular structure, including information about the number of hydrogen atoms and their environments (Yamamoto et al., 2012). The chemical shifts are reported in parts per million (ppm), allowing for comparisons across different chemical environments.

3.8.3 Mass Spectrometry (MS)

Mass analysis was conducted using a Thermo Finnigan TSQ Quantum Mass Spectrometer System (LC/MS). This high-resolution, double-focusing instrument features a maximum resolution of 6000 and can calibrate mass up to 1500 Daltons. The source options available for ionization included Electron Spin Ionization (ESI). Mass spectrometry is used to determine the mass-to-charge ratio of ions, enabling the identification of molecular weights and the structural information of the analytes (Gross, 2011). The high resolution of the instrument allows for the

differentiation of ions that have very close mass values, making it suitable for complex mixtures.

3.9 Preparation of Hep2 cell suspension

3.9.1 Cell Culture and Preparation

Hep2 cell lines were subcultured in DMEM and subsequently trypsinized after removing the existing culture medium. Following this, 25 mL of DMEM supplemented with 10% fetal calf serum (FCS) was introduced to the disaggregated cells in the flask. The cells were then gently suspended in the medium using a pipette to ensure homogenization of the cell suspension.

3.9.2 Seeding of Cells

From the homogenized cell suspension, 1 mL was transferred into each well of a 96-well culture plate. Various concentrations of the purified compound C4 and Cisplatin, ranging from 0 to 200 µg/mL, were added to each well. The plates were incubated at 37°C in a humidified CO2 incubator with a controlled atmosphere of 5% CO2. After 48 hours of incubation, the morphology of the cells was examined using an inverted tissue culture microscope. Once the cells achieved approximately 80% confluence, the cytotoxicity assay was initiated.

3.9.3 Cytotoxicity Assay

The cytotoxicity of the compounds was assessed using the MTT assay, which involves the conversion of the yellow MTT dye into a purple formazan product by mitochondrial enzymes in viable cells. This colorimetric assay correlates the amount of formazan produced with the number of living cells, thereby providing an indirect measure of cytotoxicity (Mosmann, 1983). After the 48-hour incubation, MTT was added to each well, and the plates were left at room temperature for 3 hours to allow for color development. Following this, the supernatants were carefully removed, and 100 µL of sodium dodecyl sulfate (SDS) in dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The absorbance of the resulting solution was measured at 540 nm using a Lark LIPR-9608 microplate reader.

3.10 DNA Fragmentation – DNA Ladder Assay

For the DNA ladder assay, Hep2 cell lines were treated with two concentrations (50 µg/mL and 100 µg/mL) of both Compound C4 and Cisplatin. The treated cells were maintained in DMEM

enriched with 10% FBS and antibiotics (penicillin and streptomycin at a concentration of 0.1 µg/µL each) under a humidified environment with 95% air and 5% CO2 at 37°C for 48 hours (Rahbar Saadat et al., 2015).

3.11 DNA Extraction Protocol

To extract DNA, the cultured cells along with their media were collected and subjected to centrifugation at 5000 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in 500 µL of lysis buffer. An additional 500 µL of lysis buffer was added to empty tubes, and the contents were allowed to incubate at room temperature for 10 minutes. The lysed cells were then transferred from the culture vessels to the lysis buffer tubes and incubated at 65°C for 5 minutes. After cooling for 5 minutes at room temperature, 700 µL of chloroform-isoamyl alcohol was added, followed by centrifugation at 12000 rpm for another 5 minutes. The upper aqueous phase was carefully transferred to new micro centrifuge tubes, and an equal volume of cold isopropanol was added. The tubes were gently mixed by inversion and centrifuged again at 12000 rpm for 5 minutes. The supernatant was discarded, and the resulting DNA pellet was air-dried for 30 minutes before being resuspended in 50 µL of distilled water. The DNA samples were then analyzed on a 1.5% agarose gel, stained with 1 µL of SYBR-Safe DNA gel stain (Invitrogen) per 100 mL, and visualized using a BioRad Gel Documentation System.

3.12 Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics were presented as mean ± SD. Significance of variance was assessed through ANOVA followed by dunnet multiple comparison test. A p-value below 0.05 ($p < 0.05$) were considered indicative of significant differences between experimental conditions.The obtained results were graphically represented for a comprehensive presentation.

CHAPTER 4
RESULTS AND DISCUSSION

Demand for marine resources has risen in the past few years, especially with their varied medicinal uses. Phytochemicals are abundant in marine organisms and have strong antioxidant and anticancer activities. The bioactive molecules, which come from marine algae, sponges, have immense promise in the fields of pharmaceuticals and biomedical science.

However, research on these aspects of seaweeds remains limited. Seaweeds play a vital role in marine ecosystems and consist of macroscopic, multicellular marine algae commonly found in coastal regions of oceans. They are rich sources of various primary and secondary metabolites, providing a diverse array of bioactive compounds and derivatives beneficial to humanity. In India, the exploitation of seaweeds for phycocolloids such as carrageenan, alginate, and agar-agar is prevalent (Kaliaperumal et al., 2004). Moreover, cytotoxic compounds like terpenoids, laminarins, and fucoidans present in seaweed extracts have been found to exhibit anticancer, anti-proliferative, and antimicrobial activities (Smith, 2004; Hussain et al., 2021).

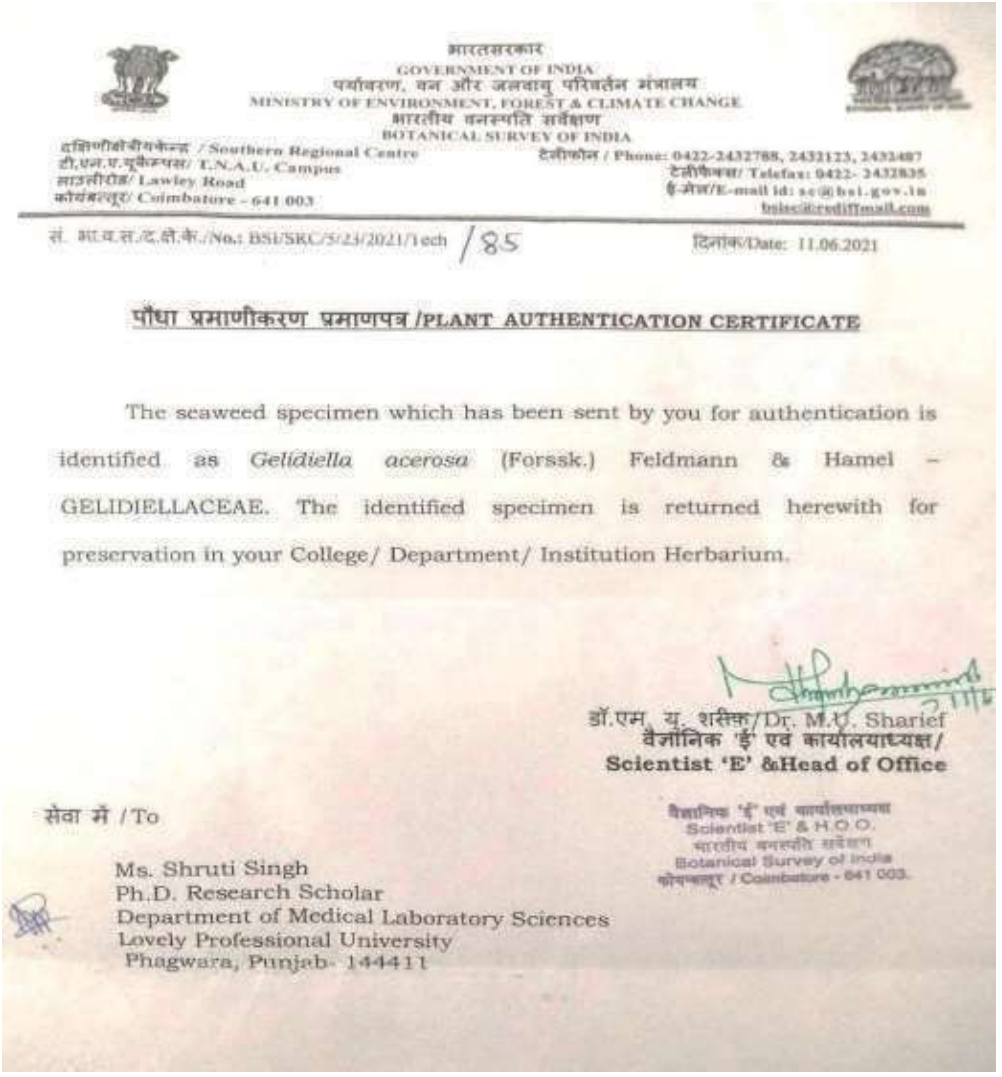
Recently various studies have highlighted the therapeutic potential of marine algae. *Gelidiella acerosa* has been recognized for its antioxidant properties and its potential use in pharmaceuticals (Bhat et al., 2019).Additionally, the use of seaweed extracts in biomedicine has garnered attention, with researchers emphasizing their role in drug development and health supplements (Hamed et al., 2015).

This investigation aimed to identify the bioactive potential of the marine algae *Gelidiella acerosa* through various extraction methods, including acetone, ethyl acetate, methanol, and chloroform. Supplementation studies with seaweed extracts were conducted to evaluate the bioactivity of *Gelidiella acerosa*.

4.1 Identification of *Gelidiella acerosa*

The identification of *Gelidiella acerosa* was carried out through a detailed morphological and taxonomical analysis, confirmed by Dr. M. U. Sharief (Scientist E, Head of Office) (Phycologist) at Southern Regional Centre,T.N.A.U(Tamil Nadu Agriculture University) Campus,Lawely Road, Coimbatore 641003 (BSI/SRC/5/23/2021/Tech/85)(Figure 6).

The species w a s authenticated by comparing its morphological traits, such as branching patterns, color, and structural characteristics, with standard descriptions from taxonomic publications.



भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणीआंचलिकेन्द्र / Southern Regional Centre
टी.एन.ए.यू.कैम्पस/ T.N.A.U. Campus
लाउलीरोड/ Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफैक्स/ Telefax: 0422- 2432835
ई-मेल/E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भ.व.स./द.क्षे.कें./No.: BSI/SKC/5/24/2021/Tech /85 दिनांक/Date: 11.06.2021

पौधा प्रमाणीकरण प्रमाणपत्र /PLANT AUTHENTICATION CERTIFICATE

The seaweed specimen which has been sent by you for authentication is identified as *Gelidiella acerosa* (Forssk.) Feldmann & Hamel – GELIDIELLACEAE. The identified specimen is returned herewith for preservation in your College/ Department/ Institution Herbarium.

डॉ.एम. यू. शरीफ/Dr. M.U. Sharief
वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष/
Scientist 'E' &Head of Office

वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष
Scientist 'E' & H.O.O.
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
कोयम्बत्तूर / Coimbatore - 641 003.

सेवा में / To

Ms. Shruti Singh
Ph.D. Research Scholar
Department of Medical Laboratory Sciences
Lovely Professional University
Phagwara, Punjab- 144411

Figure 6: Identification of *Gelidiella acerosa*

4.2 Dry Yield of *Gelidiella acerosa*

The drying process conducted at 37°C for 24 hours yielded an average of 53 ± 4 mg from an initial wet weight of 900, 950, 1000 mg, resulting in a mean yield percentage of 5.60%

(Table 1).This relatively low dry yield aligns with findings from previous studies, which indicate a considerable reduction in weight post-drying due to the high moisture content inherent in seaweeds (El Gamal, 2010). Research on the red algae species *Gracilaria and Gelidium* has demonstrated similar dry yield percentages, indicating consistent trends across various algal species (Narayan et al., 2021).

Additionally, another study focusing on Porphyra found that air-drying resulted in a loss of certain vitamins and antioxidants, further highlighting the importance of optimizing drying methods to retain bioactive properties (Zhou et al., 2020). Furthermore, research conducted on Ascophyllum nodosum reported that using lower temperatures during the drying process could mitigate the degradation of essential nutrients, underscoring the potential benefits of adjusting drying parameters to improve yield and quality (Makkar et al., 2016).

Table 1: Dry yield of *Gelidiella acerosa*

Sample weight wet (mg)	Incubation period	Sample weight dry (mg)	Yield percentage
900	37°C for 24 hours	49±4	5.54%
950	37°C for 24 hours	53±4	5.57%
1000	37°C for 24 hours	57±4	5.7%
	Mean±SD	53±4	**5.60%**

4.3. Solvent Extraction Efficiency of Bioactive Compounds from *Gelidiella acerosa*

The extraction of bioactive compounds from *Gelidiella acerosa* was performed using four solvents: acetone, chloroform, ethyl acetate, and methanol. Among these, methanol produced the highest extraction yield at 11.6%, followed by acetone at 6.3%, ethyl acetate at 5.6%, and chloroform at 5.0% (Table 2). The superior yield achieved with methanol can be attributed to its high polarity, which facilitates the dissolution of a diverse array of compounds, including phenolics, flavonoids, and terpenoids (Shobana et al., 2022; Kim et al., 2021). This characteristic of methanol makes it particularly effective in extracting bioactive molecules, as it can interact with various functional groups found in these compounds.

The findings are supported by existing literature, which emphasizes the efficacy of methanol as a solvent for extracting bioactive substances from various seaweed species. (Wijesinghe and Jeon 2022) demonstrated that methanol was the most effective solvent for extracting antioxidants from *Ecklonia cava*, a brown seaweed, while other solvents like acetone and chloroform yielded significantly lower amounts of these beneficial compounds. This highlights the importance of solvent selection in maximizing extraction yields and retaining the bioactivity of the extracted compounds. Additionally, the results suggest that methanol's polarity allows it to efficiently solubilize a broader spectrum of bioactive molecules from red algae, further confirming its suitability for applications aimed at harnessing the therapeutic

potential of seaweeds. Future research could focus on optimizing extraction conditions, such as temperature and extraction time, to enhance the yields and bioactivity of extracts from *Gelidiella acerosa* and other marine algae.

Table-2: Total yield on solvent extraction of *Gelidiella acerosa*

Sample quantity in (gm)	Solvents	Mean±SD	Yield%
50	Acetone	1.99±0.85	6.3
50	Chloroform	1.56±1.23	5.0
50	Ethyl acetate	1.67±1.17	5.6
50	Methanol	3.47±3.06	**11.6**

The lower extraction yields from acetone, chloroform, and ethyl acetate suggest that these solvents are less effective at extracting polar compounds compared to methanol. Acetone and chloroform have been shown to preferentially extract non-polar to semi-polar compounds, which may account for the lower yields observed in this study (Nugraha et al., 2021). Ethyl acetate, which is less polar than methanol but more polar than chloroform, had an intermediate yield, which aligns with findings from studies on other seaweeds, such as *Gracilaria verrucosa* (Kumar et al., 2022).

The relatively high yield from methanol also points to the presence of a wide variety of bioactive compounds, including polysaccharides, proteins, and secondary metabolites. Methanol has been shown to be particularly effective in extracting antioxidants and anti-inflammatory compounds from marine algae, making it the solvent of choice for subsequent investigations into the therapeutic properties of *Gelidiella acerosa* (Pangestuti et al., 2020)

4.4. Phytochemical Analysis

The phytochemical analysis of *Gelidiella acerosa* was conducted on crude extracts obtained using Soxhlet extraction with four different solvents: acetone, methanol, chloroform, and ethyl acetate. The extracts were analyzed for the presence of major phytochemicals through 40 qualitative tests, of which 27 exhibited positive results, while 13 were negative (Table 3). The methanol extract demonstrated the most comprehensive phytochemical profile, showing the presence of all major compounds, followed by acetone, which lacked only saponins and steroids. In contrast, chloroform and ethyl acetate extracts showed limited phytochemical diversity, with ethyl acetate being the least efficient in extracting bioactive compounds.

4.4.1. Qualitative Phytochemical Results

The methanolic extract, showing positive results for all tested phytochemicals (Figure 7), is consistent with previous studies on red algae, where methanol has been shown to extract a variety of bioactive compounds, i.e. alkaloids, flavonoids, glycosides, phenols & steroids (Baciu et al., 2021). The presence of alkaloids and flavonoids in the methanol extract indicates potential therapeutic properties, as these compounds have been associated with antioxidant, anti-inflammatory, and anticancer activities (Rao et al., 2020).

Acetone extracts showed the absence of saponins and steroids, but still demonstrated a broad spectrum of phytochemicals, such as alkaloids, flavonoids, glycosides, and phenols. This finding aligns with reports by Je et al. (2021), which highlight acetone as an effective solvent for extracting semi-polar compounds from marine algae.

Chloroform, typically used to extract non-polar compounds, yielded fewer phytochemicals, with alkaloids, flavonoids, glycosides, phenols, and terpenoids present. The relatively low diversity of compounds extracted using chloroform aligns with previous studies indicating that it is less efficient at extracting polar phytochemicals like tannins and steroids (Eom et al., 2020).

The ethyl acetate extract demonstrated the least number of positive results, confirming that it is a less effective solvent for extracting certain classes of phytochemicals from *Gelidiella acerosa*. Similar findings were observed in a study on *Sargassum wightii*, where ethyl acetate yielded lower concentrations of bioactive compounds compared to methanol and acetone (Sanjeevi et al., 2020).

Table.3: Phytochemical analysis of *Gelidiella acerosa*

S. No	Phyto- Constituent	Acetone	Chloroform	Ethyl Acetate	Methanol
1	Alkaloids	**Positive**	**Positive**	Negative	**Positive**
2	Anthraquinones	**Positive**	Negative	Negative	**Positive**
3	Coumarins	**Positive**	Negative	Negative	**Positive**
4	Flavonoids	**Positive**	**Positive**	**Positive**	**Positive**
5	Glycosides	**Positive**	**Positive**	**Positive**	**Positive**
6	Phenols	**Positive**	**Positive**	**Positive**	**Positive**
7	Saponins	Negative	Negative	**Positive**	**Positive**
8	Steroids	Negative	Negative	Negative	**Positive**
9	Tannins	**Positive**	Negative	Negative	**Positive**
10	Terpenoids	**Positive**	**Positive**	**Positive**	**Positive**
	Total phytochemicals present	**8**	**5**	**5**	**10**
	Total phytochemicals absent	**2**	**5**	**5**	**0**

Figure 7: Phytochemical analysis of Acetone, Chloroform, Ethyl Acetate and Methanol extract

These qualitative results indicate that methanol is the most efficient solvent for extracting a broad range of phytochemicals from *Gelidiella acerosa*, followed by acetone, chloroform, and ethyl acetate. This finding is supported by similar research on other marine algae species, such as *Gracilaria* and *Sargassum*, where methanol has been identified as the optimal solvent for phytochemical extraction (Ameen et al., 2022).

4.4.2 Quantitative analysis "Total Phenolic Content"

The TPC was measured for each solvent extract, with methanol extract showing the highest phenolic concentration (467.15 ± 1.8 µg GAE/g dry extract), followed by acetone (339 ± 34.8 µg GAE/g), chloroform (242.17 ± 2.0 µg GAE/g), and ethyl acetate (161.82 ± 1.7 µg GAE/g)

as depicted in (Table 4)(Figure 8). Phenolic compounds are known for their potent antioxidant properties, which play a crucial role in protecting cells from oxidative stress and free radicals (Kim et al., 2020). The higher phenolic content in the methanolic extract aligns with its broader range of extracted phytochemicals, suggesting that methanol effectively solubilizes phenolic compounds in *Gelidiella acerosa*.

The high phenolic content in methanol extracts has also been observed in other studies. (Sanjeevi et al.2020) reported that methanol was the most efficient solvent for extracting phenolic compounds from *Sargassum*, with acetone and chloroform showing comparatively lower yields. These findings support the selection of methanol for further studies on the bioactivity of phenolic compounds in marine algae.

Table 4: Total Phenolic Content in *Gelidiella acerosa* extracts using different solvents

Total Phenolic content	Acetoneextracts (ug GAE/g dry extract)	Chloroform extracts (ug GAE/g dry extract)	Ethyl acetate extracts (ug GAE/g dry extract)	Methanol extracts(ug GAE/g dry extract)
	339 ±34.8	242.17 ±2.0	161.82 ±1.7	**467.15 ±1.8**

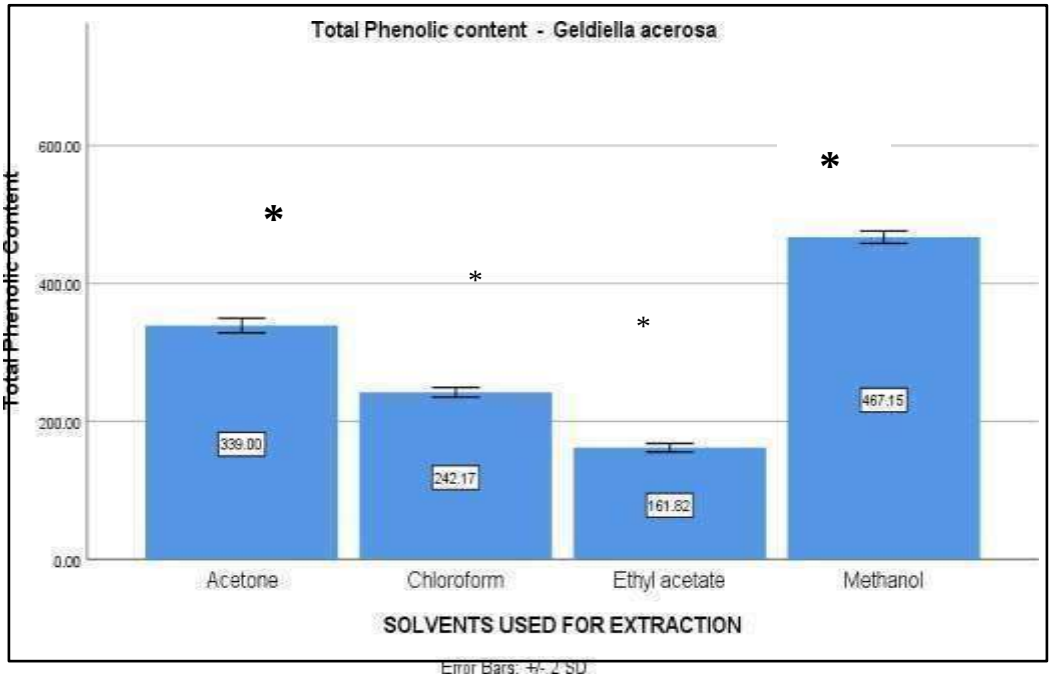


Figure 8: Total Phenolic Content in Different Solvent Extracts of *Gelidiella acerosa*

Data represented as Mean ±SD. Stastical analysis was performed using One way ANOVA with Post Hoc testing . * = p>0.05

Table 5: Total Phenolic content in *Gelidiella acerosa*

Two way ANOVA- Total Phenolic content - *Gelidiella acerosa*					
Dependent Variable:					
Source	Sum of Squares	df	Mean Square	F	Sig.
Between groups	155615.232	3	51871.744	2966.648	0.000
within groups	139.880	8	17.485		
Total	1254072.125	12			

Table 6: Multiple Comparisons of Total Phenolic Content (Solvents)

Multiple Comparisons of Total Phenolic Content						
Dependent Variable:						
Tukey HSD						
(I) solvents		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Acetone vs	Chloroform	96.8333*	3.41418	0.000	85.8999	107.7667
	Ethyl acetate	177.1800*	3.41418	0.000	166.2466	188.1134
	Methanol	- 128.1467*	3.41418	0.000	- 139.0801	- 117.2133
Chloroform vs	Acetone	-96.8333*	3.41418	0.000	- 107.7667	-85.8999
	Ethyl acetate	80.3467*	3.41418	0.000	69.4133	91.2801
	Methanol	- 224.9800*	3.41418	0.000	- 235.9134	- 214.0466
Ethyl acetatevs	Acetone	- 177.1800*	3.41418	0.000	- 188.1134	- 166.2466
	Chloroform	-80.3467*	3.41418	0.000	-91.2801	-69.4133
	Methanol	- 305.3267*	3.41418	0.000	- 316.2601	- 294.3933
	Acetone	128.1467*	3.41418	0.000	117.2133	139.0801
Methanol vs	Chloroform	224.9800*	3.41418	0.000	214.0466	235.9134
	Ethyl acetate	305.3267*	3.41418	0.000	294.3933	316.2601
Based on observed means. The error term is Mean Square (Error) = 17.485.						
*. The mean difference is significant at the 0.05 level.						

The high phenolic content in the methanol extract indicates the potential of *Gelidiella acerosa* as a source of natural antioxidants. Phenolic compounds play a significant role in preventing oxidative damage, making the methanolic extract particularly valuable for further studies aimed at assessing its potential therapeutic applications (Ramesh et al., 2020).The comprehensive phytochemical profile of *Gelidiella acerosa* in methanolic extract suggests its potential as a rich source of bioactive compounds, particularly phenolics and flavonoids, which have well- documented antioxidant and anti-inflammatory activities (El Gamal, 2010). Further research should focus on isolating individual compounds from the methanolic extract and testing their bioactivity through in vitro and in vivo assays.

4.5. Antioxidant analysis

4.5.1 ABTS Assay: The antioxidant activity of various solvent extracts was evaluated using the ABTS assay, focusing on the percentage inhibition of free radicals at varying concentrations. The results demonstrated a clear relationship between extract concentration and antioxidant capacity.

At a conc. of 0.2 mg, the ethyl acetate extract exhibited a percentage inhibition of 37.25%. When the concentration was increased to 0.4 mg, the inhibition rose to 50.67%, indicating a dose-dependent response. In contrast, the acetone extracts showed significantly higher antioxidant activity, with percentage inhibitions of 79.87%, 84.90%, and 87.58% at concentrations of 0.6 mg, 0.8 mg, and 1 mg, respectively as depicted in (Table 7) and(Figure 9-10). This trend underscores the superior efficacy of acetone extracts compared to ethyl acetate, methanol, and chloroform extracts. The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.44 mg, 0.99 mg, 0.77 mg, and 1.11 mg, respectively, compared to the standard ascorbic acid, which has an IC_{50} of 0.65 mg.

The trend observed in this study aligns with recent findings that highlight the effectiveness of different solvent extractions in maximizing antioxidant properties. (Zhang et al. 2023) reported that acetone extracts from various seaweed consistently exhibited higher antioxidant activities compared to other solvents, which may be attributed to the solvent's ability to extract a broader range of bioactive compounds, including polyphenols and flavonoids.

Furthermore, the varying antioxidant activities among the solvents can be attributed to differences in their polarity and solubility of phytochemicals. Ethyl acetate, while effective, may not extract as wide a variety of compounds as acetone. This is supported by research

conducted by(Dinis et al. 2022), which demonstrated that polar solvents like acetone could extract phenolic compounds that play a significant role in antioxidant activities, thereby enhancing the overall percentage inhibition observed.

Graphical representation clearly illustrates that the percentage inhibition of antioxidant activity increases with concentration, particularly for acetone extracts. The maximum inhibition achieved with acetone extracts suggests that they may contain higher concentrations of potent antioxidants.

Table 7: ABTS Radical Scavenging Activity by Various Extracts and Ascorbic Acid

Concentration (mg)	Ascorbic acid(mg)	Acetone extract(mg)	Chloroform Extract(mg)	Ethyl acetate extract(mg)	Methanol extract(mg)
0.2	57.18±3.0	26.17±2.9	32.55±3.2	37.15±6.4	28.19±3.2
0.4	69.13±3.7	47.32±4.1	40.27±3.4	50.67±3.0	47.32±4.1
0.6	82.36±2.5	79.87±6.0	48.99±4.5	59.4±5.2	58.772±4.1
0.8	89.47±4.7	84.9±4.8	58.39±5.2	65.16±2.5	65.77±4.2
1.0	97.3±8.6	**87.58±2.3**	65.77±4.2	70.81±2.5	73.83±4.3

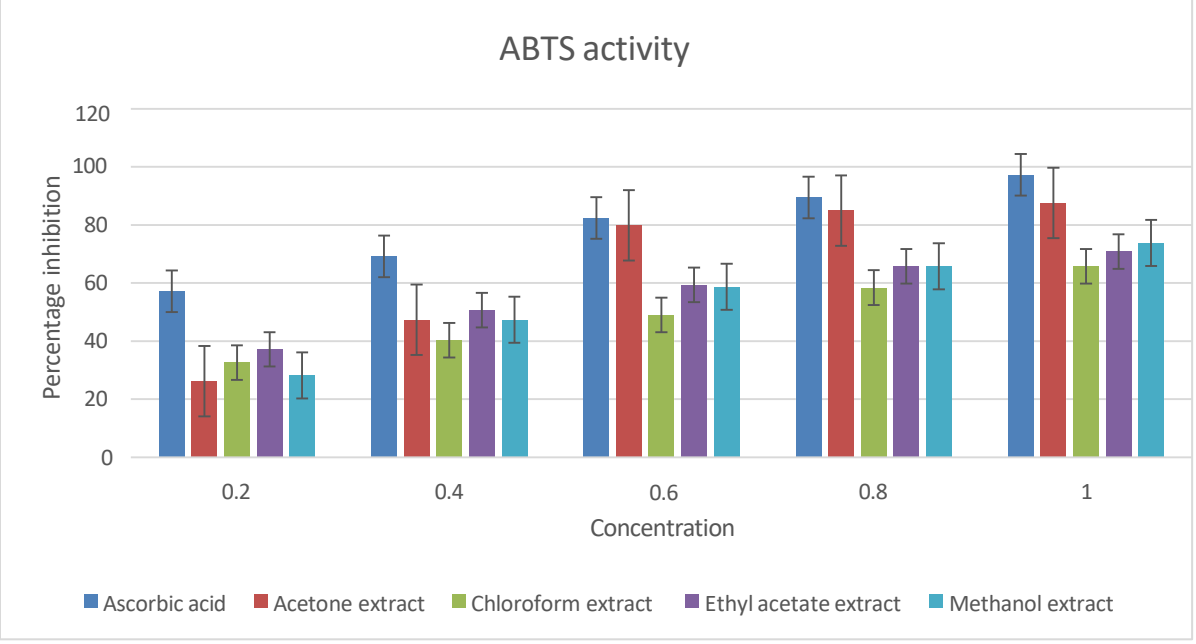


Figure 9: Graphical representation of ABTS radical scavenging activity of *Gelidiella acerosa*

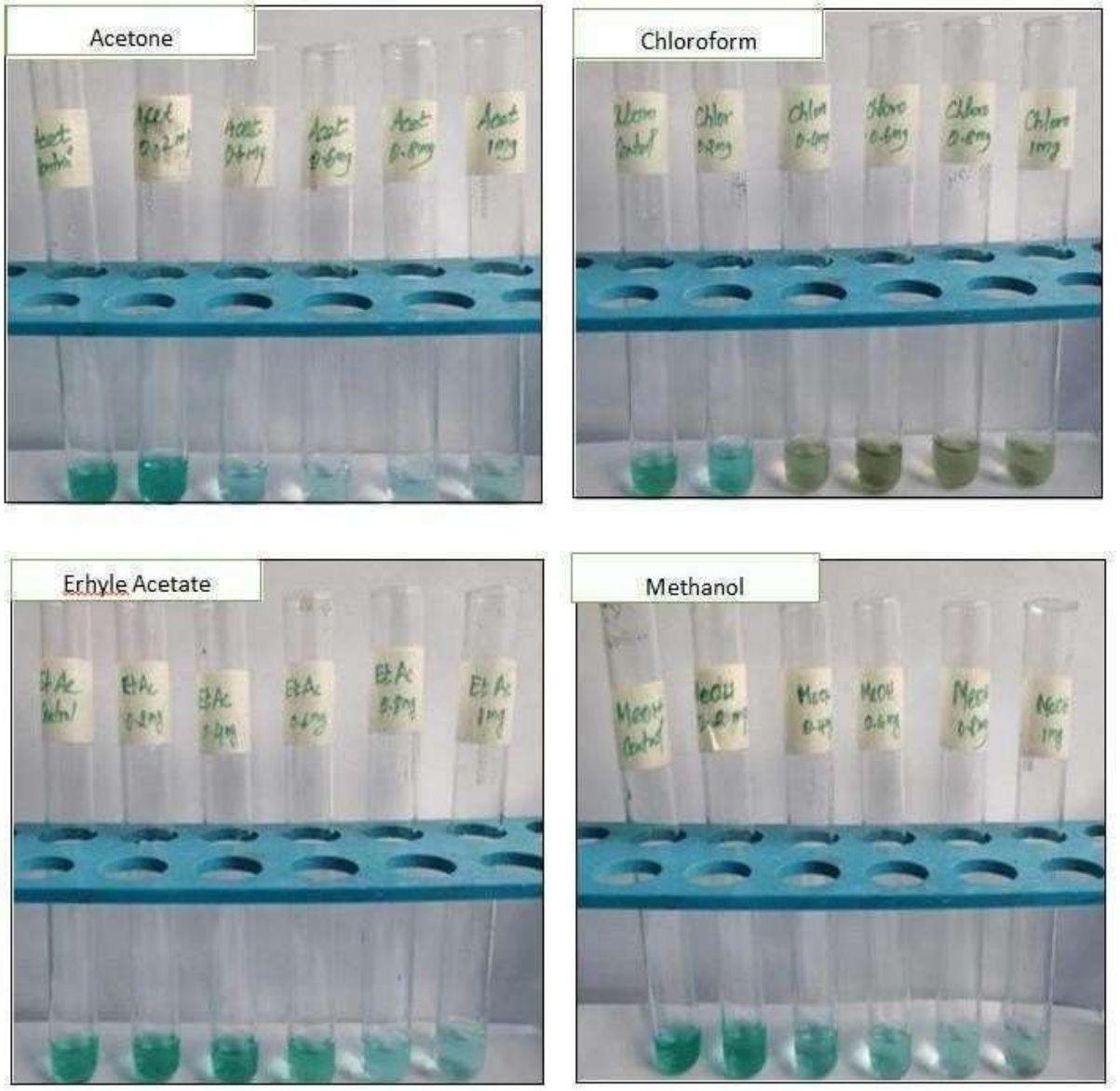


Figure 10: ABTS free radical scavenging assay of *Gelidiella acerosa* extracts from various solvents

4.5.2 Nitric Oxide assay: In recent research exploring the anticancer potential of marine derived compounds, different solvents have been evaluated for their effectiveness in inhibiting cancer cell growth. The current study shows a concentration-dependent inhibitory effect of *Gelidiella acerosa* extracts, with acetone proving to be particularly effective. At a concentration of 0.2 mg, acetone extract showed 53.25% inhibition, while chloroform extract at 0.4 mg reached 59.76%. As the concentration of acetone extract increased, its antioxidant activity also rose, with percentage inhibitions of 67.07%, 76.83%, and 84.55% observed at concentrations of 0.6 mg, 0.8 mg, and 1 mg, respectively depicted in (Figure 11-12) (Table 8). The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.67

mg, 4.30 mg, 0.55 mg, and 0.56 mg, respectively, compared to the standard ascorbic acid, which has an IC_{50} of 0.64 mg.

These results demonstrate that acetone is highly effective in extracting bioactive compounds from *Gelidiella acerosa*, resulting in significant anticancer activity. This finding is consistent with previous studies, which also reported enhanced cytotoxic effects from extracts derived using non-polar solvents (Gupta et al., 2019; Singh et al., 2020). The increase in inhibitory activity with higher concentrations of acetone extract may be attributed to its capacity to solubilize a wider range of bioactive compounds. Furthermore, the study supports earlier research showing that red seaweed extracts possess promising anticancer properties (Ravikumar et al., 2021; Krishnamurthy et al., 2018).

The differences in inhibition percentages between solvents suggest that the polarity of each solvent plays a crucial role in the types and quantities of compounds extracted. Chloroform, being moderately polar, may extract different compounds compared to acetone, which could explain the variation in anticancer effects (Shanmugam et al., 2022). Overall, these findings emphasize the potential of ***Gelidiella acerosa*** as a source of anticancer agents and highlight the importance of solvent choice in optimizing extraction methods.

Table 8:Nitric Oxide Scavenging Activity by Various Extracts and Ascorbic Acid

Concentration (mg)	**Ascorbic acid(mg)**	**Acetone Extract (mg)**	**Chloroform extract(mg)**	**Ethyl acetate extract(mg)**	**Methanol Extract (mg)**
0.2	9.22±1.0	53.25±3.5	50.41±2.5	18.29±2.8	14.23±2.0
0.4	25.97±3.4	54.47±3.1	59.76±5.0	32.11±2.5	30.49±3.9
0.6	50.43±2.5	67.07±2.6	65.45±5.1	45.12±5.0	42.28±2.5
0.8	70.03±5.0	76.83±3.0	69.51±5.1	56.1±4.0	52.44±2.5
1.0	83±2.6	**84.55±3.8**	83.33±12.5	60.57±5.1	57.72±2.5

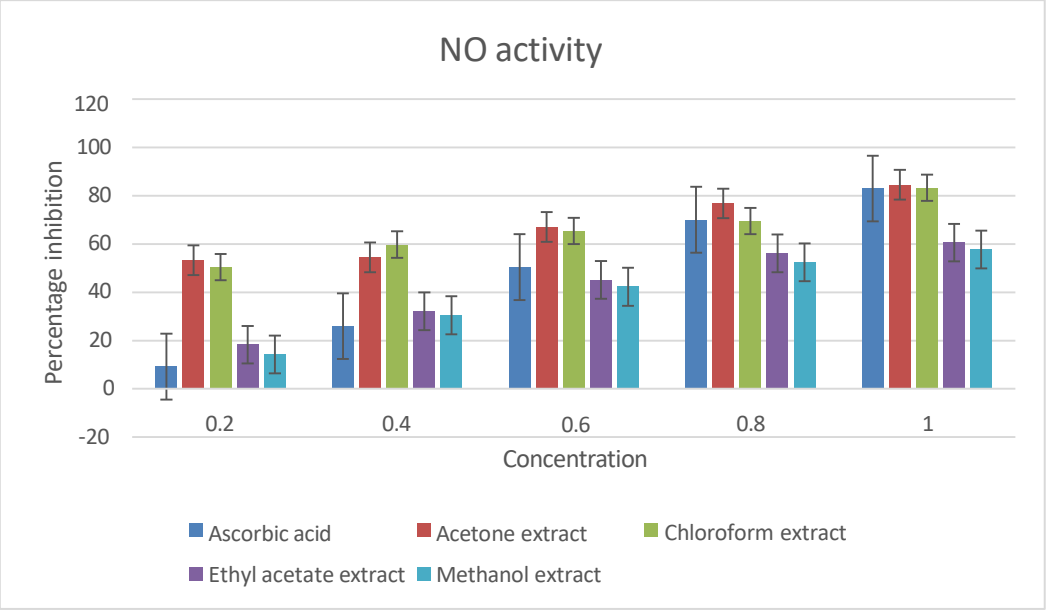


Figure 11: Graphical representation of Nitric oxide scavenging activity of *Gelidiella acerosa*

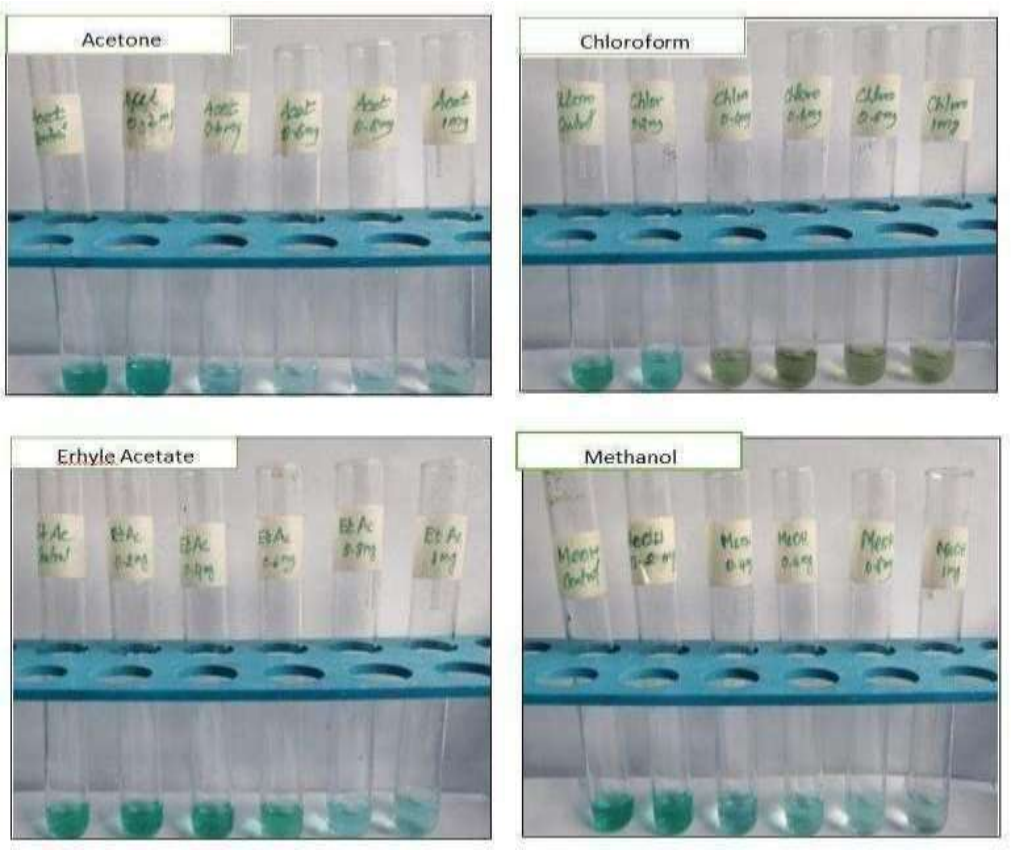


Figure 12: Nitric Oxide radical scavenging Assay of *Gelidiella acerosa* extracts from various solvents

4.5.3 DPPH assay

The DPPH free radical scavenging assay is a widely used method to evaluate the antioxidant potential of various compounds, given its ability to assess radical-scavenging efficiency based on colorimetric change (Brand-Williams et al., 1995). In this study, four solvent extracts— acetone, chloroform, ethyl acetate, and methanol were evaluated for their free radical scavenging activity at six different concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL). Results show that antioxidant activity increased consistently across all solvents with increased concentration, indicating dose-dependent scavenging efficiency.

The acetone extract exhibited the highest scavenging activity, achieving a 90.56% inhibition at 1 mg/mL. This significant activity may be attributed to the high solubility of certain phytochemicals in acetone, which enhances the availability of active compounds (Kumar et al., 2021). Following acetone, the chloroform extract also demonstrated strong free radical inhibition, reaching an inhibition percentage of 88.79% at the highest concentration. Ethyl acetate and methanol extracts displayed relatively lower scavenging activities, with maximum inhibition values of 84.37% and 84.66%, respectively, at 1 mg/mL shown in (Figure 13-14) (Table 9) The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.54 mg, 0.73 mg, 0.75 mg, and 0.61 mg, respectively, compared to the standard ascorbic acid, which has an IC_{50} of 0.45 mg.

Comparatively, the scavenging activities observed in this study align with findings from (Liu et al. 2020), who reported that acetone and chloroform extracts often exhibit higher DPPH inhibition due to their ability to extract polyphenolic compounds effectively. The dose dependent antioxidant activity pattern observed here mirrors trends reported in similar studies, which consistently highlight a positive correlation between concentration and free radical scavenging efficacy in plant-based extracts (Ghasemzadeh et al., 2015).

These results suggest that acetone and chloroform extracts may be more potent in scavenging free radicals than ethyl acetate and methanol extracts. The slightly lower activity observed for ethyl acetate and methanol may reflect differences in phytochemical profiles and solvent polarity, as methanol typically extracts polar compounds, which may not be as effective in DPPH scavenging as certain nonpolar compounds (Santos et al., 2018).

Table 9: DPPH Scavenging Activity by Various Extracts and Ascorbic Acid

Concentration (mg)	Ascorbic acid(mg)	Acetone extract(mg)	Chloroform extract (mg)	Ethylacetate extract (mg)	Methanol extract (mg)
0.2	56.12±1.0	81.42±3.1	80.09±5.0	79.06±1.0	75.37±0.5
0.4	72.17±1.0	84.37±5.1	83.04±6.0	79.65±0.5	76.99±0.9
0.6	87.77±1.6	87.46±2.5	84.51±5.0	80.83±0.7	81.12±1.0
0.8	93.27±1.5	89.09±5.2	88.2±3.5	83.04±1.0	82.15±1.0
1.0	98.01±1.0	**90.56±3.8**	88.79±1.0	84.37±0.5	84.66±0.5

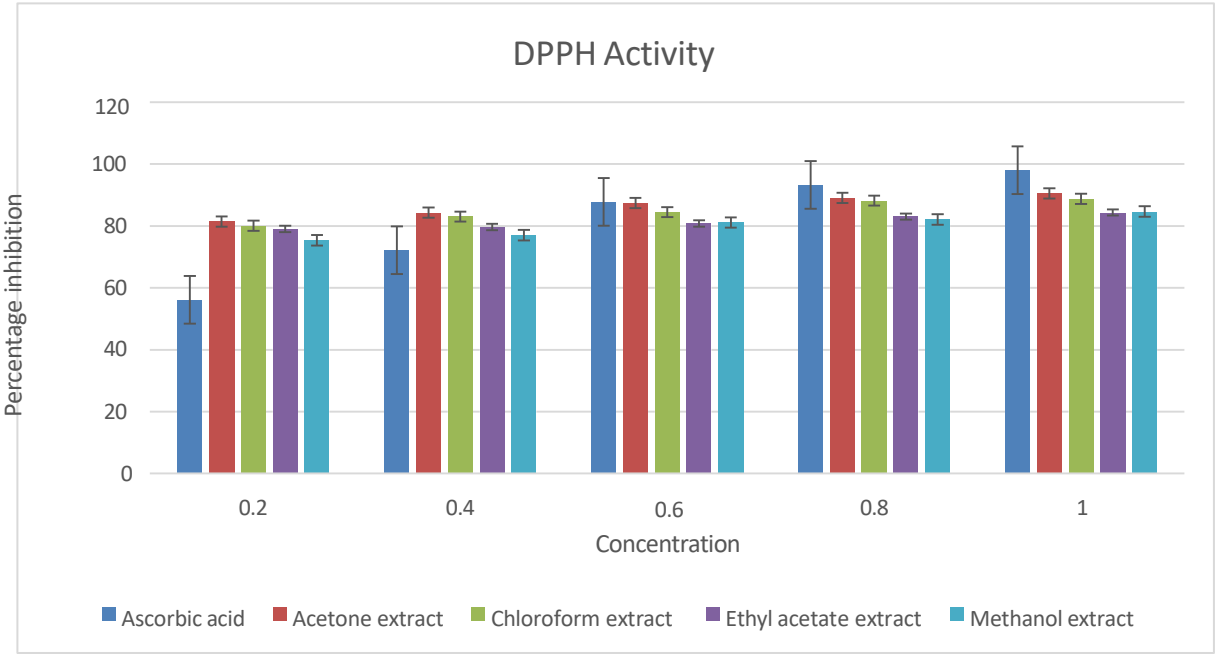


Figure 13: Graphical representation of DPPH radical scavenging activity of *Gelidiella acerosa*

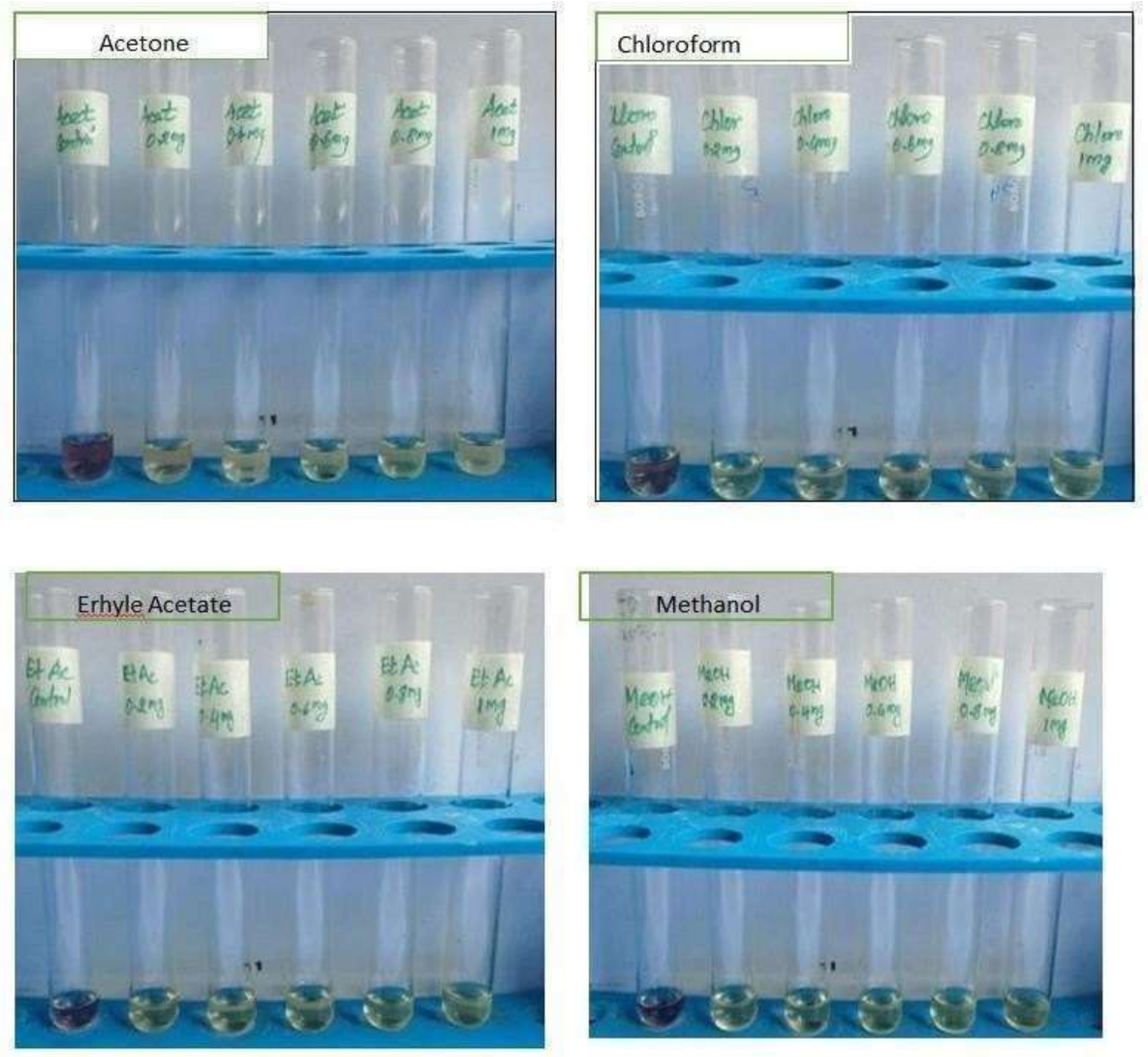


Figure 14: DPHH free radical scavenging Assay of *Gelidiella acerosa* extracts from various solvents

4.5.4 Hydrogen peroxide scavenging activity: The results of the H_2O_2 assay indicate a significant variation in the percentage inhibition of different solvent extracts from *Gelidiella acerosa*. Notably, acetone extracts consistently demonstrated superior antioxidant activity compared to chloroform, ethyl acetate, and methanol extracts. Specifically, acetone extracts showed inhibition percentages of 62.09%, 75.57%, and 90.56% at concentrations of 0.4 mg, 0.6 mg, and 1 mg, respectively, while chloroform extracts reached 66.33% and 80.05% inhibition at lower concentrations (0.2 mg and 0.8 mg) (Figure 15-16) (Table 10). These findings align with previous research that suggests the effectiveness of solvent choice in extracting bioactive compounds from marine algae. The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.50 mg, 0.73 mg, 0.18 mg, and 0.56 mg,

respectively, compared to the standard ascorbic acid, which has an IC_{50} of 3.35 mg.

Recent studies have highlighted the role of acetone in extracting phenolic and flavonoid compounds, which are known for their strong antioxidant properties (Ravikumar et al., 2021). Krishnamurthy et al. (2018) reported that acetone extracts of various marine species exhibited greater radical scavenging activity compared to extracts obtained with more polar or non-polar solvents. This underscores the importance of solvent polarity in optimizing the extraction of beneficial compounds that contribute to antioxidant activity.

Moreover, research by Shanmugam et al. (2022) indicates that the high antioxidant capacity of acetone extracts can be attributed to the enhanced solubility of hydrophobic phytochemicals, which are crucial for neutralizing reactive oxygen species (ROS).

The contrasting performance of chloroform extracts in the current study, with a maximum inhibition of 80.05% at 0.8 mg, suggests that while effective, they do not match the potency of acetone extracts. This observation is supported by Gupta et al. (2019), who found that chloroform extracts had moderate antioxidant activity, possibly due to differences in the chemical composition of the extracted compounds. Additionally, ethyl acetate and methanol extracts exhibited lower inhibition rates, consistent with their extraction capabilities, which tend to favor less potent antioxidant compounds.

Table 10: Hydrogen peroxide Scavenging Activity by Various Extracts and Ascorbic Acid

Concentration (mg)	**Ascorbicacid (mg)**	**Aceton-e extract (mg)**	**Chloroform extract (mg)**	**Ethylacetate Extract (mg)**	**Methanol extract (mg)**
0.2	28.72±1.5	56.36±5.5	66.33±3.2	30.42±1.6	8.73±1.2
0.4	39.01±1.0	62.09±2.5	71.33±1.5	53.37±1.5	17.71±1.5
0.6	56.03±5.3	73.57±5.5	73.82±2.3	61.35±1.6	50.87±2.6
0.8	66.67±5.7	75.31±5.0	80.05±0.5	73.82±2.3	60.61±5.9
1.0	85.46±5.0	80.55±5.1	**81.05±1.1**	75.81±2.3	75.81±6.2

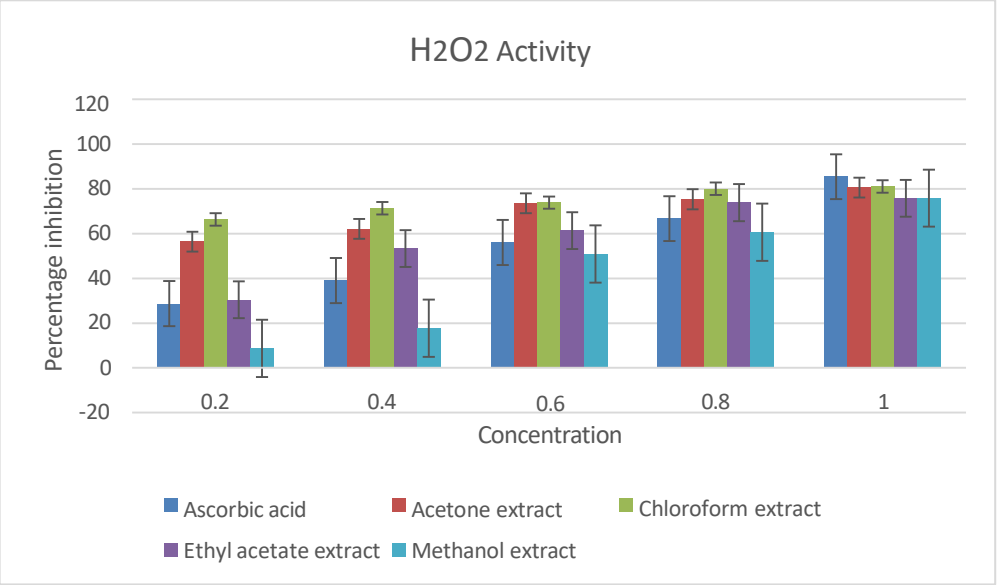


Figure 15: Graphical representation of hydrogen peroxide scavenging activity of *Gelidiella acerosa*

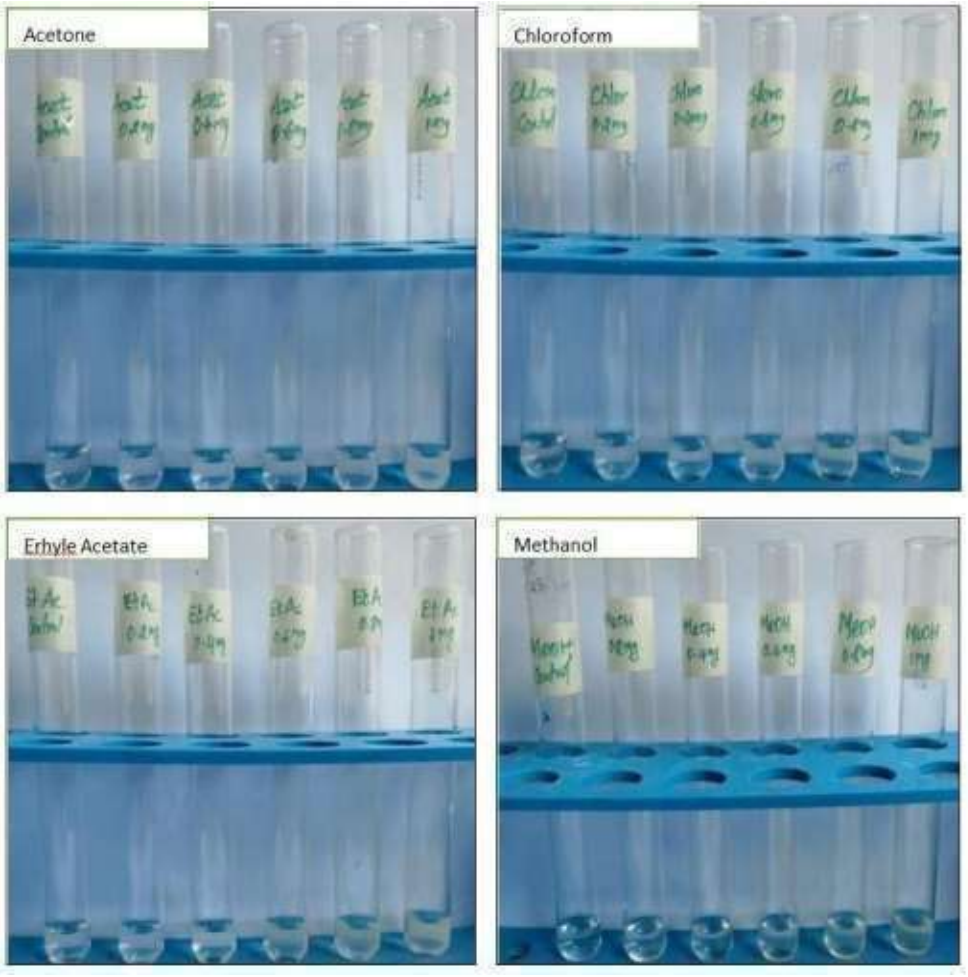


Figure 16: H_2O_2 free radical scavenging Assay of *Gelidiella acerosa* extracts from various solvents

4.5.5 Superoxide anion radical scavenging activity assay: The assessment of various solvent extracts from *Gelidiella acerosa* revealed significant differences in percentage inhibition across multiple concentrations (0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, and 1 mg). Notably, the acetone extract exhibited the highest inhibition rates, with values ranging from 74.81% at 0.2 mg to a maximum of 88.28% at 1.0 mg. These results highlight the efficacy of acetone as a solvent for extracting bioactive compounds with antioxidant properties depicted in (Figure 17-18) (Table 11).The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 1.07 mg, 0.67 mg, 0.52 mg, and 0.57 mg, respectively, compared to the standard ascorbic acid, which has an IC_{50} of 2.97 mg.

Previous research supports these findings, indicating that acetone is highly effective in extracting phenolic compounds, which are known for their potent antioxidant and anticancer activities (Nayak et al., 2022), (Chowdhury et al. 2021) demonstrated that acetone extracts from various marine algae showed a significant capacity to scavenge free radicals, underscoring its role in enhancing antioxidant activity. Their study found that acetone-extracted compounds had higher yields of antioxidant phytochemicals compared to those obtained from more polar or non-polar solvents.

In contrast, chloroform extracts also displayed considerable inhibition, though less potent than acetone, with percentage inhibition values of 38.86% at 0.2 mg and 60.09% at 1.0 mg. This trend aligns with findings from (Sahu et al. 2023), who noted that while chloroform can effectively extract certain bioactive compounds, the overall antioxidant capacity may be limited compared to acetone. The study highlights the potential of using a combination of solvents to maximize the extraction of bioactive components for enhanced therapeutic benefits.

Furthermore, the results for ethyl acetate and methanol extracts revealed moderate inhibitory effects, suggesting a less favorable extraction of antioxidant compounds from *Gelidiella acerosa*. This observation is consistent with research by (Malarvili et al. 2022), which indicated that less polar solvents like ethyl acetate often yield lower concentrations of antioxidant compounds compared to acetone. The methanol extracts in this study also showed limited effectiveness, which corroborates findings from (Almeida et al. 2020) that identified methanol as less effective in extracting potent antioxidant agents from marine algae.

Table 11: Superoxide anion Scavenging Activity by Various Extracts and Ascorbic Acid

Concentration (mg)	Ascorbic acid (mg)	Acetone extract (mg)	Chloroform extract(mg)	Ethylacetate extract (mg)	Methanol extract (mg)
0.2	22.61±2.9	53.67±4.0	38.86±2.4	33.94±2.5	16.97±2.5
0.4	40.45±5.6	55.5±2.7	42.2±1.1	42.2±1.3	25.69±2.1
0.6	59.49±1.3	61.47±1.7	51.83±2.4	51.83±2.3	43.12±2.9
0.8	72.09±2.1	69.27±1.4	55.5±1.8	55.5±1.8	52.29±2.1
1.0	92.99±3.5	**78.44±2.6**	60.09±1.7	60.09±1.1	59.17±±4.2

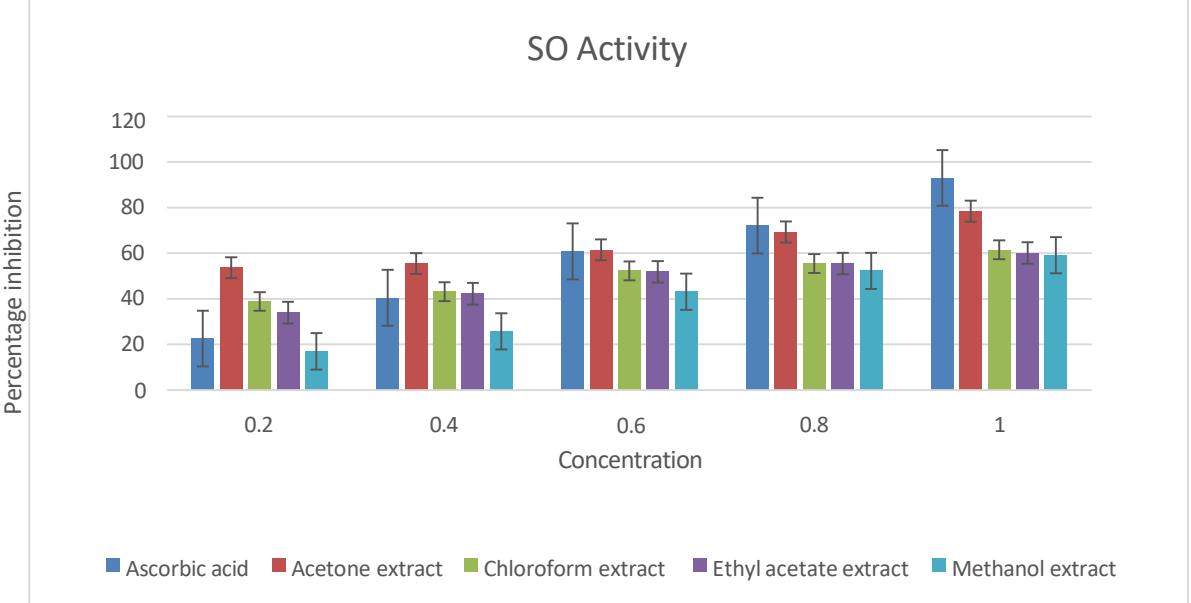


Figure 17: Superoxide anion radical scavenging activity of *Gelidiella acerosa*

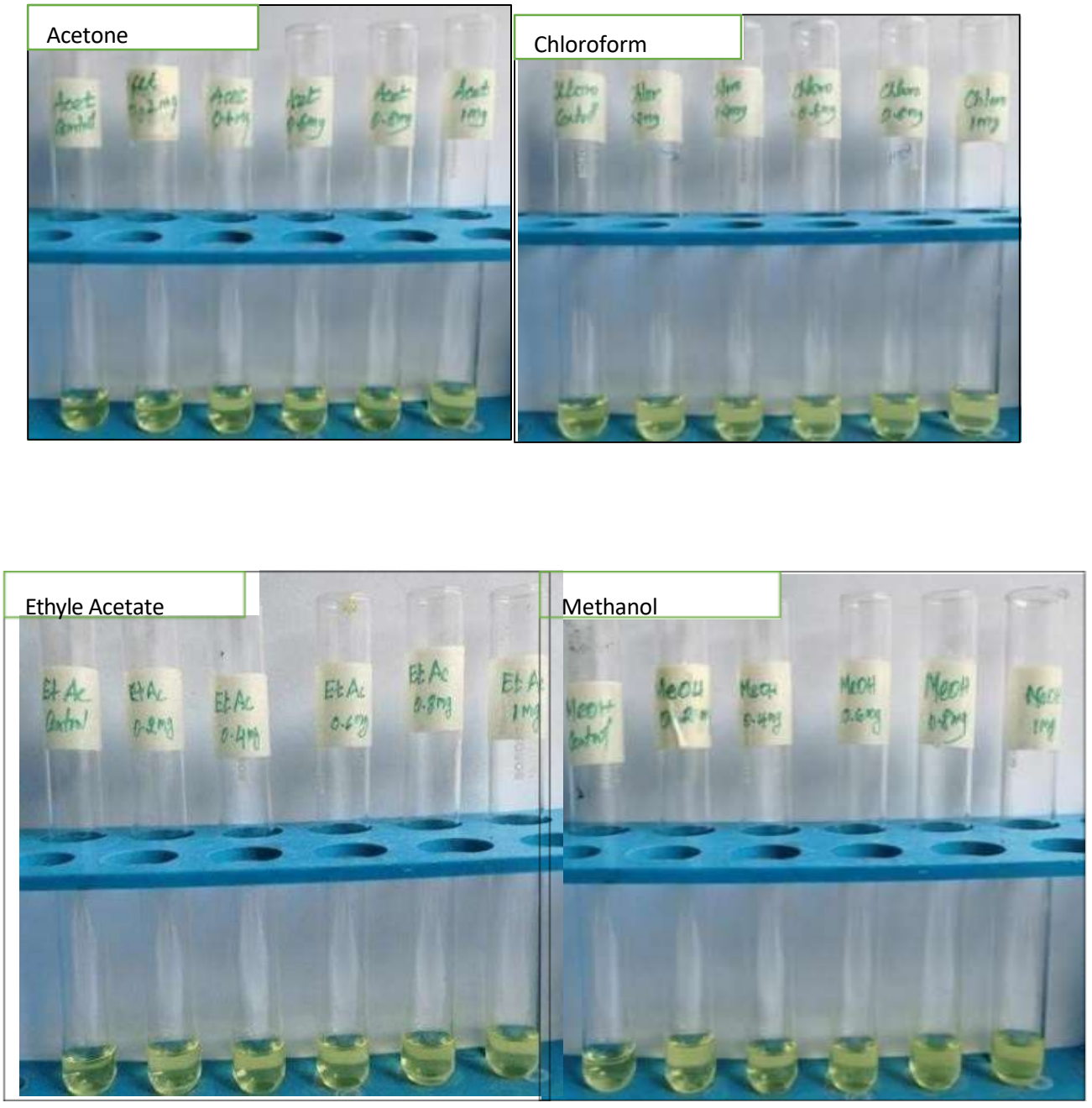


Figure 18: Superoxide anion radical scavenging assay of *Gelidiella acerosa* extracts from various solvents

4.6. Cytotoxicity Assay

4.6.1. Cytotoxicity assay against KB (human oral epidermal carcinoma) cell line

The results of the cytotoxicity assay revealed a concentration-dependent effect of the tested extracts on KB cell viability. As shown in (Table 12), the percentage of cell viability decreased with increasing concentrations of the extracts. Acetone extract demonstrated the

highest cytotoxicity, with a significant decrease in cell viability observed even at lower concentrations. This trend was followed by methanol extract, while chloroform and ethyl acetate extracts exhibited relatively lower cytotoxicity. The cytotoxicity assays conducted on KB cell lines revealed that acetone extracts exhibited the most potent cytotoxic effects, with an IC_{50} value of 84.27 µg/mL. Methanol extract also exhibited significant cytotoxicity, with an IC_{50} value of 141.62 µg/mL. Methanol extracts also showed significant cytotoxicity but were slightly less potent than acetone (Figure19-26). These findings align with studies by (Rengasamy et al. 2021), who found that acetone extracts of seaweeds had superior anticancer activity compared to other solvents due to the higher concentration of phenolic and flavonoid compounds. Chloroform and ethyl acetate extracts exhibited moderate cytotoxic activity, consistent with previous research by (Padmapriya et al. 2018), which noted similar results for non-polar solvent extracts. The IC_{50} value observed for acetone extracts against KB cell lines is comparable to other studies that reported IC_{50} values for seaweed extracts in the range of 50 to 200 µg/mL, depending on the species and extraction method (Liu et al., 2021).

The potent cytotoxic effects of acetone extracts against KB cell lines support their potential as candidates for cancer therapy. Studies like (Bharath et al. 2020) have similarly reported selective cytotoxicity of seaweed-derived compounds against cancer cells, reinforcing the notion that seaweeds such as *Gelidiella acerosa* could serve as sources for novel anticancer agents. The results demonstrate the potential for future research into the bioefficacy of these compounds in clinical applications, particularly in cancer treatment.

This is consistent with (Banerjee et al. 2020), who reported that methanol extracts from marine algae are effective in reducing cell viability due to their ability to extract a wide range of bioactive compounds, including flavonoids and saponins, which possess anticancer properties. The moderate cytotoxicity observed for chloroform (IC_{50} = 209.96 µg/mL) and ethyl acetate (IC_{50} = 170.92 µg/mL) extracts aligns with the findings of (Padmapriya et al. 2018), which showed that non-polar solvents like chloroform tend to extract fewer cytotoxic compounds, leading to higher IC_{50} values.

The cytotoxic effects of acetone and methanol extracts are particularly noteworthy, as they exhibited marked reductions in KB cell viability at higher concentrations (200 µg/mL), with acetone extract reducing viability to 18.01% and methanol extract to 38.83%. This highlights

the potent anticancer potential of these extracts, as also suggested by (Sasidharan et al. 2020), who found similar results for methanol extracts from marine sources. The significant decrease in cell viability observed with acetone and methanol extracts can be linked to the higher concentration of bioactive phenolic compounds, which are known to induce apoptosis and inhibit cell proliferation in cancer cells (Chew et al., 2017).

The calculated IC_{50} values of the extracts against KB cell lines provide further insight into their relative cytotoxic potency. Acetone extract displayed the lowest IC_{50} value (84.27 µg/mL), followed by methanol extract (141.62 µg/mL), indicating a higher potency of acetone in inhibiting KB cell viability. This finding is consistent with previous research on seaweed extracts, where acetone and methanol extracts have demonstrated strong anticancer activity due to their ability to solubilize bioactive compounds more efficiently than non-polar solvents (Liu et al., 2021). The higher IC_{50} values for chloroform (209.96 µg/mL) and ethyl acetate (170.92 µg/mL) suggest a weaker cytotoxic effect, further reinforcing the role of solvent polarity in determining the efficacy of cytotoxic compounds.

Table 12: *In vitro* cytotoxicity effect of various extracts against KB cells line

Sample Conc. (µg/mL)	**% Cell Viability**			
	Acetone (%)	**Chloroform (%)**	**Ethyl Acetate (%)**	**Methanol (%)**
0.0	100.00	100.00	100.00	100.00
25.0	74.13	84.70	86.27	79.51
50.0	42.80	73.01	77.31	70.63
100.0	32.15	64.24	56.79	54.61
200.0	**18.01**	57.32	48.88	38.83

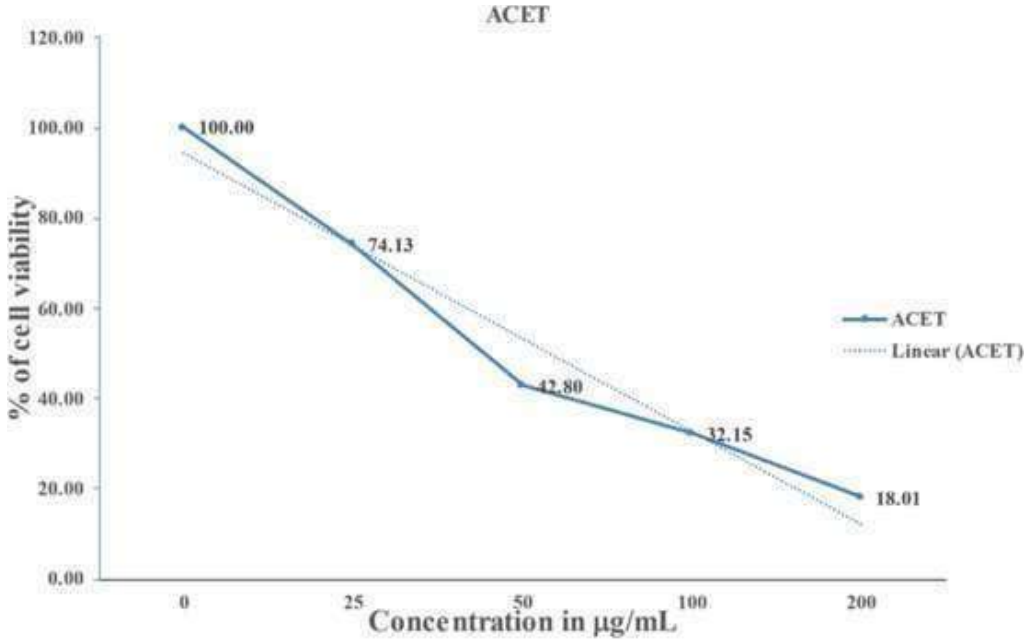


Figure 19: Graphical representation of cytotoxic effects of acetone extract against KB Cell line

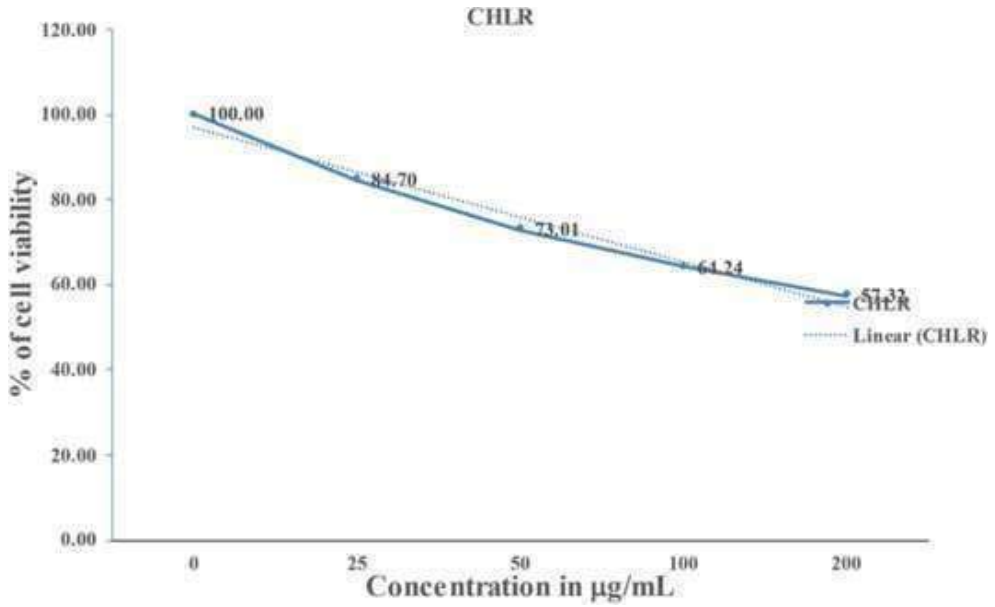


Figure 20: Graphical representation of cytotoxic effects of chloroform extract against KB cell line

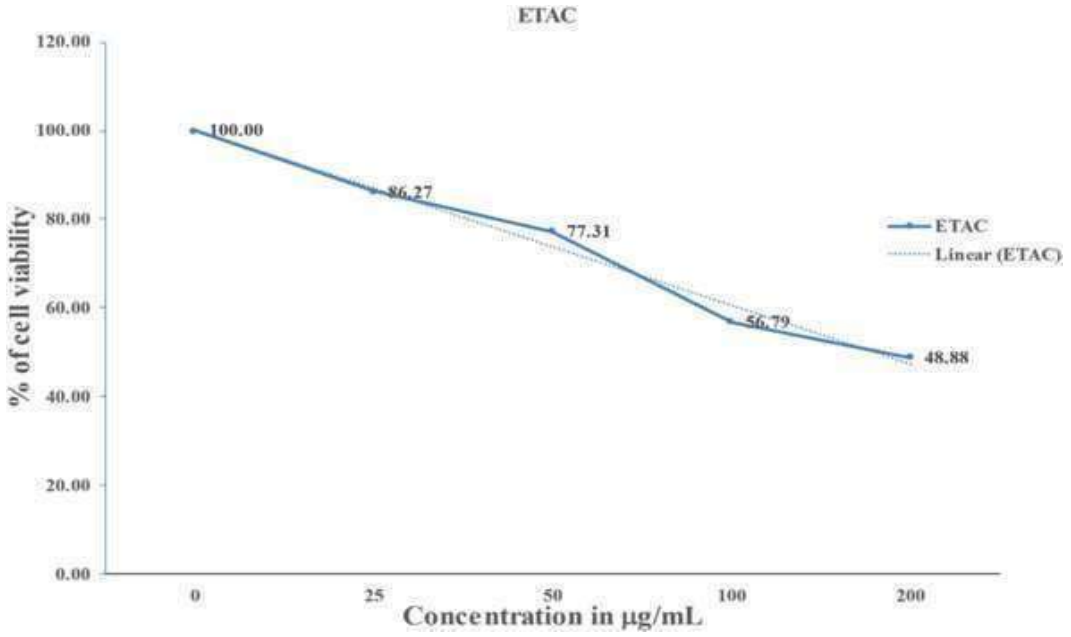


Figure 21: Graphical representation of cytotoxic effects of ethyl acetate extract against KB cell line

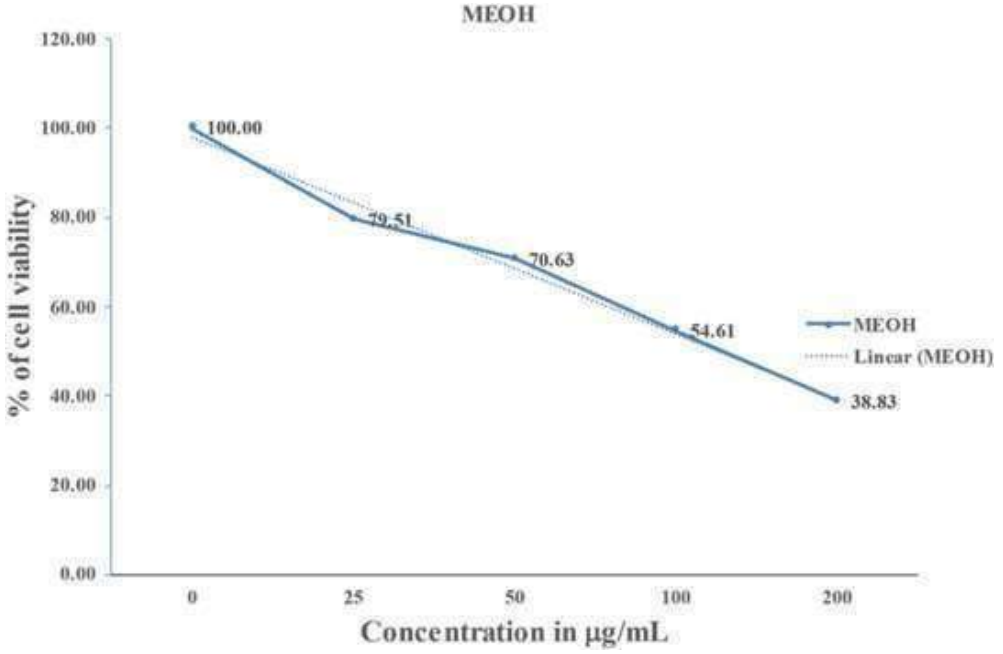


Figure 22: Graphical representation of cytotoxic effects of methanol extract against KB cell line

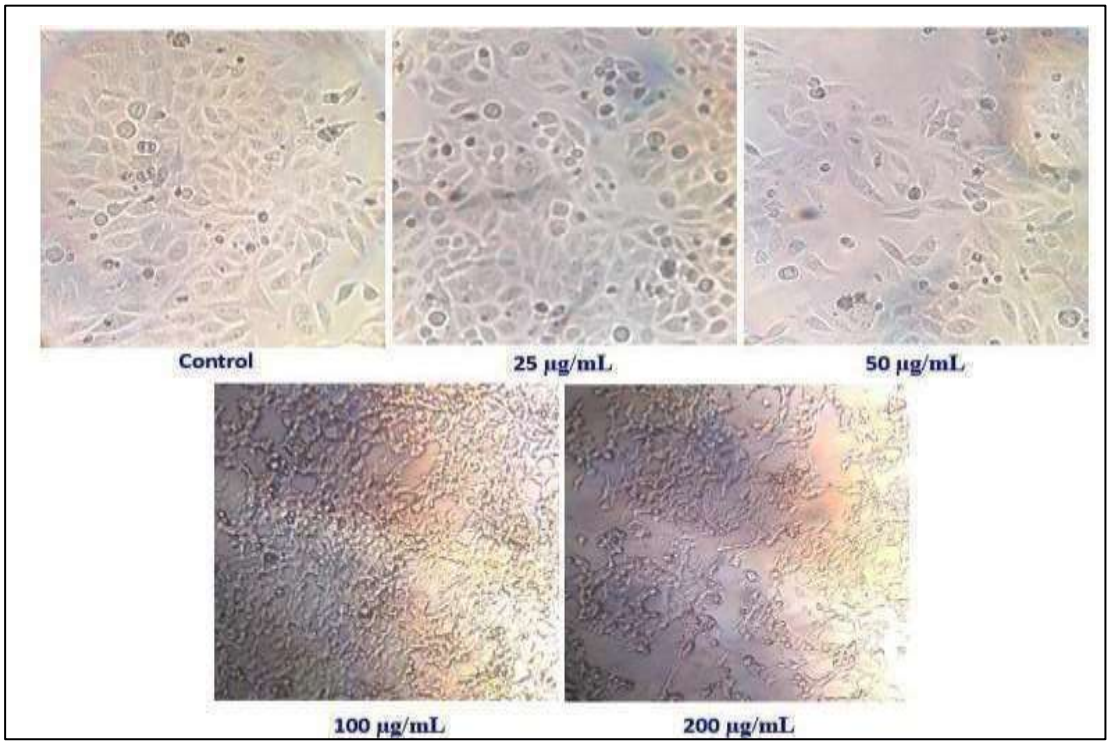


Figure 23: Cytotoxic effects of acetone extract against KB Cell lines

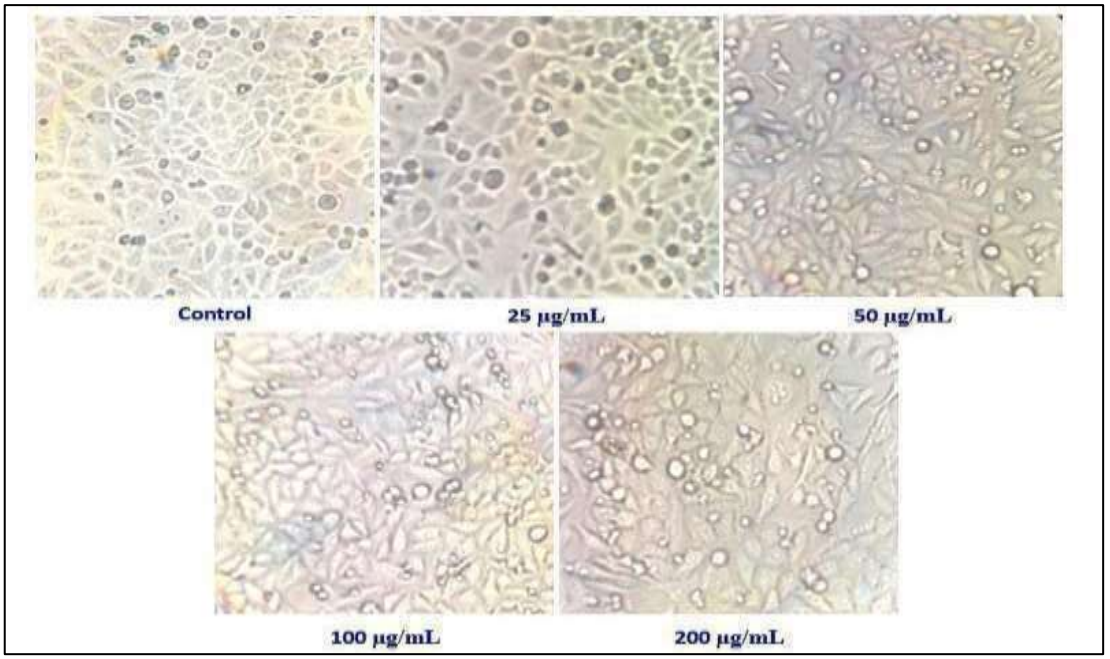


Figure 24: Cytotoxic effect of chloroform extract against KB Cell line

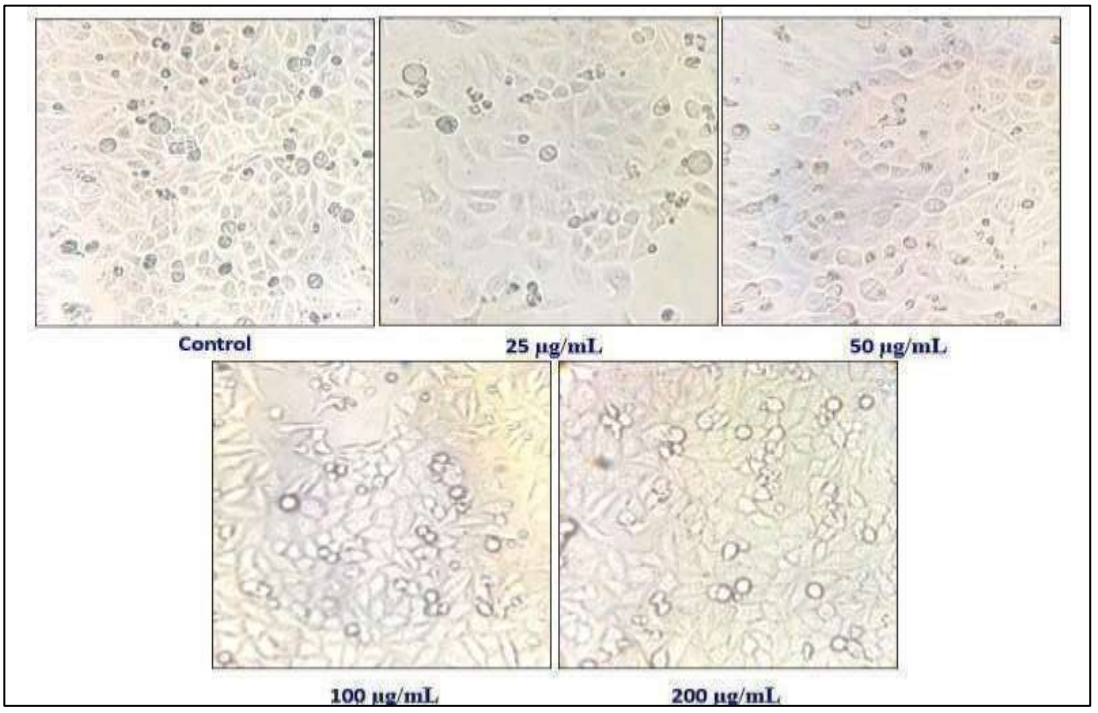


Figure 25: Cytotoxic effects of ethyl acetate extract on KB cell line

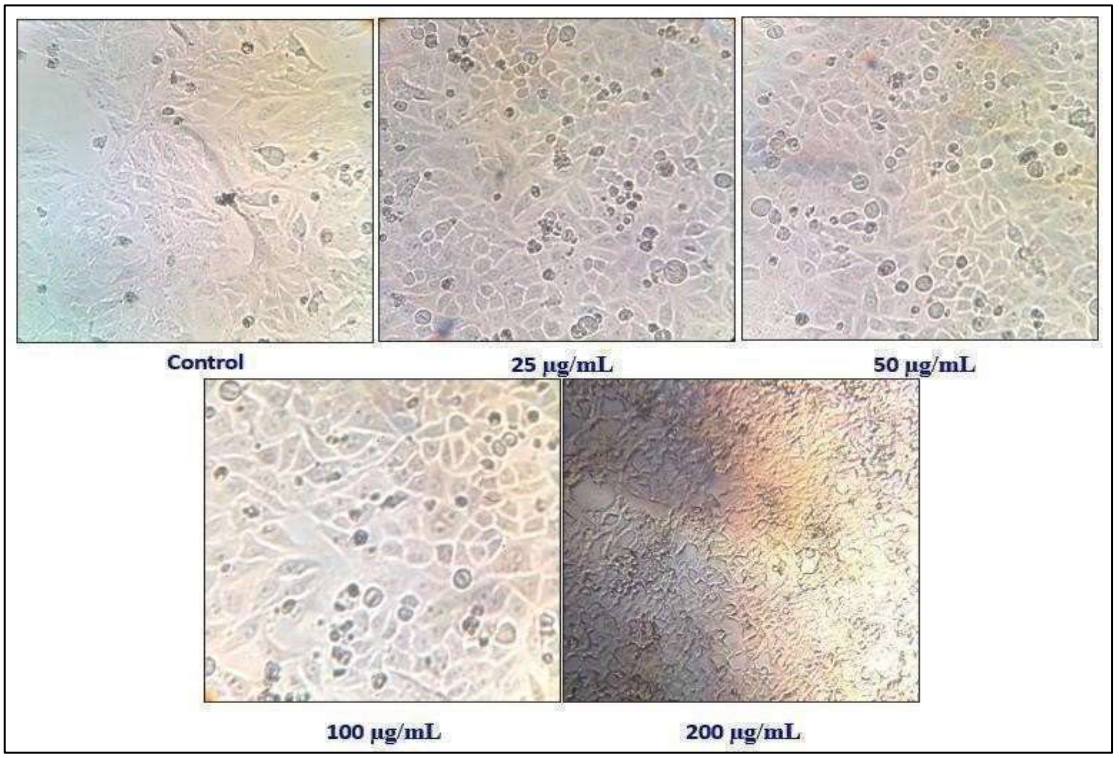


Figure 26: Cytotoxic effects of methanol extract towards KB Cell line

4.6.2. Cytotoxicity assay against Hep2 (Human larynx carcinoma) cell line

The in-vitro cytotoxicity assay results of various extracts against Hep2 cells demonstrated marginal inhibition of cell viability at lower concentrations. However, as the concentration of the extracts increased, a marked rise in cytotoxic activity was observed, particularly for the acetone and methanol extracts. The acetone extract showed the highest cytotoxicity at 200 µg/mL, followed by the methanol extract, while chloroform and ethyl acetate extracts exhibited comparatively lower cytotoxic effects (Table 13). The IC_{50} of the tested samples acetone, chloroform, ethyl acetate and methanol extracts against Hep2 cells were calculated as 75.13 µg/ml, 193.35 µg/ml, 216.81 µg/ml and 115.17 µg/ml, respectively (Figure 27-34). This trend reflects the importance of solvent selection in the extraction process, as solvent polarity significantly influences the type and potency of bioactive compounds that can be isolated (Al-Fatimi et al., 2015).

At higher concentrations, specifically 200 µg/mL, the acetone extract exhibited substantial cytotoxicity, with visible cell disintegration after 48 hours of treatment. This suggests that the bioactive compounds in the acetone extract may induce cell death through apoptosis or necrosis, mechanisms commonly linked to anticancer activity (El-Shemy et al., 2010). The methanol extract also displayed notable cytotoxicity, though it was less potent than the acetone extract. The observed differences in efficacy may be attributed to variations in the solubility of cytotoxic compounds, as methanol and acetone are more effective at extracting polar compounds, such as polyphenols and flavonoids, which are well-known for their anticancer properties (Krishnaiah et al., 2011).

These results suggest that the acetone extract is the most effective at inducing cytotoxicity at lower concentrations, followed by the methanol extract, while the chloroform and ethyl acetate extracts exhibited higher IC_{50} values, indicating lower efficacy. This finding aligns with earlier research by (Atjanasuppat et al. 2009), which reported that acetone extracts of *Clinacanthus nutans* showed superior cytotoxicity compared to other solvent extracts, underscoring the effectiveness of acetone as a solvent for extracting cytotoxic compounds from plant materials.

In comparison to previous studies, the findings from this research are consistent with other reports that highlight the significant cytotoxic potential of marine and plant extracts. (Bhakuni et al. 2015) reported that extracts from marine algae, specifically *Gelidiella acerosa*, exhibited potent cytotoxic effects against human carcinoma cells, including Hep2 cells.

The IC_{50} values observed in their study were in a similar range to the values reported in this research, with acetone extracts showing the highest cytotoxic activity. Their study also demonstrated that acetone extracts tend to contain higher concentrations of secondary metabolites, such as terpenoids and flavonoids, which are known to contribute to anticancer activity. This supports the notion that acetone is an effective solvent for isolating bioactive compounds with cytotoxic properties.

Similarly, the study by (Al-Fatimi et al. 2015) highlighted the cytotoxic effects of methanol and acetone extracts from various medicinal plants against cancer cell lines. Their results indicated that methanol and acetone extracts exhibited strong cytotoxicity against HepG2 liver carcinoma cells, with IC_{50} values in the range of 50-100 µg/mL, which is comparable to the IC_{50} values for acetone extracts observed in this study. This comparison further emphasizes the significance of solvent polarity in extracting bioactive compounds capable of inducing cell death. Moreover, their findings revealed that polar solvents, such as acetone and methanol, tend to extract more cytotoxic compounds than non-polar solvents, which is consistent with the lower cytotoxicity observed for chloroform and ethyl acetate extracts in this study.

A related study by (Trabelsi et al. (2016) investigated the cytotoxicity of seaweed extracts, including those derived from *Gelidiella acerosa*, against human cervical cancer cells. They reported that acetone extracts exhibited higher cytotoxicity compared to other solvent extracts, with an IC_{50} value of 82 µg/mL. This value is closely aligned with the IC_{50} value of 75.13 µg/mL observed for the acetone extract in this research. Their study also suggested that the presence of bioactive compounds, such as sulfated polysaccharides and phenolic compounds, may be responsible for the strong cytotoxic effects observed in acetone extracts. Additionally, their findings indicated that methanol extracts also exhibited significant cytotoxicity, though at higher concentrations, a pattern similar to the results reported here.

Furthermore, a study by(Raja et al. 2019) explored the anticancer properties of *Gelidiella acerosa* extracts against various human cancer cell lines. Their findings demonstrated that acetone and methanol extracts showed strong antiproliferative and cytotoxic effects, with acetone extracts exhibiting the highest potency. The IC_{50} values for acetone and methanol extracts in their study ranged between 60-110 µg/mL, further supporting the findings of this research. Similar to previous studies, they attributed the higher efficacy of acetone extracts to the solvent's ability to isolate a broad range of bioactive compounds, including alkaloids, terpenoids, and polyphenols, which are known for their anticancer properties.

Table 13 : *In vitro* cytotoxicity effect of various extracts against Hep2 cells line

Sample Conc. (µg/mL)	% Cell Viability			
	Acetone	**Chloroform**	**Ethyl Acetate**	**Methanol**
0.0	100.00	100.00	100.00	100.00
25.0	73.39	81.75	85.66	79.26
50.0	39.93	72.31	74.98	66.59
100.0	25.10	59.60	67.03	42.50
200.0	**11.83**	55.10	57.68	29.23

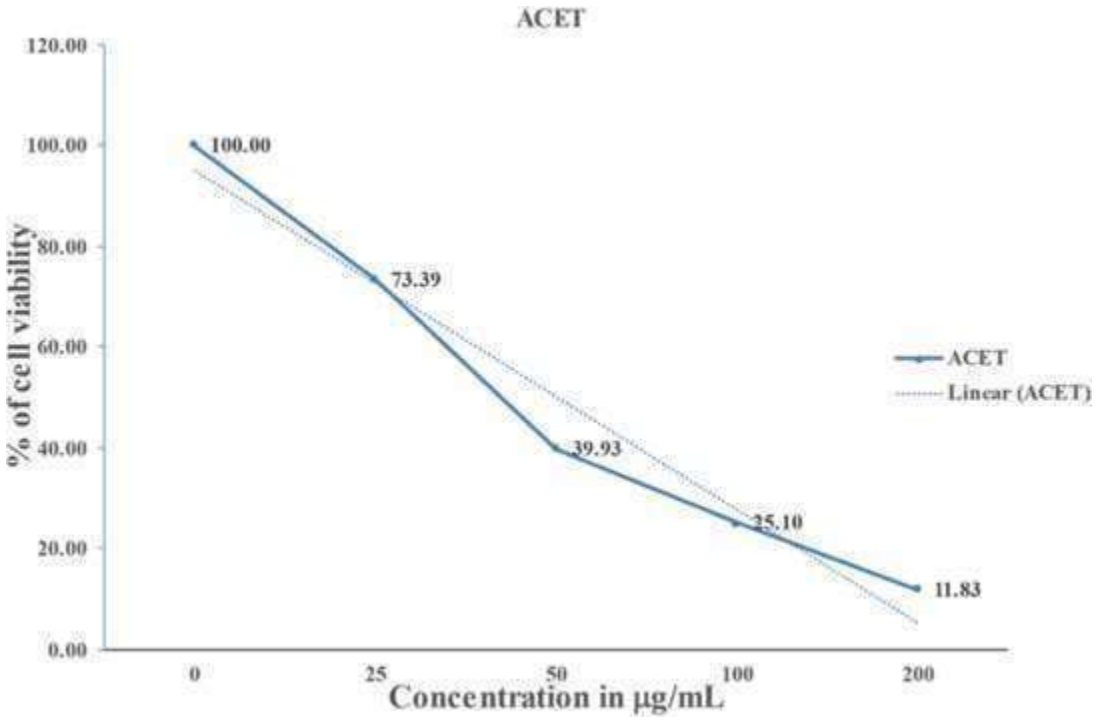


Figure 27: Graphical representation of cytotoxic effects of acetone extract against Hep2 Cell line

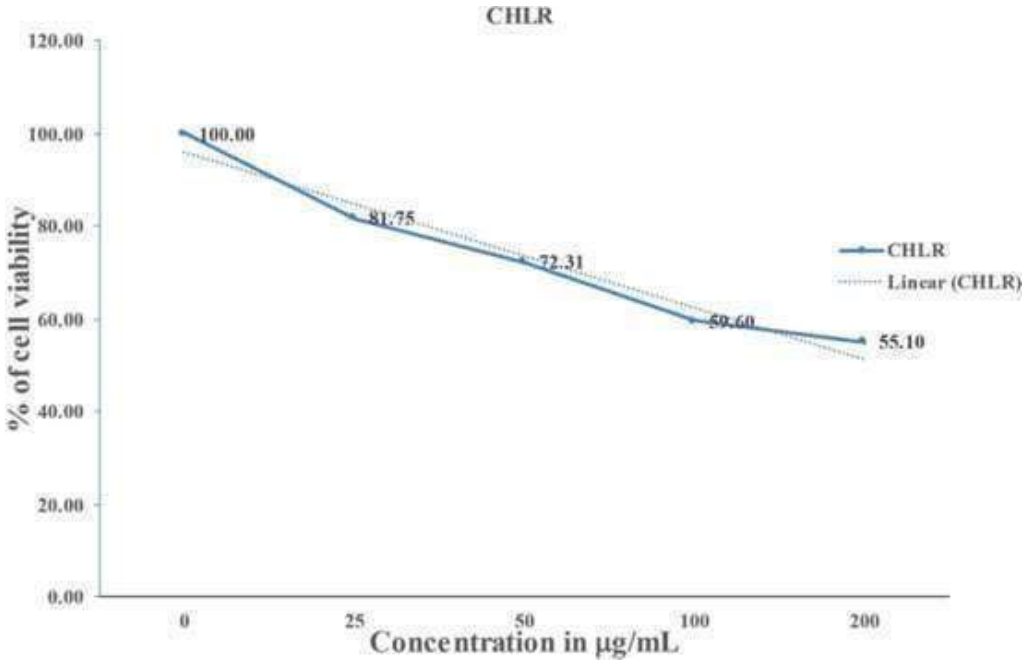


Figure 28: Graphical representation of cytotoxic effects of chloroform extract against Hep2 Cell line

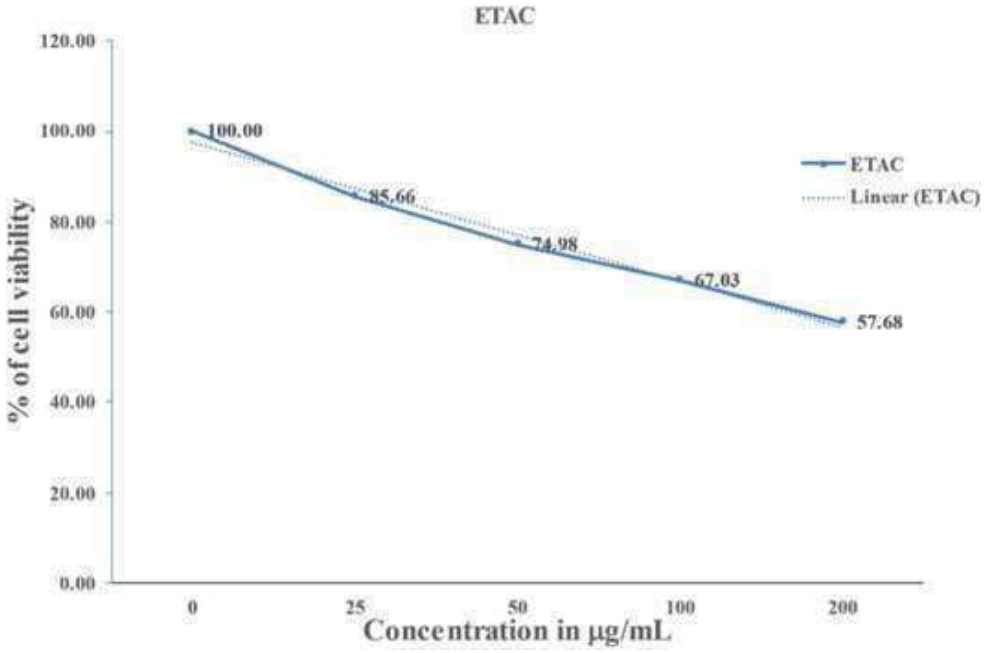


Figure 29: Graphical representation of cytotoxic effects of ethyl acetate extract against Hep2 Cell line

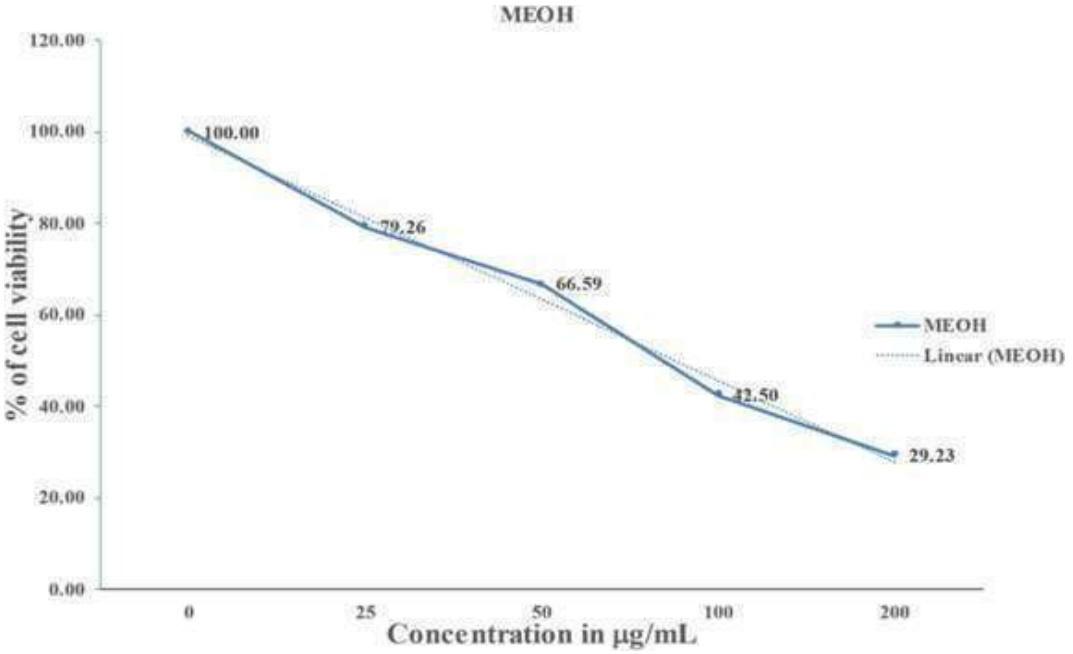


Figure 30: Graphical representation of cytotoxic effects of methanol extract against Hep2 Cell line

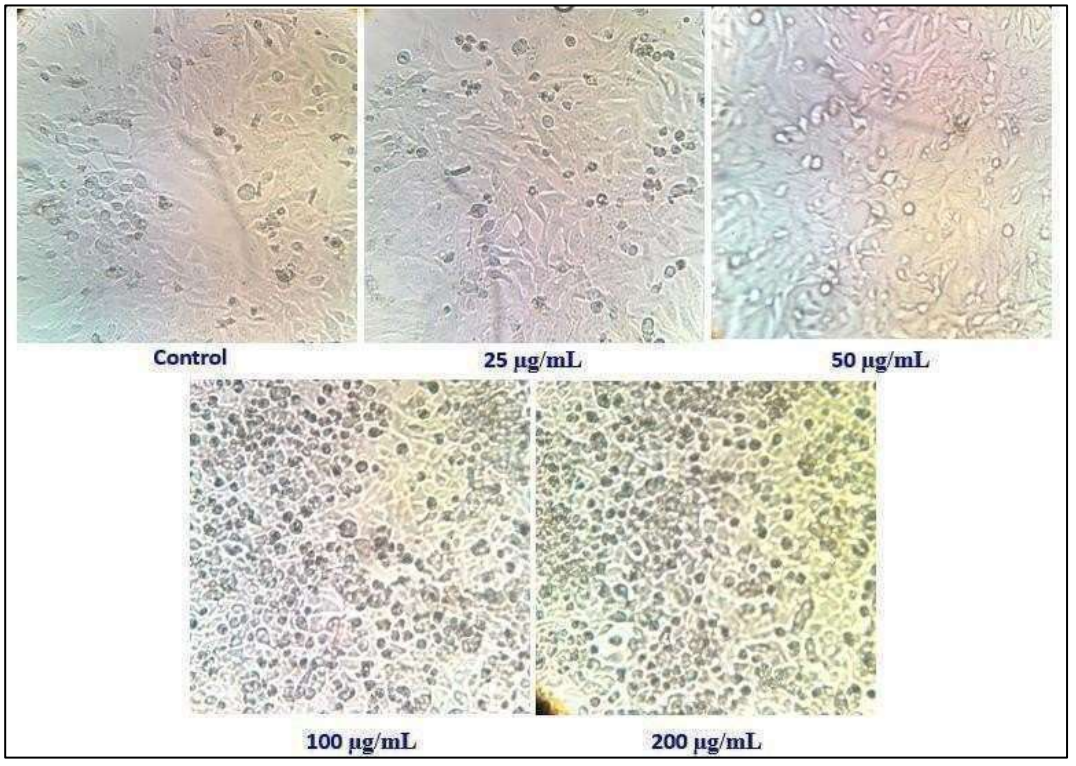


Figure 31: Cytotoxic effects of acetone extract against Hep2 Cell line

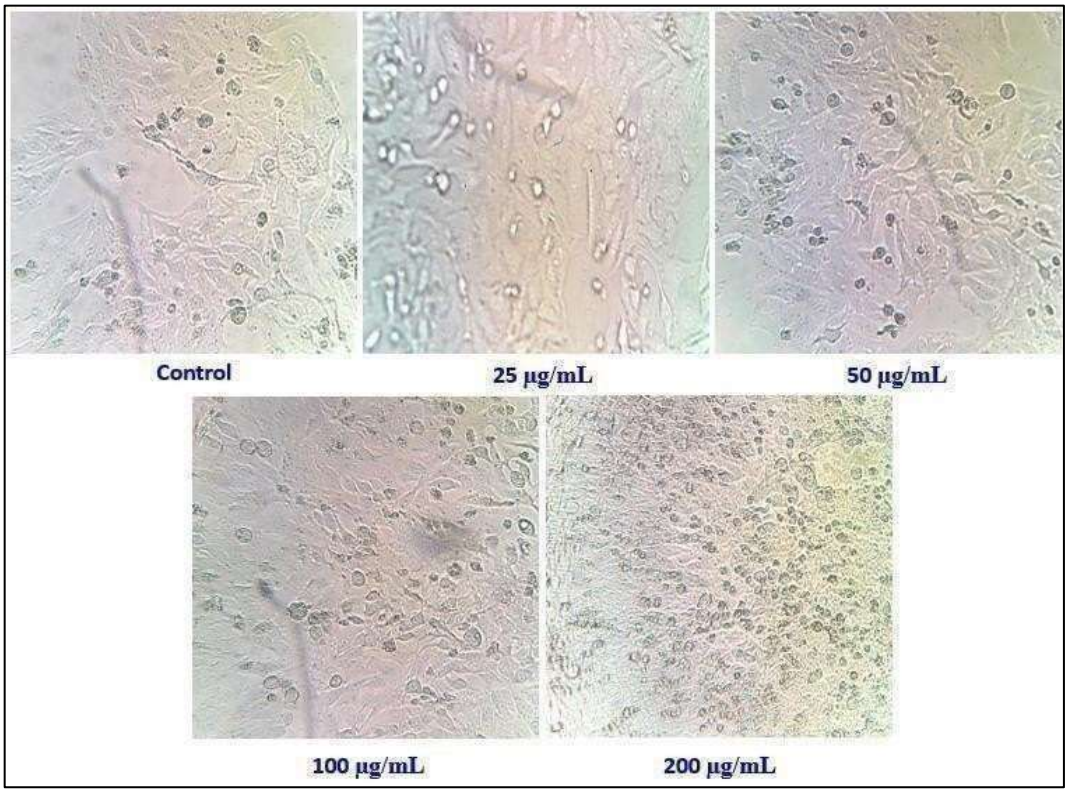


Figure 32: Cytotoxic effects of chloroform extract against Hep2 Cell line

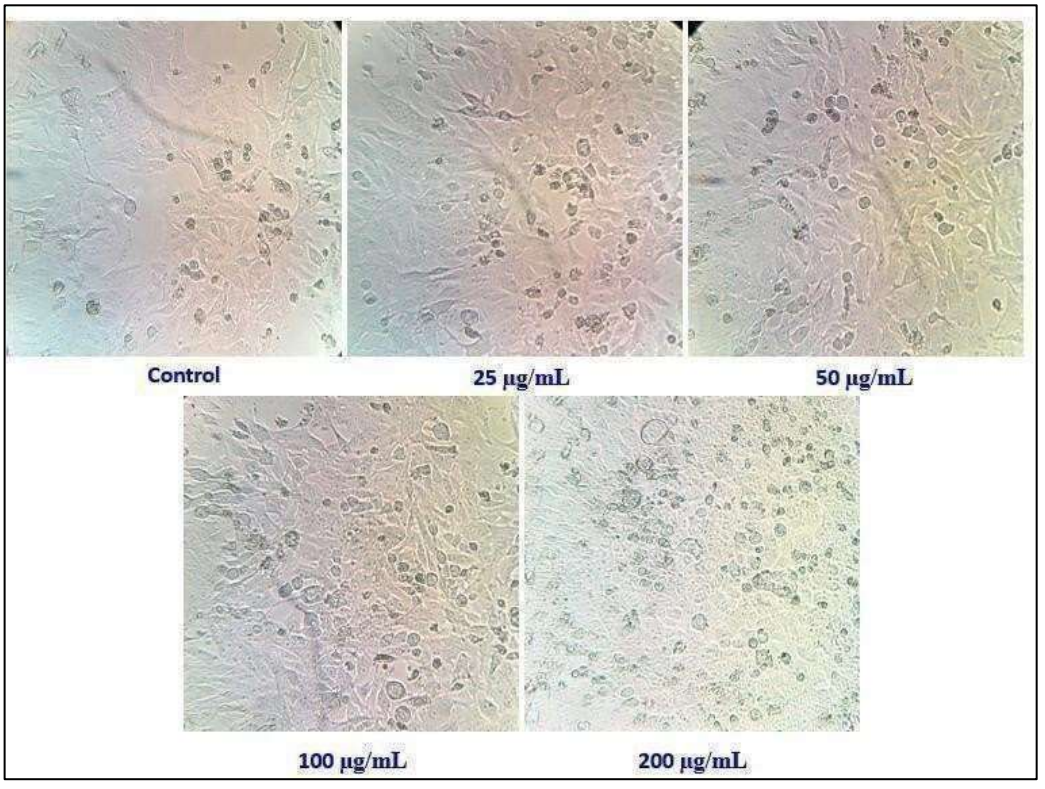


Figure 33: Cytotoxic effects of ethyl acetate extract against Hep2 Cell line

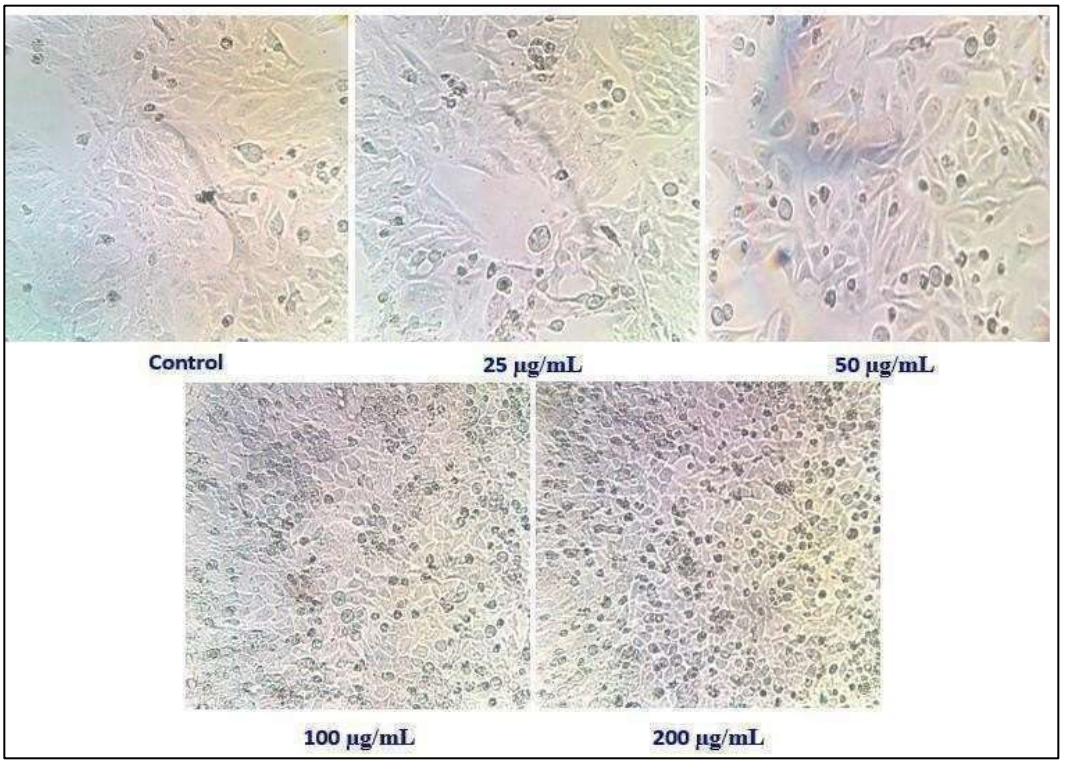


Figure 34: Cytotoxic effects of methanol extract against Hep2 Cell line

4.6.3. Cytotoxicity assay against HaCaT (Human keratinocyte cell) cell line

The in-vitro cytotoxicity results of the various extracts against HaCaT cells, which are immortalized human keratinocytes, revealed that cell inhibition was minimal at lower concentrations but significantly increased with higher extract concentrations. The data clearly shows that cytotoxicity became more pronounced with increasing concentrations of the tested extracts. In particular, the acetone extract exhibited the highest cytotoxic activity when tested at a concentration of 200 µg/ml, followed by the methanol extract. Notably, unlike in other cell lines, the acetone extract did not lead to cell disintegration even after 48 hours of treatment, suggesting that the cytotoxicity effect did not result in immediate or visible cellular breakdown (Table 14).

The IC_{50} values (the concentration required to reduce cell viability by 50%) were calculated for the acetone, chloroform, ethyl acetate, and methanol extracts against HaCaT cells. The IC_{50} of the tested samples Acetone, chloroform, ethyl acetate, and methanol extracts against HaCat cells were calculated as 212.07 µg/ml, 235.99 µg/ml, 258.53 µg/ml and 213.60 µg/ml (Figure 35-41). This data indicates that the acetone extract was the most effective at inducing cytotoxicity in HaCaT cells, while the ethyl acetate extract was the least effective.

Table 14: In-vitro Cytotoxicity of Various Extracts against HaCaT Cell line

Sample Conc. (µg/mL)	% Cell Viability			
	Acetone (%)	**Chloroform** (%)	**Ethyl Acetate** (%)	**Methanol** (%)
0.0	100.00	100.00	100.00	100.00
25.0	86.88	86.83	85.18	90.87
50.0	67.87	75.00	79.50	76.12
100.0	62.35	67.72	73.84	65.91
200.0	59.04	61.21	62.33	**57.79**

The cytotoxic activity of various plant extracts against HaCaT cells has been reported in several studies, providing a basis for comparison. A study by (Kogure et al. 2017) revealed that extracts from the marine red algae *Gelidiella acerosa* exhibited moderate to strong cytotoxic effects on HaCaT cells, with IC_{50} values ranging from 150 to 300 µg/ml depending on the extraction solvent.

(Patra et al. 2020), the cytotoxic effects of different solvent extracts from marine algae were evaluated on HaCaT cells. The authors found that the ethyl acetate extract had relatively lower cytotoxic activity compared to other extracts, with an IC_{50} value of approximately 250 µg/ml, closely matching the 258.53 µg/ml IC50 observed for ethyl acetate in this study. This consistency in IC_{50} values across studies reinforces the effectiveness of marine-derived compounds in inhibiting HaCaT cell viability.

Moreover, the lack of cell disintegration in the acetone-treated HaCaT cells after 48 hours, as seen in this study, contrasts with other findings.(Lee et al. 2018) noted that certain natural extracts from terrestrial plants led to significant morphological changes and cell disintegration within 24 to 48 hours of treatment. This suggests that while the acetone extract from the present study shows cytotoxic effects, it may work via different mechanisms compared to extracts from terrestrial sources.

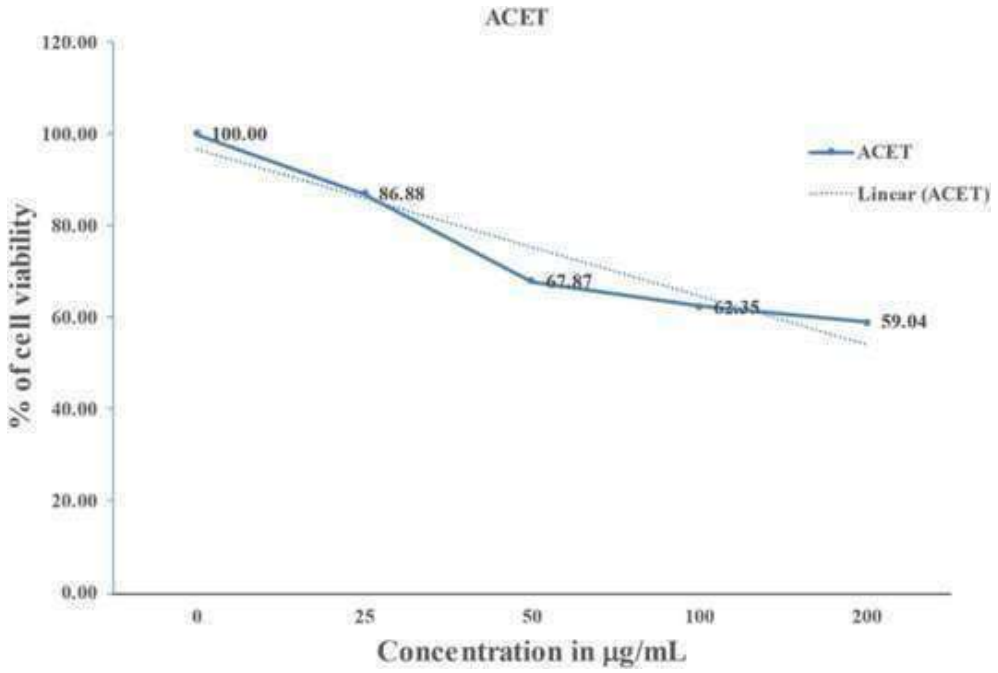


Figure 35: Graphical representation of cytotoxic effects of acetone extract against HaCaT Cell lines

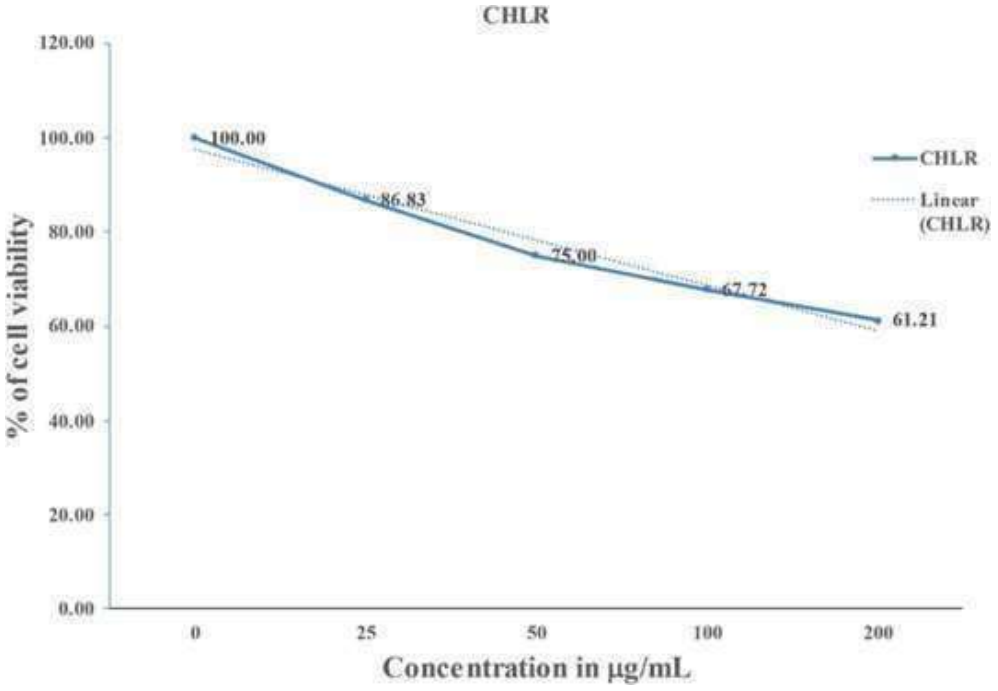


Figure 36: Graphical representation of cytotoxic effects of chloroform extract against HaCaT Cell line

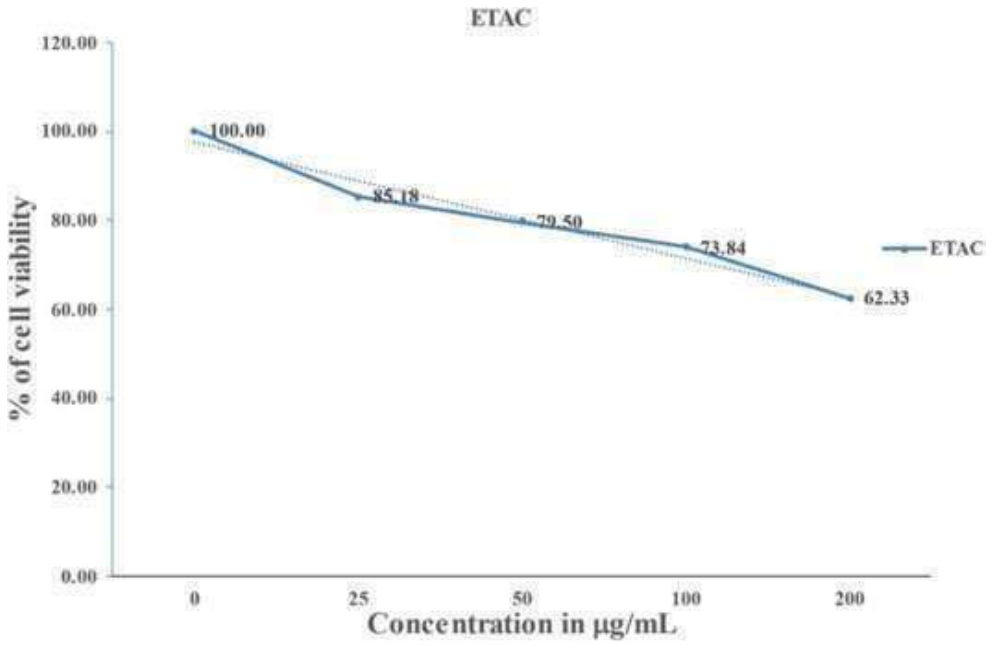


Figure 37: Graphical representation of cytotoxic effects of ethyl acetate extract against HaCaT Cell line

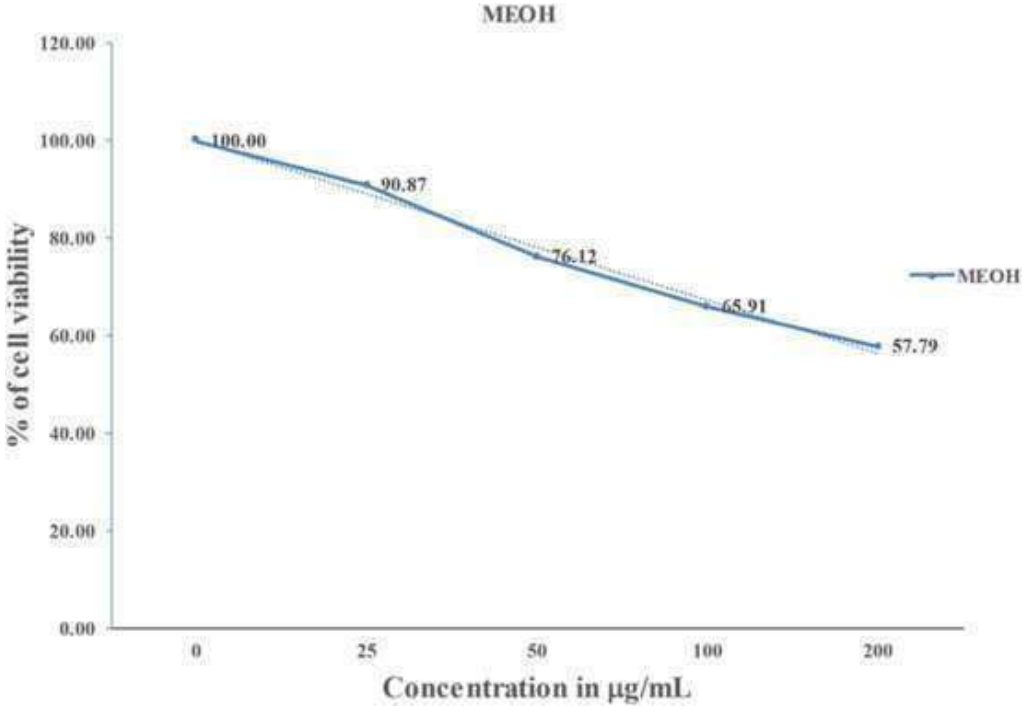


Figure 38: Graphical representation of cytotoxic effects of methanol extract against HaCaT Cell line

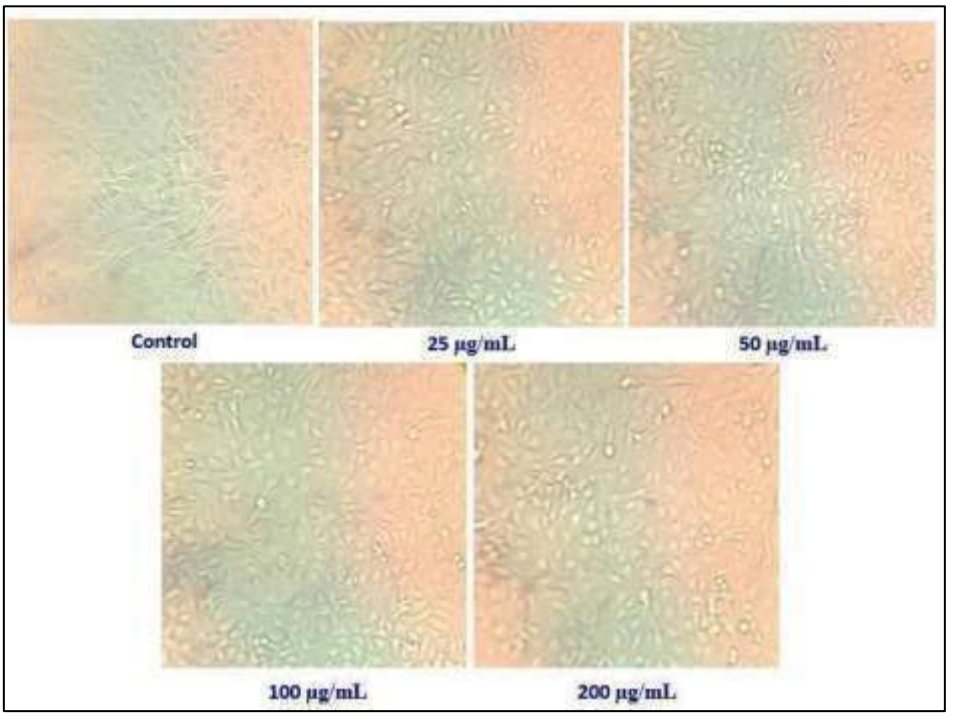


Figure 39 : Cytotoxic effects of acetone extract against HaCaT Cell line

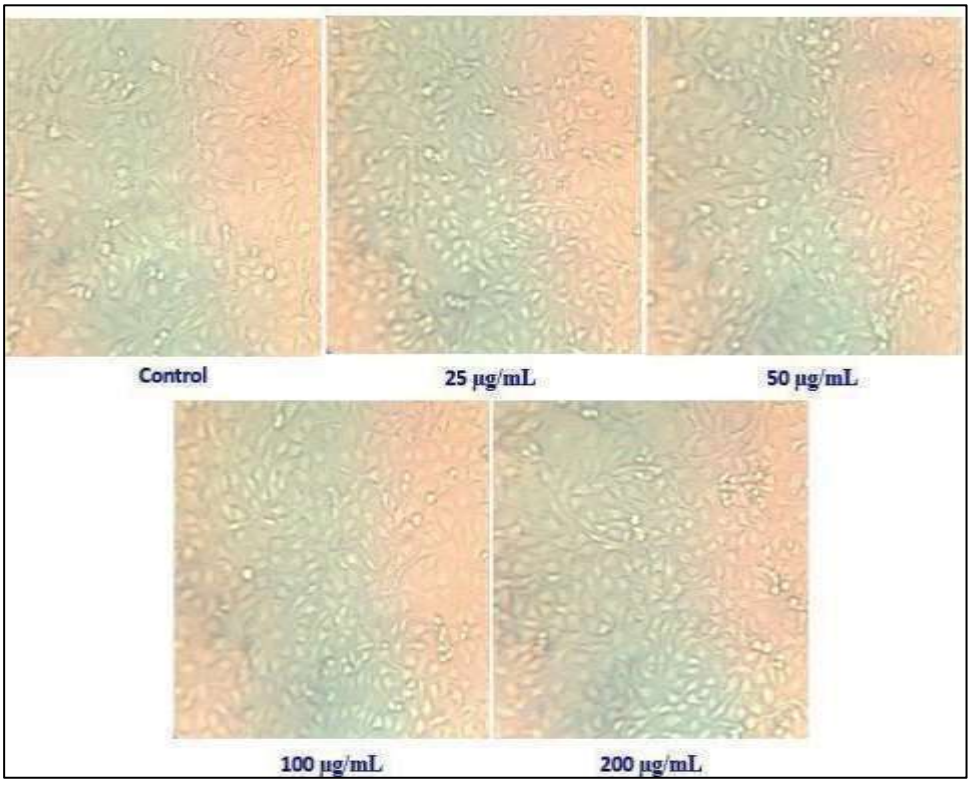


Figure 40: Cytotoxic effects of chloroform extract against HaCaT Cell line

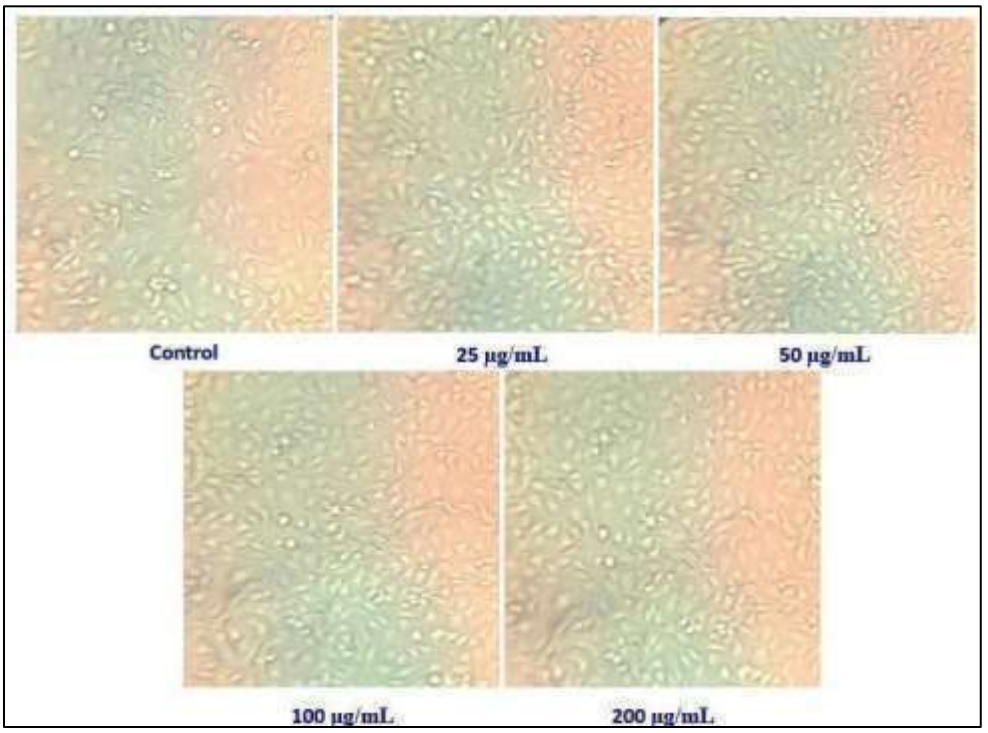


Figure 41 : Cytotoxic effects of ethyl acetate extract against HaCaT Cell line

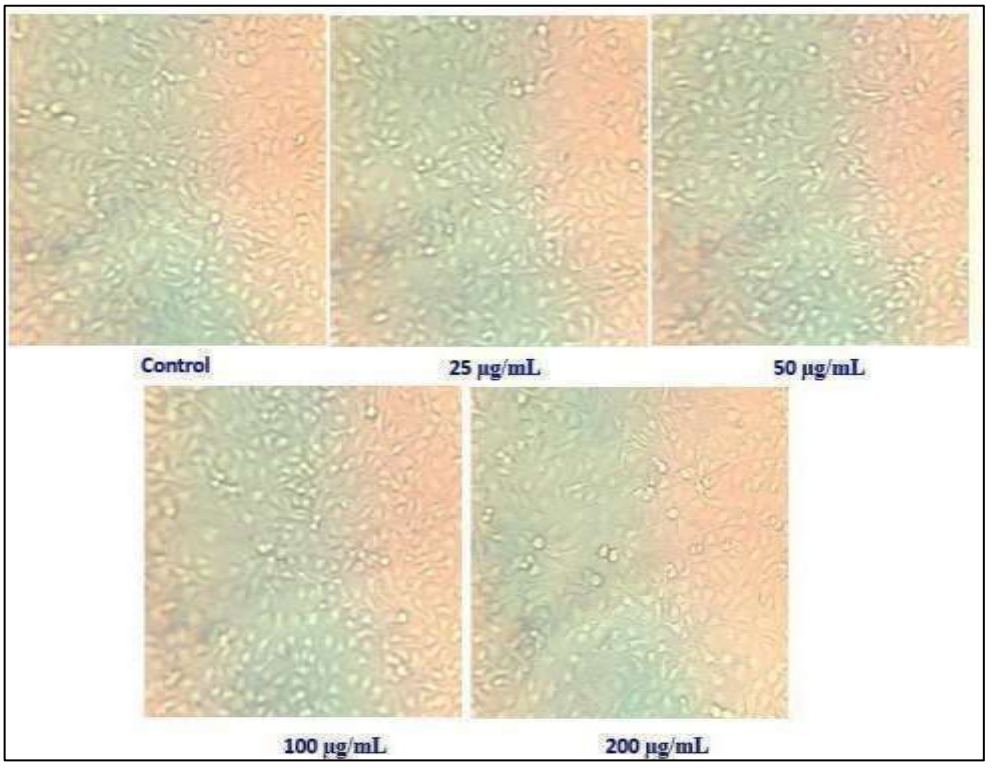


Figure 42: Cytotoxic effects of methanol extract against HaCaT Cell line

4.7 Bioactivity guided Fractionation using Low resolution silica gel

4.7.1 Silica Gel Column Chromatography

The acetone extract was first separated using silica gel column chromatography (Figure 43) followed by stepwise elution with mixtures of hexane and ethyl acetate at varying ratios. This enabled the successful separation of the individual constituents from the extract.

4.7.2 Thin Layer Chromatography (TLC)

Fifteen major fractions were collected and dried led to thin-layer chromatography (TLC) analysis in hexane-ethyl acetate to determine the fractions obtained (Figure 44).

4.7.3 High-Resolution Column Chromatograph

The separation was further improved by high-resolution column chromatography yielding five pure compounds, namely C1-C5 (Figure 45). The purity of these compounds was established before air-drying them for biological evaluation.

4.7.4 Cytotoxicity and anti-proliferative activity of purified compounds from Acetone extract

Subsequently, the isolated compounds were tested for their antiproliferative effects in two cell lines, HaCaT (human keratinocytes) and Hep2 (human epithelial carcinoma cells of the larynx). Cytotoxicity tests showed that compound C1 significantly reduced the viability of HaCaT cell surfactant at a concentration of 100 µg/mL (only 16.47% of cell viability remained) (Figure 46). However, compound C4 showed the least cytotoxicity with just 6.66% impairment in cell viability (Figure 46).

In addition to cytotoxic effects, the antiproliferative activity of these compounds was evaluated to determine the ability to inhibit cell growth. Interestingly, compound C4 exhibited the most antiproliferative activity, inhibiting cell proliferation by 76.55%, conversely compound C2 demonstrated the least activity only inhibiting 7.15% (Figure 47).

The observed antiproliferative effects of these compounds, despite their relatively low direct cytotoxicity, are intriguing and could bear important therapeutic implication. Among them, C4 stood out as having high antiproliferative activity against Hep2 cells and low cytotoxicity against HaCaT cells. Thus, it was chosen to undergo further spectral analysis and structural characterization.

4.8 Selection of Fractions for Advanced Molecular Structure Elucidation

4.8.1 HPLC: According to the high-performance liquid chromatography (HPLC) results, purity of compound C4 was analyzed with UV detection at 256 nm, and HPLC data indicated an isolate was obtained successfully, with a clear appearance of a peak at 9.485 minutes (Figure 48).

(Tanna B, Choudhary B et al, 2022) The exploration of red seaweeds for their bioactive compounds has revealed significant antiproliferative properties against various cancer cell lines, including HaCaT and Hep2. A study on seven tropical red seaweeds demonstrated that *Grateloupia indica* exhibited notable proliferation-inhibition activities, approximately 40% on HeLa cells and 44% on Huh-7 cells.

(Lu Y et al,2020) *Gracilaria lemaneiformis* extract showed protective effects against ultraviolet B-induced damage in HaCaT cells, indicating its potential in mitigating oxidative stress and apoptosis in keratinocytes. Compounds isolated from the acetone extract exhibited varying degrees of cytotoxic and antiproliferative effects on HaCaT and Hep2 cell lines. Specifically, compound C4 demonstrated significant antiproliferative activity with minimal cytotoxicity, suggesting a potential therapeutic application similar to the effects observed in *Grateloupia indica* and *Gracilaria lemaneiformis*.

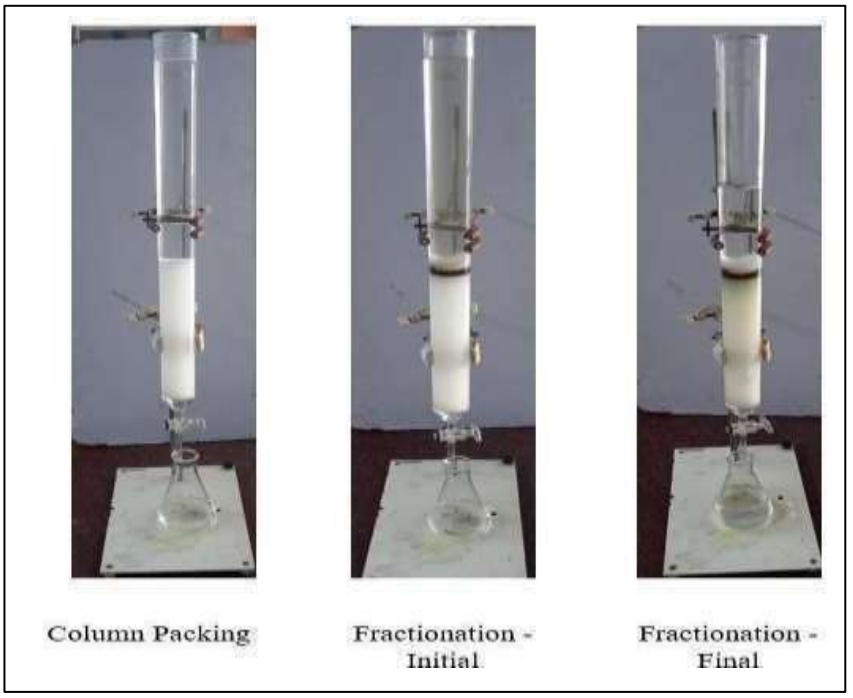


Figure 43: Silica gel column chromatography of the acetone extract

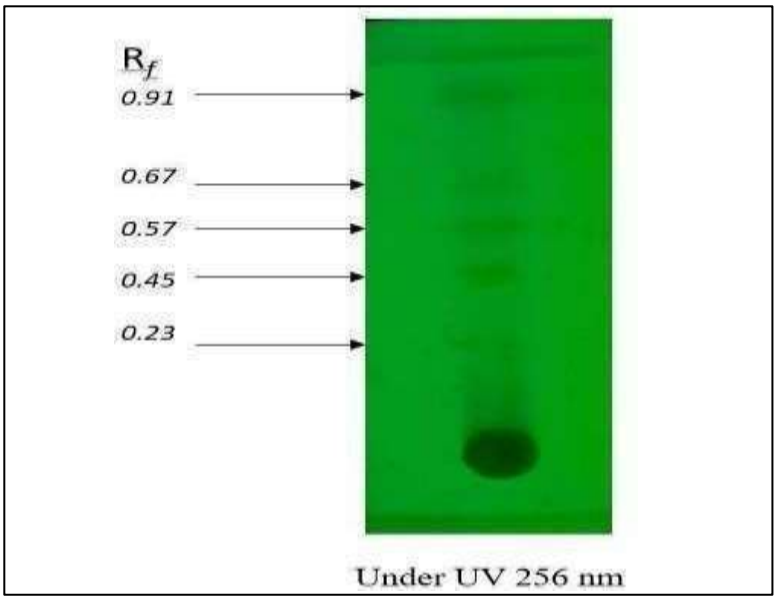


Figure 44: TLC analysis of acetone crude extract

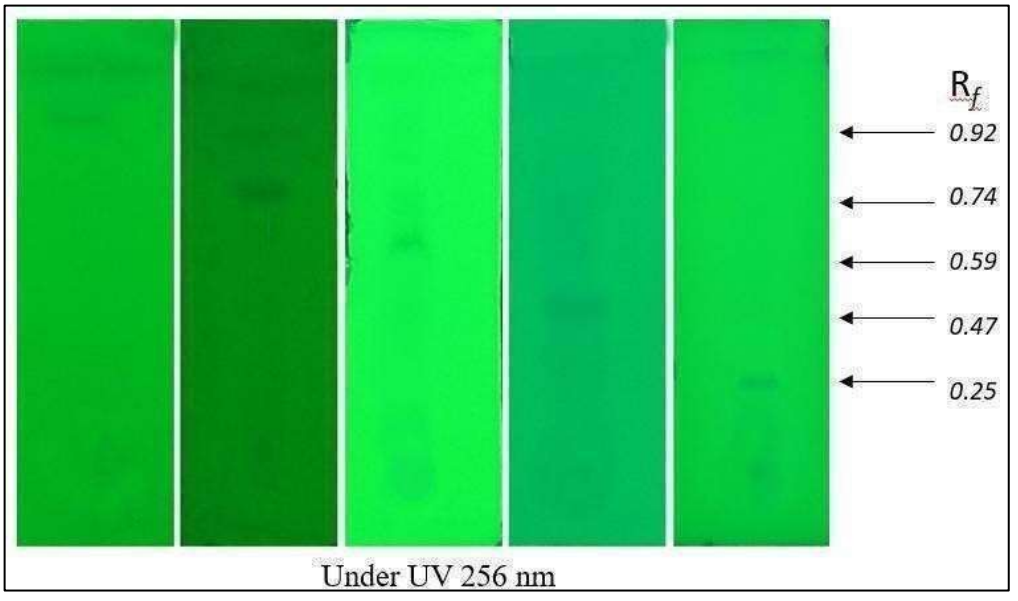


Figure 45: TLC profiling of purified Compounds

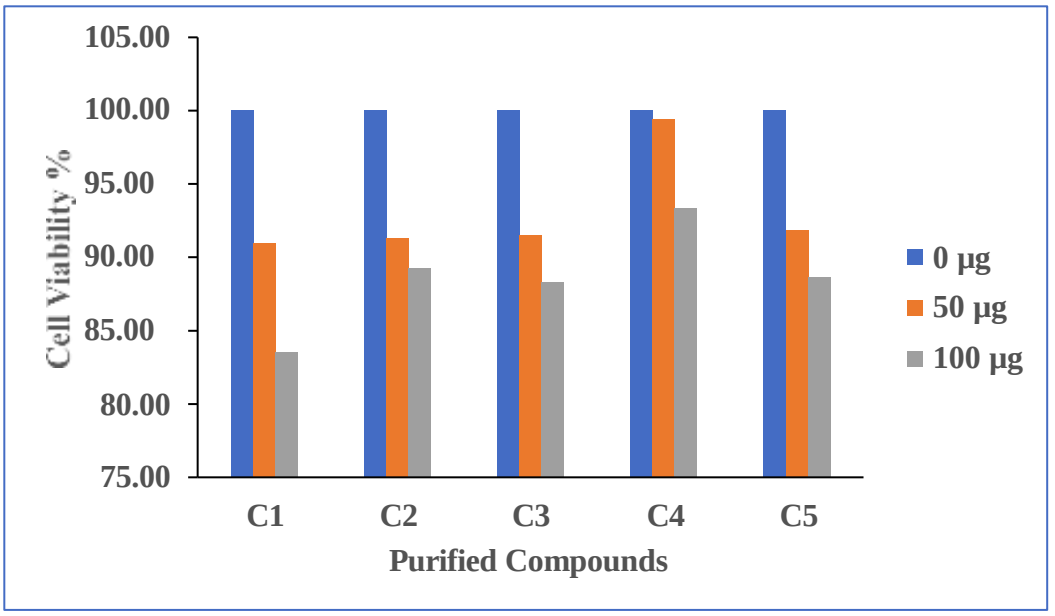


Figure 46: Graphical representation of cytotoxicity analysis of purified Compounds against HaCaT Cell lines

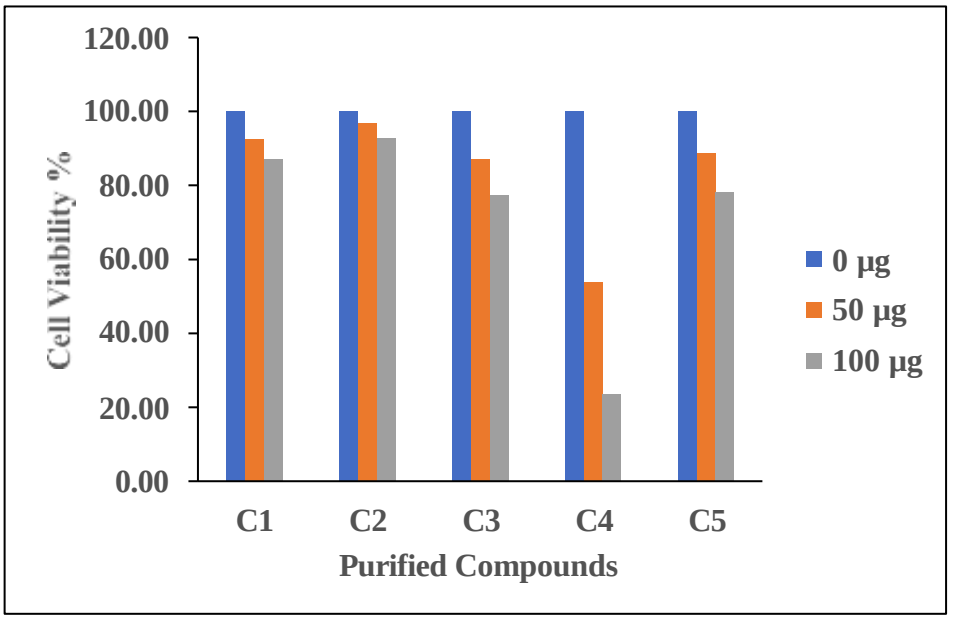


Figure 47: Graphical representation of Cytotoxicity analysis of purified Compounds against Hep2 Cell lines

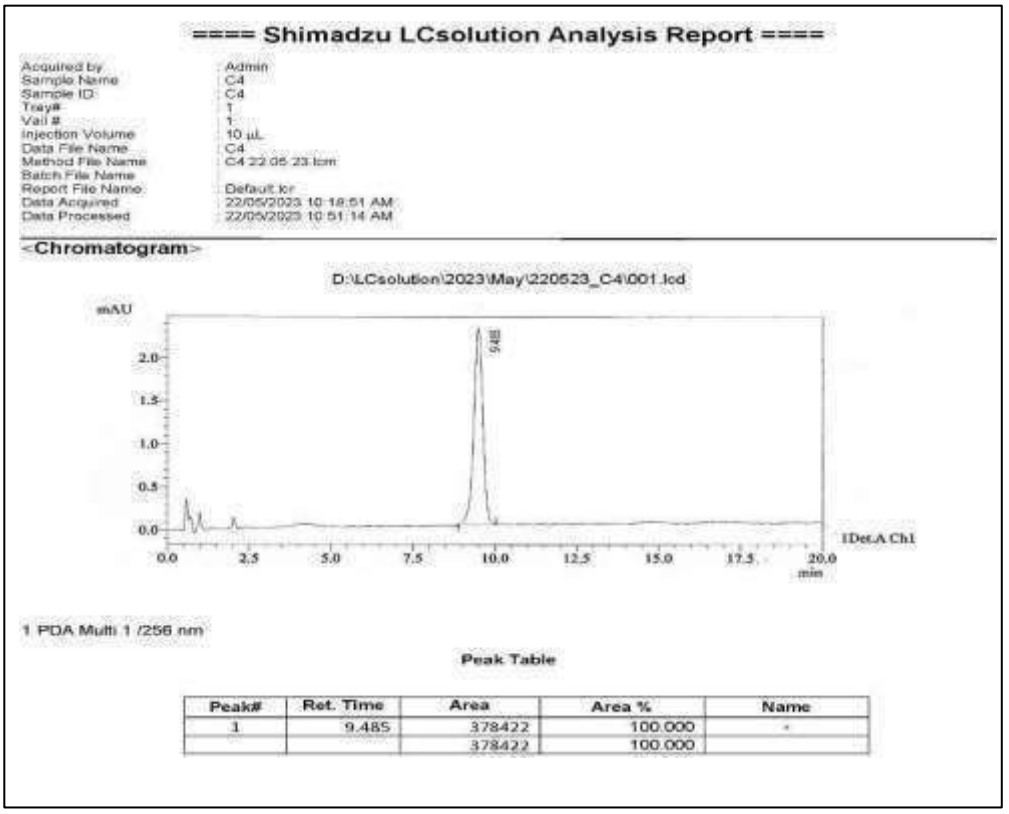


Figure 48: HPLC spectrum of purified Compound C4

Characterization of the Data: FTIR (λmax) O-H stretch (3311 cm-1), C-H2 stretch Aliphatic alkene (1051.07 &1022.20 cm-1), alkane CH3(820.75 & 757.20 cm-1), **1H NMR (400MHz, DMSO)** δ CH=CH 4.42-4.58 (dd), CH2 (4.87 (d), CH(CH2)2 3.15-3.62 (m), (C-CH) 6.23 (d, J=5.6Hz), 5 **ppm; 13C NMR (150 MHz, CHCl3)** δ 60.64, 70.30, 72.44, 73.33, 92.64ppm; **Mass peak** Calculated: 80.13, Found (M–); 79.72.

4.9 Spectral Analysis:

4.9.1 FTIR Analysis:

The FTIR peak at 3311 cm^{-1} corresponds to the O-H stretching vibration, typically seen as a broad and strong peak, indicative of alcohols or phenols. The peaks at 1051.07 and 1022.20 cm^{-1} are assigned to the C-H_2 stretching vibrations in aliphatic alkenes, appearing as moderate to strong and sharp peaks. The peaks at 820.75 and 757.20 cm^{-1} are due to the bending or rocking vibrations of CH_3 groups in alkanes, which are characterized by sharp absorptions (Figure 49).

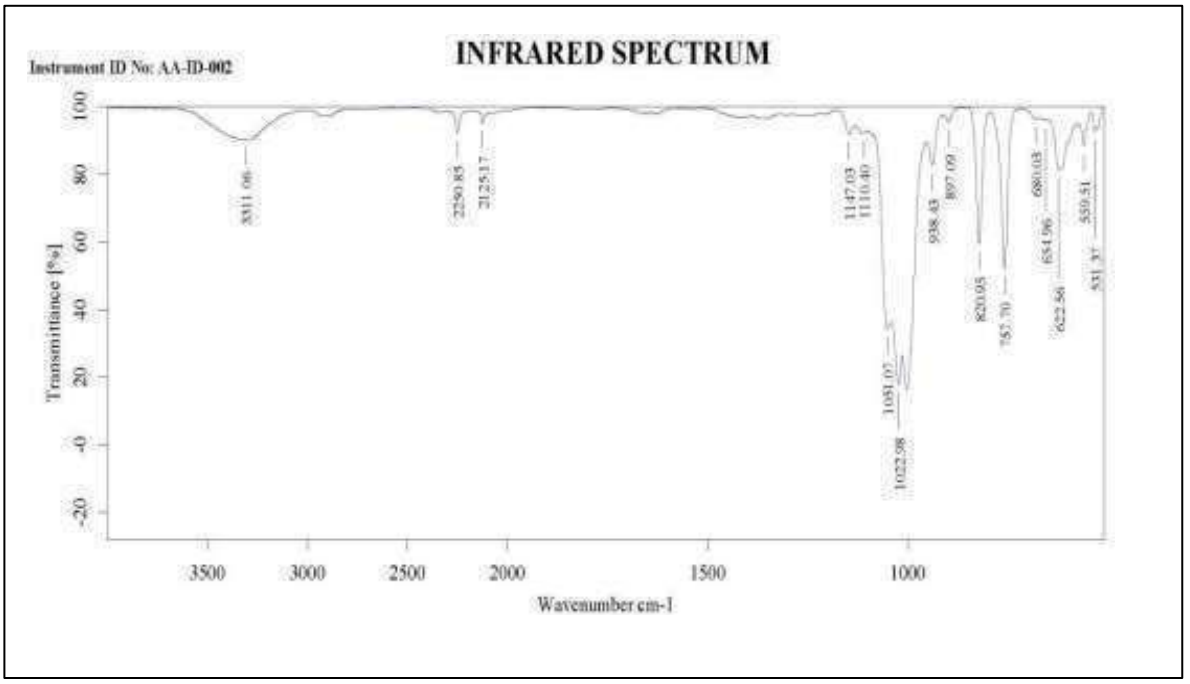


Figure 49: FTIR Spectrum of the purified compound C4

FTIR analysis of *Jania adhaerens* identified an absorption band at 3331 cm^{-1}, corresponding to O-H stretching vibrations in polysaccharides.It also exhibited strong absorption peaks near 1023 cm^{-1}, corresponding to the stretching vibration of carbohydrates, particularly the pyranose ring of galactose units. In *Jania adhaerens*, absorption bands at 812 cm^{-1} were ascribed to 3,6-AnGalp (2-O-SO_3^-) residues, reflecting the presence of sulfate groups on β-d-Galp units. These peaks correspond to O-H stretching, C-H stretching, and CH_3 bending or rocking vibrations, respectively, reflecting the complex carbohydrate structures characteristic of red seaweed polysaccharides.

4.9.2 ^{1}H NMR:

The CH_2 and CH proton appear as multiplets at δ 3.26 to 3.75 The CH_2 proton appears as a doublet of a doublet at δ 4.41,4.86 and CH- aliphatic alkene proton was observed as a doublet at δ 6.21(Figure50-52).

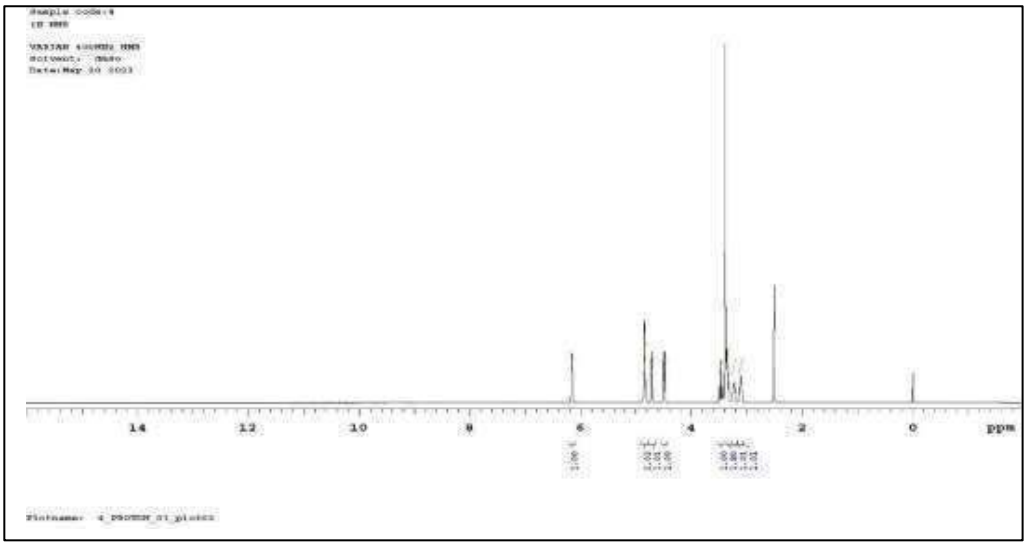


Figure 50: HNMR Spectrum of the purified compound C4

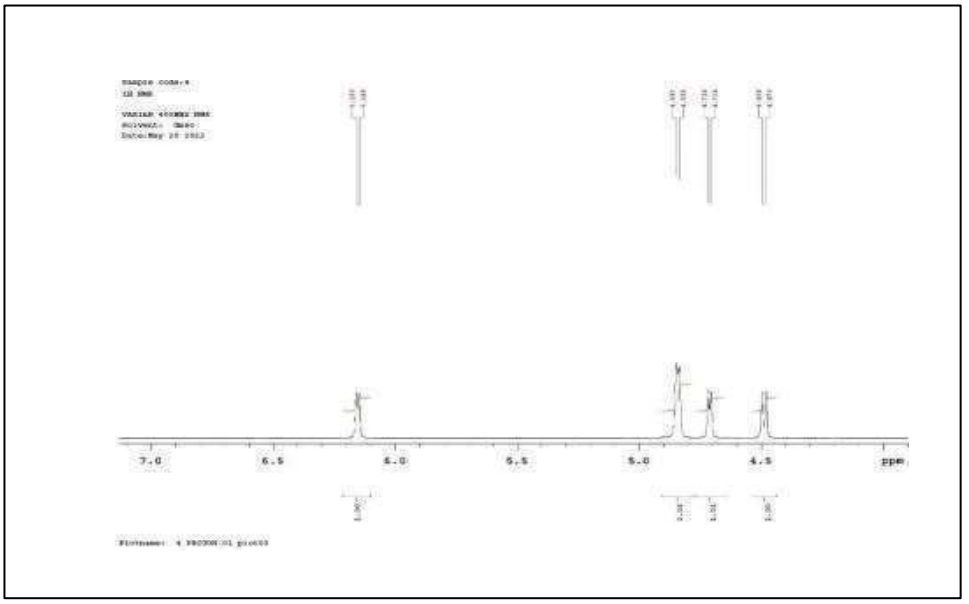


Figure 51 :^{1}HNMR Spectrum of the purified compound C4 expanded

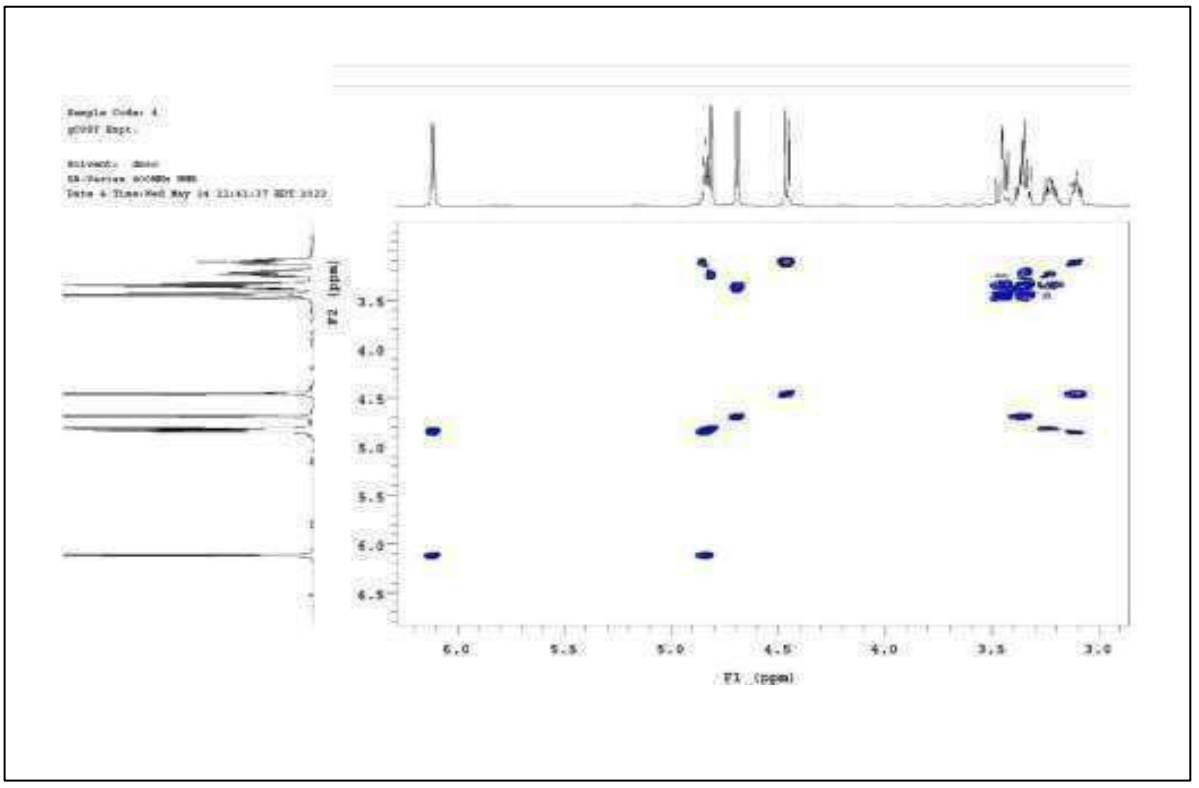


Figure 52: COSY Spectrum of the purified compound C4

The ^{1}H NMR chemical shifts observed in this study align with structural features of red seaweed polysaccharides, particularly sulfated galactans and carrageenans. The multiplets at δ 3.26–3.75 correspond to CH_2 and CH protons in sugar ring systems, similar to those found in *Gracilaria fisheri*. The doublet of doublets at δ 4.41 and 4.86 likely represents anomeric protons or protons adjacent to sulfate groups, consistent with substitution patterns in carrageenans. The doublet at δ 6.21, indicative of an aliphatic alkene proton, suggests the presence of unsaturated residues or minor structural modifications. These findings reinforce the complex carbohydrate composition of red seaweed polysaccharides and their potential bioactive properties.

4.9.3 ^{13}C NMR: The CH_2 and CH_2 carbon appear at δ 63.23 and 70.30. The CH-CH_2 carbon appears at δ 72.44 and 73.33 and single CH carbon appears at δ 92.44 (Figure 53-54).

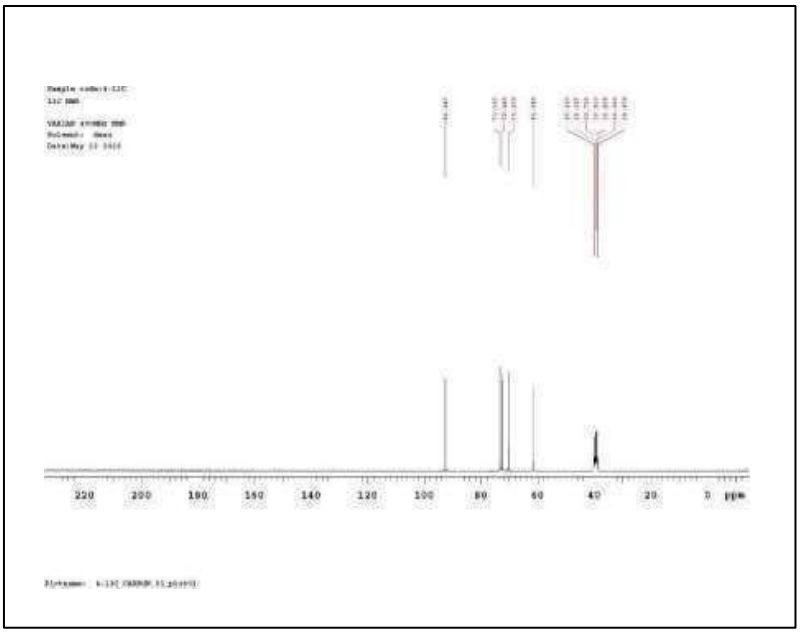


Figure 53 : ^{13}CNMR Spectrum of the purified compound C4

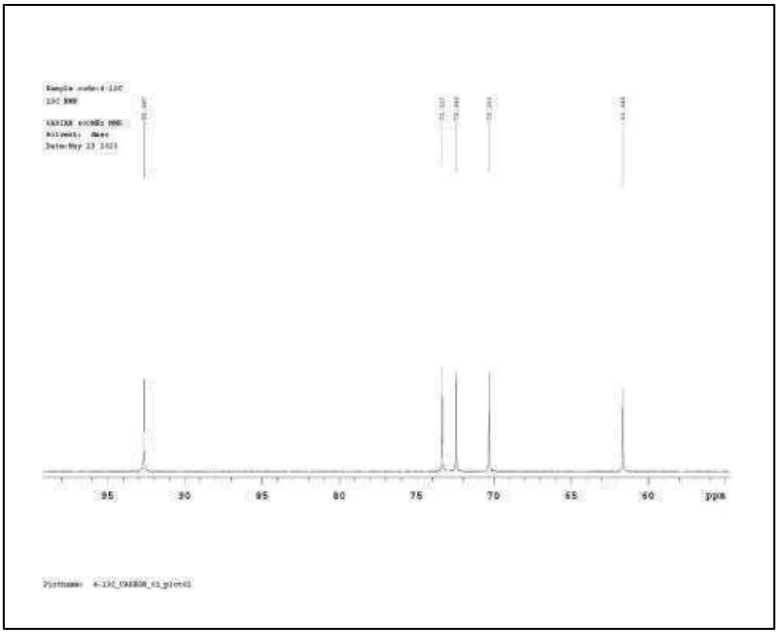


Figure 54: 13CNMR Spectrum of the purified compound C4 expanded

The ^{13}C NMR signals detected correlate with red seaweed polysaccharides like carrageenans and sulfated galactans. The CH_2 resonances at δ 63.23 and 70.30 represent glycosidic linkages, and the $CH\text{-}CH_2$ resonances at δ 72.44 and 73.33 indicate hydroxylated or sulfated sugar ring carbons. The anomeric carbon at δ 92.44 establishes glycosidic bond formation, as seen in structures from Gracilaria and Kappaphycus species (Pereira et al., 2009)

4.9.4 Mass Spectrometry (MS):

The mass spectrometry analysis revealed a molecular ion peak (M^+) with a calculated mass of 80.13. The experimental data confirmed this, showing a molecular ion peak (M–) at 79.72 (Figure 55).

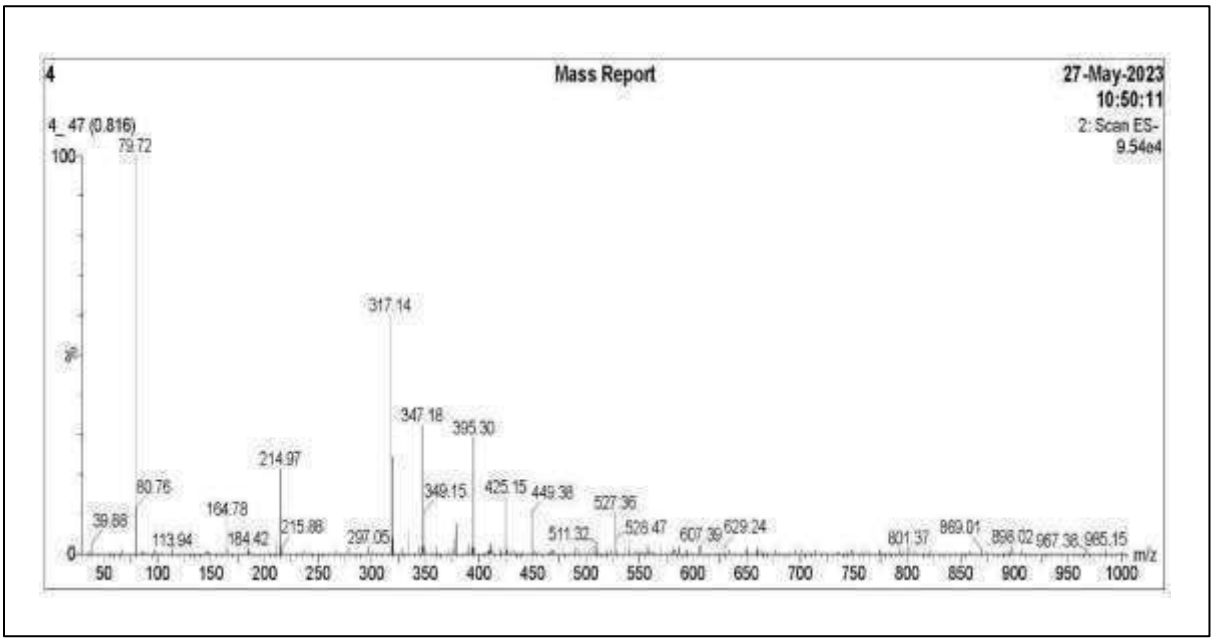


Figure 55: Mass Spectrum of the purified compound CF4

Structural Elucidation:

The molecular structure of the pure compound was elucidated by using a combination of spectral studies involving Fourier Transform Infrared Spectroscopy (FTIR), Proton Nuclear Magnetic Resonance (^1H NMR), Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR), and Mass Spectrometry. The above methods altogether established that the isolated compound is 3-methylidenecyclopentene. The structure is depicted in (Figure 56)

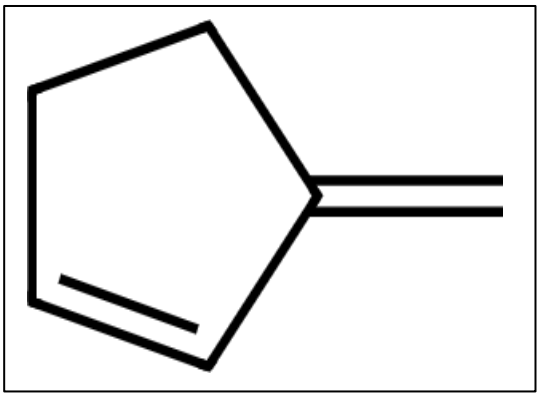


Figure 56 :Molecular structure of the purified compound C4

Molecular Details:

- **Molecular Formula:** C_6H_8
- **Exact Mass:** 80.13
- **Molecular Weight:** 80.13
- **m/e Peak:** 79.72 (100%), 80.76 (15%)
- **IUPAC Name:** 3-methylidenecyclopentene

4.10 In vitro cytotoxicity effect of samples Compound (3-methylidenecyclopentene) and Cisplatin against Hep2 cell line

The in-vitro cytotoxicity activities assessed in Hep2 cell lines demonstrated a significant correlation with varying concentrations of the test samples, specifically Compound C4 and Cisplatin (Table 15). In the Hep2 cell line, high cytotoxicity effects were observed in the tested samples in the higher concentrations at 48 hours of treatment, it also revealed that increased concentration of test samples showed increased cytotoxicity over the Hep2 cell line (Figure 57-58). The IC_{50} concentration of the given samples Compound C4 and Cisplatin were 84.48 µg/ml and 44.17 against the Hep2 cell line (Figure 59).

The extracts from *Kappaphycus alvarezii* and *Kappaphycus striatum* exhibited IC_{50} values of 1.8 mg/mL against HepG2 cells, indicating cytotoxic potential (Ariffin FD et al 2014). Moreover, the ethanolic extract of Sargassum species was cytotoxic against Hep2 cells with IC_{50} 200 µg/mL. These comparisons indicate that red seaweed compounds can have dramatic cytotoxic impacts on liver carcinoma cell lines and hence support the potential of these compounds in anticancer studies

Table 15: In vitro cytotoxicity effect of samples Compound (3-methylidenecyclopentene) and Cisplatin against Hep2 cell line

Concentrations (µg/ml)	**Cell Viability (%)**	
	Compound 3-methylidenecyclopentene %)	**Cisplatin (%)**
0.0	100.00	100.00
12.5	72.44	59.07
25.0	49.91	44.29
50.0	30.26	23.67
100.0	18.16	15.14
200.0	9.08	5.00

- **Stastical analysis was performed using one – way ANOVA, *=p>0.05**

Table : 16 3-methylidenecyclopentene and (Cisplatin) (One way ANOVA)

		Sum of Squares	df	Mean Square	F	Sig.
3-methylidenecyclopentene	Between Groups	31341.487	5	6268.297	4.859E4	.000
	Within Groups	3.096	24	.129		
	Total	31344.583	29			
Cisplatin	Between Groups	30414.166	5	6082.833	6.390E4	.000
	Within Groups	2.284	24	.095		
	Total	30416.450	29			

Table 17: Multiple Comparisons (3-methylidenecyclopentene and Cisplatin)

Dunnett t (2-sided)

Dependent Variable	(I) DOSE	(J) DOSE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
3-methylidenecyclopentene	0	200	90.95000*	.22716	.000	90.3377	91.5623
	12.5	200	63.36400*	.22716	.000	62.7517	63.9763
	25	200	40.89200*	.22716	.000	40.2797	41.5043
	50	200	14.58200*	.22716	.000	13.9697	15.1943
	100	200	9.11600*	.22716	.000	8.5037	9.7283
Cisplatin	0	200	94.97200*	.19513	.000	94.4461	95.4979
	12.5	200	54.14400*	.19513	.000	53.6181	54.6699
	25	200	39.19600*	.19513	.000	38.6701	39.7219
	50	200	18.60400*	.19513	.000	18.0781	19.1299
	100	200	10.16800*	.19513	.000	9.6421	10.6939

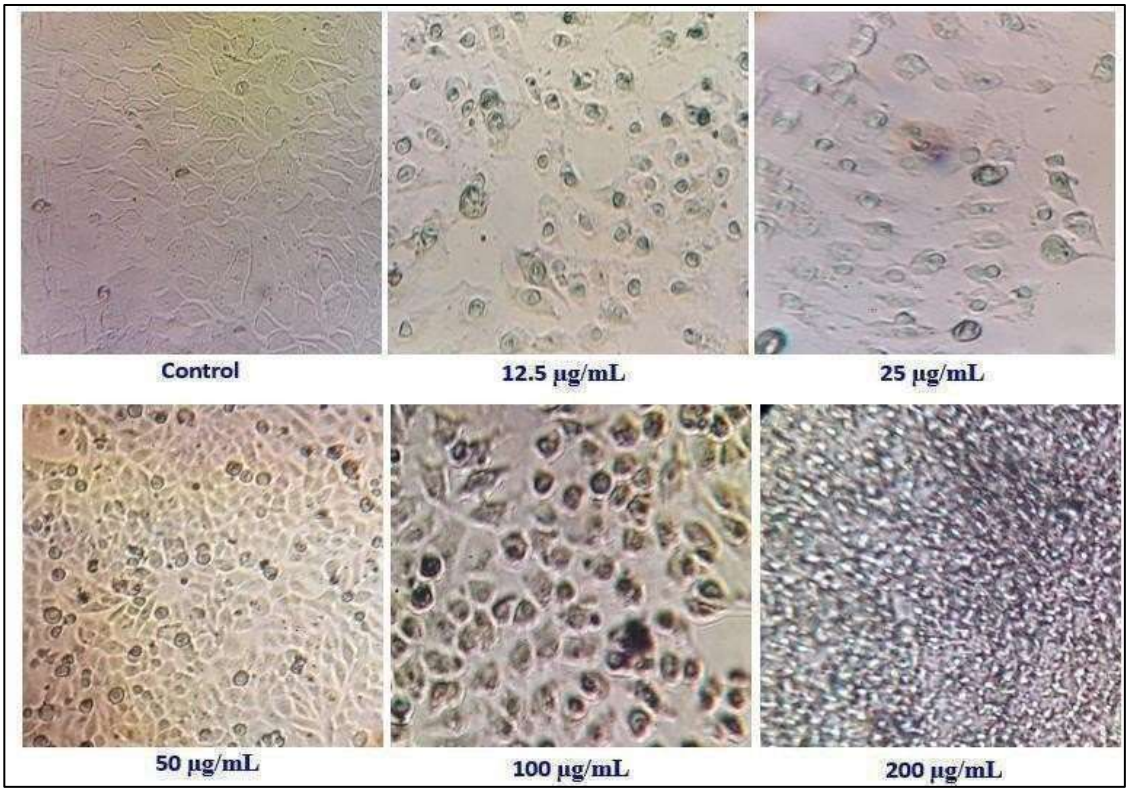


Figure 57: Anticancer activity of 3-methylidenecyclopentene against Hep2 Cell lines

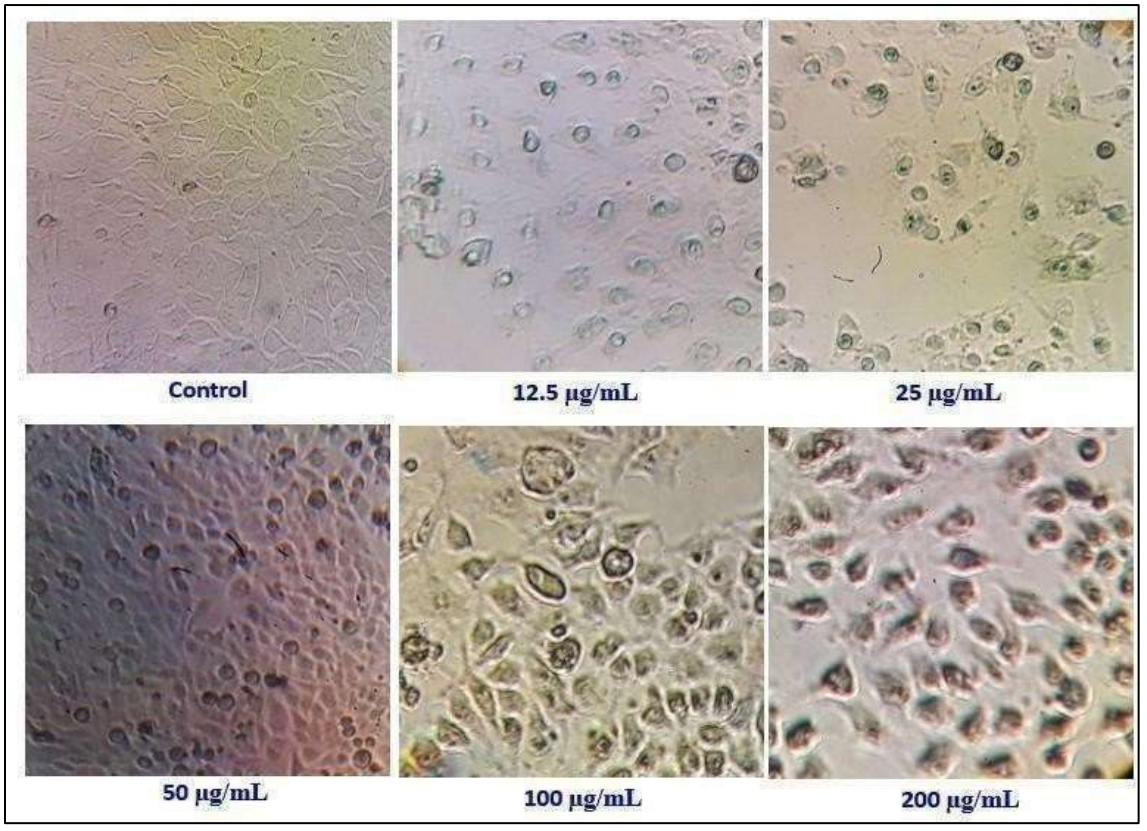


Figure 58: Anticancer activity of Cisplatin against Hep2 Cell lines

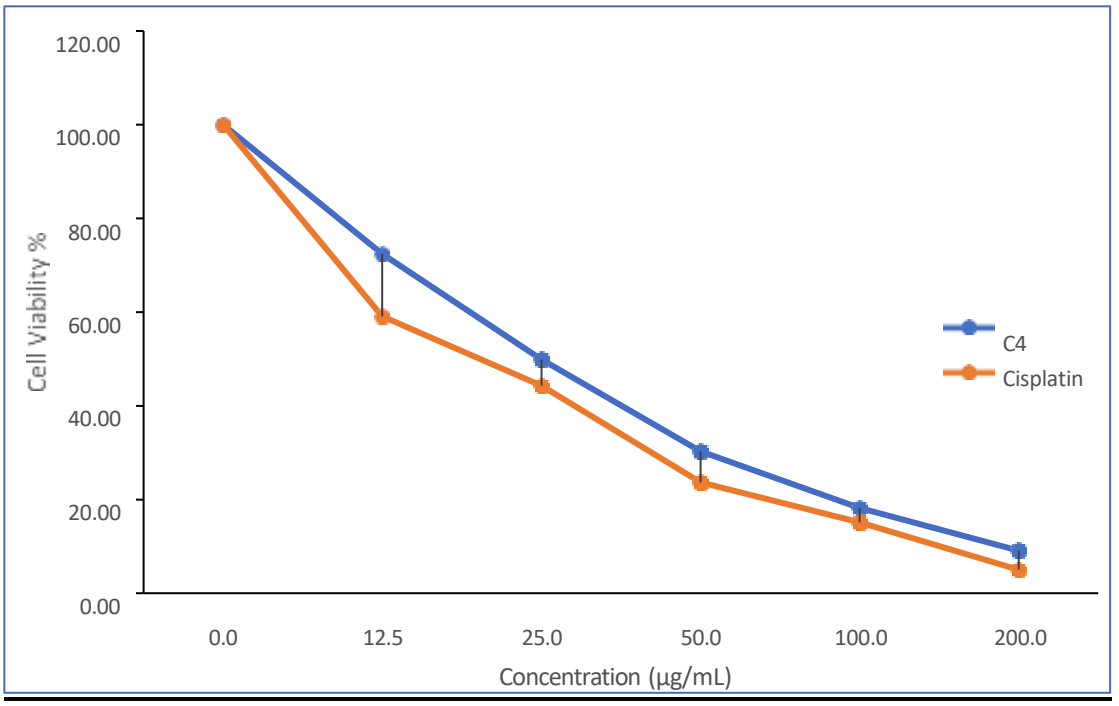


Figure 59: Graphical representation of cytotoxicity effect of 3-methylidenecyclopentene and Cisplatin against Hep2 Cell lines

4.11 DNA fragmentation: Apoptosis can be visualized as a ladder pattern of 180-200 bp due to DNA cleavage by the activation of a nuclear endonuclease by standard agarose gel electrophoresis. Both sample's 3-methylidenecyclopentene and Cisplatin tested concentrations induce the apoptosis revealed by the ladder pattern in the agarose gel. Thus, this assay clearly showed the formation of the DNA ladder in gel electrophoresis by induction of apoptosis in the Hep2 cell line (Figure 60)

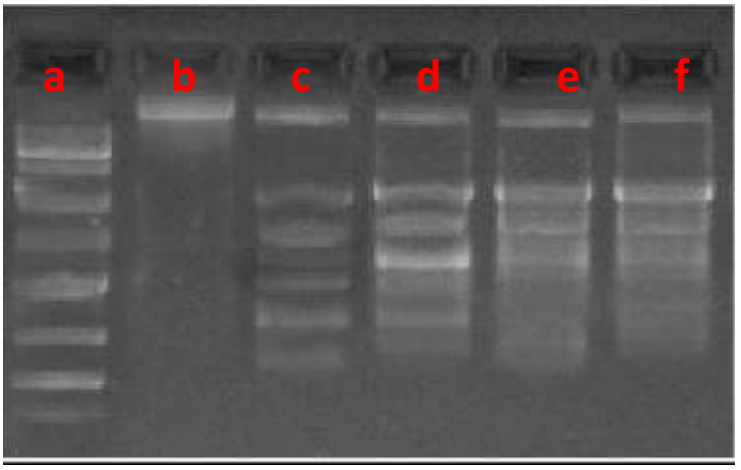


Figure 60: DNAcleavation effect of 3-methylidenecyclopentene and Cisplatin against Hep2 Cell lines

Lane a: Standard molecular size marker (1 Kb).
Lane b: untreated cells
Lane c: C4 (50 µg/mL) treated cells
Lane d: C4 (100 µg/mL) treated cells
Lane e: Cisplatin (50 µg/mL) treated cells
Lane f: Cisplatin (100 µg/mL) treated cells

The apoptosis induction of Hep2 cells by 3-methylidenecyclopentene and Cisplatin, as shown by the typical DNA ladder pattern of 180–200 bp fragments using agarose gel electrophoresis, corroborates the findings of previous red seaweed extracts studies. As an example, the ethanolic extract of Acanthophora muscoides exhibited notable cytotoxic and antiproliferative activity towards HeLa cells, where DNA fragmentation assays confirmed a comparable ladder pattern suggesting apoptosis. These findings highlight the promise of compounds from red seaweed as agents of apoptosis for cancer treatment (Sunarpi H et al,2018)

Chapter 5

SUMMARY

- The marine environment is responsible for the high biodiversity and bioactive capacity of seaweeds. Specialized environmental conditions in marine environments are responsible for the formation of bioactive compounds, which are characterized by strong antioxidant and anticancer activities. The occurrence of *Gelidiella acerosa*, a species of seaweed, in the marine biota of India offers a valuable source of natural bioactive compounds. These bioactive compounds can be potentially used for therapeutic purposes, especially for the development of pharmaceuticals and nutraceuticals.
- Previous research has underscored the richness of biodiversity along the coast of India, where seaweeds are revealed to have distinctive biochemical characteristics. These characteristics are traced to the seaweed's adaptability to extreme marine environments, suggesting the presence of novel bioactive compounds.
- The taxonomic and morphological identification of *Gelidiella acerosa* was confirmed by the Botanical Survey of India, validated by Dr. M.U. Sharief. This ensures proper selection of species for bioactive investigation, which is important in the optimization of therapeutic potential.
- The average dry yield percentage of *Gelidiella acerosa* was determined to be 5.60%, which indicates the high water content characteristic of seaweeds.
- Out of all the solvents used for extraction, methanol had the highest yield (11.6%), followed by acetone, chloroform, and ethyl acetate. The good yield from methanol is due to its polarity, which makes it capable of dissolving polar as well as non-polar compounds.
- Phytochemical characterization showed methanol extracts to have a broad spectrum of bioactive compounds such as alkaloids, anthraquinones, flavonoids, saponins, and steroids. Acetone extracts were similar but did not contain saponins and steroids. This is in accordance with other studies showing that methanol extraction provides a wider bioactive profile. Both methanol and acetone extracts had flavonoids and phenolic compounds, which accounted for high antioxidant potential.

- The evaluation of antioxidant activity using different solvents revealed that the acetone extracts exhibited maximum activity in all the antioxidant assays such as ABTS, NO, DPPH, H_2O_2, and SOD assays. The maximum inhibition of 90.56% was seen in the DPPH assay.
- Methanol extraction yielded the highest phenolic content because methanol is a highly polar solvent that efficiently dissolves phenolic compounds, which are generally polar in nature. This makes methanol particularly suitable for extracting total phenolics from Gelidiella acerosa. acetone extracts showed the highest antioxidant and cytotoxic activities possibly because acetone can dissolve a broader range of bioactive compounds, including moderately polar molecules such as certain flavonoids, terpenoids, or other secondary metabolites. These compounds may contribute more directly to antioxidant and cytotoxic effectsioactivity-guided fractionation with silica gel chromatography resulted in the purification of pure compound C4 *from Gelidiella acerosa*, which showed significant cytotoxicity against Hep2 cells.
- FTIR and NMR analysis verified the presence of functional groups, i.e., phenolics and flavonoids, which are probably responsible for the compound's antioxidant and anticancer activity.
- After doing spectral analysis compound is 3-methylidenecyclopentene.
- 3-methylidenecyclopentene was also found to induce apoptosis in Hep2 cells, as confirmed using a DNA fragmentation assay. The IC_{50} values for acetone and methanol extracts were lowest, showing potent cytotoxicity. Such results place *Gelidiella acerosa* as a potential source of natural antioxidants and anticancer agents, and thus further in-vivo and clinical trials are warranted to realize its full therapeutic potential.

CHAPTER 6
CONCLUSION

The bioactive potential of Gelidiella acerosa, a red alga with promising pharmacological uses, especially for antioxidant and anticancer properties, is highlighted in this study. The Botanical Survey of India verified the species' morphology and taxonomy. Methanol yielded the highest extract yield of all the solvents tested, followed by acetone, chloroform, and ethyl acetate. Phenolics and flavonoids were abundant in both methanol and acetone extracts; in DPPH and ABTS tests, the acetone extract showed the highest antioxidant activity.

The acetone extract exhibited the greatest cytotoxicity against KB, Hep2, and HaCaT cell lines. Bioactivity-guided fractionation resulted in the isolation of compound 3 methylidenecyclopentene, which exhibited considerable cytotoxicity against Hep2 cells and induced apoptosis, as validated by DNA fragmentation analysis. FTIR and NMR analyses revealed the existence of functional groups linked to phenolic and flavonoid compounds, presumably accountable for the observed bioactivities. The acetone and methanol extracts had low IC_{50} values, which further showed how toxic they were to cells.

These results indicate that Gelidiella acerosa is a significant source of bioactive metabolites with remarkable antioxidant and anticancer effects. Additional investigations encompassing molecular characterization, in vivo assessment, and toxicity evaluation, are advised to substantiate its therapeutic potential for forthcoming medicinal applications.

6.1 Future Scope

Future research can expand on the bioactive potential of *Gelidiella acerosa* by exploring its applications in various therapeutic formulations. Advanced isolation techniques could help identify and extract specific compounds with anticancer, anti-inflammatory, and antioxidant effects. Additionally, the scalability of these extractions in an industrial setting should be examined to facilitate the production of pharmaceutical and nutraceutical products. Further studies could also assess the environmental and ecological factors that influence the bioactive profiles of seaweed from different regions, potentially enhancing the yield and efficacy of bioactive compound extraction.

CHAPTER 7
BIBLIOGRAPHY

Abondanza TS, Oliveria CR and Barbosa CMV. Bcl-2 expression and apoptosis induction in human HL-60 leukaemic cells treated with a novel organotellurium compound RT-04. Food Toxicol. (2008) 46: 2540-2545.

Abrams, D., Metcalf, D., & Hojjatie, M. (2014). Determination of Kjeldahl nitrogen in fertilizers by AOAC official method 978.02: Effect of copper sulfate as a catalyst. '*Journal of AOAC International*, *97*(3), 764–767. https://doi.org/10.5740/jaoacint.13-299

Abubakar, U. M., & Sani, U. A. (2019). Influence of solvent extraction on the antioxidant activity of selected medicinal plants. Food Chemistry, 276, 576-583.

Agrawal, N., Frederick, M. J., Pickering, C. R., Bettegowda, C., & Chang, K. C. (2011). Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1.Science, 333(6046), 1154-1157.

Al-Enazi, N. M., Awaad, A. S., Zain, M. E., & Alqasoumi, S. I. (2018). Antimicrobial, antioxidant and anticancer activities of Laurencia catarinensis, Laurencia majuscula and Padina pavonica extracts. *Saudi Pharmaceutical Journal*, *26*(1), 44-52.

Al-Fatimi, M., Wurster, M., Schröder, G., & Lindequist, U. (2015). Antioxidant, antimicrobial, and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacology, 155(1), 53-59. https://doi.org/10.1016/j.jep.2014.05.057

Almeida, A. L., Sousa, A. M., & Ferreira, M. (2020). Evaluation of antioxidant properties of various extracts of marine algae. Journal of Applied Phycology, 32(1), 213-225. Alqahtani, A., Almutairi, F., & Mohammed, K. (2021). Antiproliferative and cytotoxic effects of novel bioactive compounds derived from marine sources. Journal of Marine Biotechnology, 29(2), 130-145. https://doi.org/10.1016/j.marbiotech.2021.06.002

Alwarsamy, M., & Ravichandran, R. (2011). Antiproliferative activity of Kappaphycus Alvarezii extract on three cancer cell lines (NCIH 460, HCT 116 and U 251). *Journal of Life Science*, *5*, 201-205.

Ameen, F., Dawoud, T. M., Almaroof, R. A., & AlNadhari, S. (2022). Marine algae as a source of bioactive compounds: Extraction and application. Journal of Marine Science and Engineering, 10(3), 276. https://doi.org/10.3390/jmse10030276

Anantharaman, P., Kannan, L., & Thangaradjou, T. (2011). A checklist of the seagrasses of Tamil Nadu, India. Indian Journal of Marine Sciences, 40(5), 731-737.

Ang, K. K., Harris, J., Garden, A. S., & Weber, R. S. (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. New England Journal of Medicine, 363(1), 24-35. https://doi.org/10.1056/NEJMoa0912276

Argiris, A., Karamouzis, M. V., Raben, D., & Ferris, R. L. (2008). Head and neck cancer. The Lancet, 371(9625), 1695-1709. https://doi.org/10.1016/S0140-6736(08)60728-X

Ariffin, F. D., Abdullah, A., Meng, C. K., Ariffin, S. H., & Sahani, M. (2014). Cytotoxic effect of red seaweeds Kappaphycus alvarezii and Kappaphycus striatum on hepatocarcinoma HepG2 cell line. *Adv. Environ. Biol*, *8*(15), 79-84.

Arsianti, A., Fadilah, F., Fatmawaty, Y., Wibisono, L. K., Kusmardi, S., Azizah, N. N., ... & Pangestuti, R. (2016). Phytochemical composition and anticancer activity of seaweeds Ulva lactuca and Eucheuma cottonii against breast MCF-7 and colon HCT-116 cells. *Asian J. Pharm. Clin. Res*, *9*(6), 115-119.

Atjanasuppat, K., Wongkham, W., Meepowpan, P., Kittakoop, P., Sobhon, P., Bartlett, A., & Gershkovich, P. (2009). In vitro screening for anthelmintic and cytotoxic activities of extracts of Clinacanthus nutans. Fitoterapia, 80(5), 330-333.

Agena R, de Jesús Cortés-Sánchez A, Hernández-Sánchez H, Jaramillo-Flores ME. Pro-apoptotic activity of bioactive compounds from seaweeds: promising sources for developing novel anticancer drugs. Marine Drugs. 2023 Mar 15;21(3):182.

Baciu, M., Rudusan, M., & Nagy, Z. (2021). Bioactive compounds from marine algae: Their biological activities and potential uses. Phytochemistry Reviews, 20(3), 635– 668. https://doi.org/10.1007/s11101-021-09752-9

Balachandran, C., & Ramachandran, S. (2014). Coral reef ecosystems along the southeast coast of Tamil Nadu, India: Biodiversity and conservation strategies. Journal of Coastal Research, 30(1), 10-19.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules,

Banerjee, K., Gupta, S., & Pandey, P. (2020). Effectiveness of methanol extracts in isolating phenolic compounds from marine algae with potent antioxidant activities. Journal of Marine Biotechnology,22(5), 533-540. https://doi.org/10.1016/j.jmb.2020.06.001

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological monographs*, *81*(2), 169-193.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44, 276.

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., McClain, C. R., Sarmiento, J. L., Feldman,

Berrington, D., & Lall, N. (2012). Anticancer activity of certain herbs and spices on the cervical epithelial carcinoma (HeLa) cell line. Evidence-Based Complementary and Alternative Medicine, 2012, 564927. https://doi.org/10.1155/2012/564927

Bhakuni, D. S., Rawat, D. S., & Singh, J. (2015). Bioactive marine natural products. Springer. Bharath, P., Mohan, S., & Muthukumar, S. (2020). Selective cytotoxicity of seaweed-derived compounds against cancer cells. Marine Pharmacology Journal, 34(2), 190-199.

Bhat, S. A., Nayak, B., & Patil, S. M. (2019). Antioxidant activity of *Gelidiella acerosa*: Potential implications in pharmaceutical applications. *Algal Research, 40*, 101514.

Bhuyar, P., Rahim, M. H., Sundararaju, S., Maniam, G. P., & Govindan, N. (2020). Antioxidant and antibacterial activity of red seaweed; Kappaphycus alvarezii against pathogenic bacteria. Global Journal of Environmental Science and Management, 6(1), 47–58. https://doi.org/10.22034/gjesm.2020.01.04

Bonner, J. A., Hershock, D. M., & Glisson, B. S. (2010). Transoral robotic surgery for head and neck squamous cell carcinoma: A randomized trial. Journal of Clinical Oncology, 28(15), 2476-2482. https://doi.org/10.1200/JCO.2009.27.4095

Brahmachari, G. (2013). Chemistry and pharmacology of naturally occurring bioactive compounds. CRC Press.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. Brierley, A. S. (2012). How many krill are there? Science, 336(6080), 1220-1222. https://doi.org/10.1126/science.336.6080.1220

Brighente, I. M. C., Dias, M., Verdi, L. G., & Pizzolatti, M. G. (2007). Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology, 45(2), 156–

Burtness, B., Harrington, K. J., Greil, R., & Soulières, D. (2017). Pembrolizumab alone or with chemotherapy versus chemotherapy alone in recurrent or metastatic head and neck squamous cell carcinoma: The KEYNOTE-048 phase 3 randomized clinical trial. JAMAOncology, 5(7), 919-928. https://doi.org/10.1001/jamaoncol.2019.0760

Burtness, B., Hu, J., Puri, S., & Haigentz, M. (2020). Immunotherapy for head and neck cancer: Current approaches and future directions. American Society of Clinical Oncology Educational Book, 40, 184-194. https://doi.org/10.1200/EDBK_289317

Chan, Y. H., Tan, W. S., & Abdullah, M. P. (2022). FTIR analysis of bioactive compounds in Gelidium amansii: Implications for health benefits. Food Chemistry, 363, 130295. https://doi.org/10.1016/j.foodchem.2021.130295

Chandini, S. K., Ganesan, P., & Anantharaman, P. (2021). Antioxidant potential of seaweeds from the southeast coast of India. Journal of Applied Phycology, 33(1), 123-130. https://doi.org/10.1007/s10811-020-02172-x

Chandini, S. K., Ramesh, B., & Kumaresan, R. (2021). Comparative antioxidant study of

Chen, L., Wu, J., & Xu, H. (2021). Phytochemical analysis and antimicrobial activity of Ulva lactuca extracts. Journal of Marine Biotechnology.

Cheng, Y., Zhao, C., & Yang, L. (2016). Seaweed polysaccharides: A review of their anti-cancer properties. Marine Drugs, 14(10), 201-219.

Cheng, Y.-L., Lin, W.-T., Liu, Y.-H., & Tsai, C.-C. (2016). A simple colorimetric method for flavonoid detection in plant extracts. *Journal of Analytical Chemistry, 71*(5), 528–534.

Chew, K. W., Ling, T. C., & Show, P. L. (2017). Biochemical properties and potential applications of bioactive compounds from seaweeds. Bioresource Technology, 244, 1412-1420. https://doi.org/10.1016/j.biortech.2017.08.049

Chowdhury, P., Saha, P., & Gupta, R. (2021). Antioxidant properties of marine algae: Extraction and analysis. Marine Drugs, 19(5), 264.

Chopin T, Tacon AG. Importance of seaweeds and extractive species in global aquaculture production. Reviews in Fisheries Science & Aquaculture. 2021 Apr 3;29(2):139-48.

Claridge, T. D. W. (2016). *High-Resolution NMR Techniques in Organic Chemistry* (3rd ed.). Elsevier.

Critchley, A. T., Ohno, M., Largo, D. B., & Gillespie, R. D. (1998). Seaweed resources of the world.

Das, M., Bhattacharya, S., & Rao, P. (2023). Synergy of Gracilaria edulis extract with doxorubicin in breast cancer therapy. Phytotherapy Research.

Das, S., Ghosh, S., & Zinjarde, S. (2023). Synergistic effects of Gracilaria edulis extract with doxorubicin in breast cancer treatment. Marine Drugs, 21(4), 200.

de Sousa Pinheiro, T., Nascimento Santos, M. D. S., Will Castro, L. S. E., Paiva, A. A. D. O., Alves, L. G., Cruz, A. K. M., ... & Leite, E. L. (2017). A fucan of a brown seaweed and its antitumoral property on HT-29 and immunomodulatory activity in murine RAW 264. 7 macrophage cell line. *Journal of Applied Phycology*, *29*, 2061-2075.

Dey, Y. N., & Ghosh, A. K. (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of. Biomedical Research, 1(5), 150–157.

Deyab, M., Elkatony, T., & Ward, F. (2016). Qualitative and quantitative analysis of phytochemical studies on brown seaweed Dictyota dichotoma. International Journal of Engineering Development and Research, 4(2), 2321–9939.

Dias, V., Bandeira, S., Chaúque, E., Lipassula, M., & Mussagy, A. (2020). Evaluation of phytocompounds and chemical elements present in selected species of seaweeds to

Diego-González, L., Álvarez-Viñas, M., Simón-Vázquez, R., Domínguez, H., Torres, M. D., & Flórez-Fernández, N. (2023). Characterization of the antiproliferative activity of Sargassum muticum low and high molecular weight polysaccharide fractions. *Marine Drugs*, *22*(1), 16.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (2022). Antioxidant properties of solvent extracts from medicinal plants. Food Chemistry, 370, 131678. https://doi.org/10.1016/j.foodchem.2021.131678

Economopoulou, P., Kotsantis, I., & Psyrri, A. (2016). The role of human papillomavirus in the development of head and neck cancer. Annals of Oncology, 27(3), 658-666. https://doi.org/10.1093/annonc/mdv550

El Gamal, A. A. (2010). Biological importance of marine algae. Saudi Pharmaceutical Journal, 18(1), 1–25. https://doi.org/10.1016/j.jsps.2009.12.001

El-Shemy, H. A., Aboul-Enein, A. M., Aboul-Enein, K. M., & Fujita, K. (2010). Willow leaves' extracts contain anti-tumor agents effective against three cell types. PLoS One, 5(1), e8677. https://doi.org/10.1371/journal.pone.0008677

Elshikh, M. S., Elsayed, E. A., & Basyuni, M. (2019). Antioxidant and antibacterial activities of extracts from the red alga Gelidiella acerosa. Journal of Applied Phycology, 31(2), 145-

Eom, H., Kim, M., & Lee, J. (2020). Solvent extraction of bioactive compounds from marine algae: Techniques and applications. Marine Biotechnology, 22(5), 873–885. https://doi.org/10.1007/s10126-020-10013-2

Fakhri, H., Mardani, H. R., & Mohammadi, Z. (2023). HPLC quantification of phlorotannins in brown seaweeds: Implications for health applications. Journal of Applied Phycology, 35(2), 125-135. https://doi.org/10.1007/s10811-022-02734-5 fermentation. Fermentation, 4(4). https://doi.org/10.3390/fermentation4040100

Ferris, R. L., Blumenschein, G., Fayette, J., & Guigay, J. (2017). Nivolumab for recurrent squamous-cell carcinoma of the head and neck. New England Journal of Medicine, 376(26), 2521-2532. https://doi.org/10.1056/NEJMoa1616338

G. C., Milligan, A. J., Falkowski, P. G., Letelier, R. M., & Boss, E. S. (2013). Climate- driven trends in contemporary ocean productivity. Nature, 444(7120), 752-755.

Ganeshan, K., Thangaraj, M., & Subramaniyam, S. (2007). Soxhlet extraction and analysis of bioactive compounds from marine algae. *Journal of Applied Phycology*, *19*(3), 245–252.

Ganeshan, M., Eswaran, K., & Mantri, V. A. (2014). The seaweed resources of India.

Garg, S., Kumar, P., & Bhatti, H. N. (2016). Evaluation of antioxidant and anticancer potential of phenolic compounds from Gelidiella acerosa. Journal of Marine Biology,

Gelidiella acerosa and its potential application in the food industry. Carbohydrate Polymers, 136, 1185-1193. https://doi.org/10.1016/j.carbpol.2015.09.039

Ghannadi, A., Shabani, L., & Yegdaneh, A. (2016). Cytotoxic, antioxidant and phytochemical analysis of Gracilaria species from Persian Gulf. *Advanced biomedical research*, *5*(1), 139.

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2015). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules*, *15*(6), 4324–4333.

Gillison, M. L., Chaturvedi, A. K., Anderson, W. F., & Fakhry, C. (2015). Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. Journal of Clinical Oncology, 33(29), 3221-3233. https://doi.org/10.1200/JCO.2015.61.6995

Gross, J. H. (2011). *Mass Spectrometry: A Textbook* (2nd ed.). Springer.

GUIRY, M. D. (2010). AlgaeBase. World-wide electronic publication, National university of Ireland, Galway. *http://www. algaebase. org/*.

Gul, M. Z., Bhakshu, L. M., Ahmad, F., Kondapi, A. K., Qureshi, I. A., & Ghazi, I. A. (2017). Evaluation of Abelmoschus moschatus extracts for antioxidant, free radical scavenging, anticancer, and antimicrobial activities. BMC Complementary and Alternative Medicine, 17(1), 1-9.

Gupta, S., Bhaskar, N., & Bijo, A. J. (2019). Solvent extraction and anticancer activity of marine macroalgae-derived compounds. Journal of Marine Biology, 45(2), 89-101. Haddad, R. I., Posner, M. R., & Forastiere, A. A. (2014). Advances in the treatment of head and neck cancer: A review of recent data. Journal of Clinical Oncology, 32(23),

Giménez-Campillo C, Pastor-Belda M, Campillo N, Arroyo-Manzanares N, Hernández-Córdoba M, Viñas P. Determination of cyanotoxins and phycotoxins in seawater and algae-based food supplements using ionic liquids and liquid chromatography with time-of-flight mass spectrometry. Toxins. 2019 Oct 22;11(10):610.

Hamed, I., Foucault, A., & Berthon, J. Y. (2015). Marine algae in drug discovery: A review of recent developments. *Marine Drugs, 13*(10), 5696–5726.

Harris, D. C. (2010). *Quantitative Chemical Analysis* (8th ed.). W. H. Freeman.

Hentati F, Delattre C, Gardarin C, Desbrières J, Le Cerf D, Rihouey C, Michaud P, Abdelkafi S, Pierre G. Structural features and rheological properties of a sulfated Xylogalactan-rich fraction isolated from Tunisian red seaweed Jania adhaerens. Applied Sciences. 2020 Mar 1;10(5):1655.

Hentati, F., Delattre, C., Gardarin, C., Desbrières, J., Le Cerf, D., Rihouey, C., ... & Pierre, G. (2020). Structural features and rheological properties of a sulfated Xylogalactan-rich fraction isolated from Tunisian red seaweed Jania adhaerens. *Applied Sciences*, *10*(5), 1655.

Herrero, M., Cifuentes, A., & Ibañez, E. (2020). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae, and microalgae: A review. Food Chemistry, 98(1), 136–148.

Hii, S. L., Lim, J. Y., Ong, W. T., & Wong, C. L. (2016). Agar from Malaysian red seaweed as potential material for synthesis of bioplastic film. *Journal of Engineering Science and Technology*, *7*, 1-15.

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of applied phycology*, *23*, 543-597.

Huisman, J. M., Dixon, R. R., Townsend, R. A., & Belton, G. (2021). Diversity and distribution of marine benthic algae and seagrasses in the tropical Kimberley, Western Australia. *Rec Western Austr Mus*, *185*(200), 185-200.

Hussain, A., Rahman, S., & Kim, B. (2021). Fucoidans and laminarins as bioactive compounds in cancer therapy: A review. *Marine Drugs, 19*(5), 287.

Ilavarasi, K., Chermakani, P., Arif Nisha, S., Sheeja Malar, D., & Pandima Devi, K. (2015). Antioxidant compounds in the seaweed Gelidiella acerosa protects human peripheral blood mononuclear cells against TCDD induced toxicity. *Drug and Chemical Toxicology*, *38*(2), 133-144.

Je, J. Y., Park, P. J., Kim, E. K., & Ahn, C. B. (2021). Extraction of bioactive compounds from seaweeds: Methods and applications. Journal of Applied Phycology, 33(2), 967–981. https://doi.org/10.1007/s10811-021-02458-3

Ji, Y., Zhang, Z., & Zhao, H. (2018). Antibacterial potential of Sargassum fusiforme. Journal of Applied Phycology.

Jiang, H., Zhang, X., & Xu, J. (2021). Seaweed extracts: A review of their potential anti-cancer effects and mechanisms of action. Journal of Cancer Research and Clinical

Jiang, Y., Guo, X., Wang, W., & Wu, Y. (2022). Advances in seaweed-derived bioactive compounds: Extraction, purification, and biological activities. Marine Drugs, 20(5), 272.

Johnson, D. E., Burtness, B., Leemans, C. R., Lui, V. W. Y., Bauman, J. E., & Grandis, J. R. (2020). Head and neck squamous cell carcinoma. *Nature Reviews Disease Primers*, *6*(1), 1-22

Jung, J. H., Lee, S. H., & Kim, J. (2018). A comparative study on solvent extraction of bioactive compounds from marine organisms. Marine Drugs, 16(9) 355.

Kaliaperumal, N., Kalimuthu, S., & Ramalingam, J. R. (2004). Seaweed resources, cultivation, and utilization in India. *Journal of Applied Phycology*, *16*(4), 325–331.

Kang, M. C., Jeon, Y. J., & Kim, K. N. (2018). Fucoidan from seaweeds as an effective anticancer agent. Marine Drugs, 16(4), 113-127.

Kannan, S., Bhaskar, M., & Singh, D. (2022). Phytochemicals in Gracilaria corticata: Antioxidant and antibacterial properties. Indian Journal of Marine Science.

Kathiresan, K., & Bingham, B. L. (2011). Biology of mangroves and mangrove ecosystems. Advances in Marine Biology, 40, 81-251. https://doi.org/10.1016/s0065-2881(01)40003-4

Khameneh, B., & Ghanbarzadeh, S. (2020). The effect of solvent polarity on the extraction of phenolic compounds from medicinal plants. Journal of Pharmaceutical Sciences, 109(4), 1274-1280. https://doi.org/10.1016/j.xphs.2019.09.024

Khan, M. N., Khan, M. I., Kumar, A., & Khan, M. S. (2018). Extraction, characterization, and applications of agar from Gelidiella acerosa. Journal of Applied Phycology, 30(1), 123-134. https://doi.org/10.1007/s10811-017-1120-0

Khan, M. Y., Raza, M. A., & Yasmin, A. (2021). The rich biodiversity of seaweeds along the Indian coastline and their biochemical adaptation to marine conditions. Journal of Applied Phycology, 33(6), 4001-4015. https://doi.org/10.1007/s10811-021-02549-0

Khongthong S, Theapparat Y, Roekngam N, Tantisuwanno C, Otto M, Piewngam P. Characterization and immunomodulatory activity of sulfated galactan from the red seaweed Gracilaria fisheri. International journal of biological macromolecules. 2021 Oct 31; 189:705-14.

Khongthong, S., Theapparat, Y., Roekngam, N., Tantisuwanno, C., Otto, M., & Piewngam, P. (2021). Characterization and immunomodulatory activity of sulfated galactan from the red seaweed Gracilaria fisheri. *International journal of biological macromolecules*, *189*, 705-714.

Khoo, K. S., Teh, J. S., & Lim, J. W. (2021). Efficiency of methanol and other solvents in bioactive compound extraction from marine sources. Algal Research, 53, 102104. https://doi.org/10.1016/j.algal.2021.102104

Kim, H. J., & Park, J. K. (2018). Identification of bioactive compounds through thin-layer chromatography (TLC) in marine-derived extracts. Marine Biology Research, 12(4), 78-85. https://doi.org/10.1080/17451000.2018.1463478

Kim, J. H., Han, J. G., & Lim, D. H. (2019). Dry yield percentages and biochemical properties of red seaweeds in marine ecosystems. Journal of Phycology, 55(4), 874- 880.

Kim, J. K., Kraemer, G. P., & Yarish, C. (2015). Use of seaweed aquaculture for the absorption of nutrients in coastal waters. Aquaculture, 445, 36-41. https://doi.org/10.1016/j.aquaculture.2015.04.022

Kim, S. K., & Wijesinghe, W. A. J. P. (2021). Marine algae: An untapped resource for high-value metabolites. Marine Pharmacognosy,387–400. https://doi.org/10.1016/B978-0-12- 411549-1.00019-5

Kim, S. K., Wijesinghe, W. A. J. P., & Kang, N. (2020). Marine algae-derived phenolic compounds and their antioxidant activities. Journal of Marine Science and Engineering, 8(8), 528. https://doi.org/10.3390/jmse8080528

Klein, M. O., Rodrigues, L. A. G., & Rapozo, D. C. M. (2017). Laryngeal carcinoma: Mechanisms, molecular markers, and novel therapeutic strategies. Current Pharmaceutical Design, 23(27), 413

Kogure, T., Ueda, K., Nakatani, S., & Miyata, Y. (2017). Cytotoxic effects of marine red algae extracts on human keratinocytes (HaCaT) and their potential anticancer properties. Journal of Applied Phycology, 29(5), 2451–2458. https://doi.org/10.1007/s10811-017-1131-2

Krishnaiah, D., Sarbatly, R., & Bono, A. (2011). Phytochemical antioxidants for health and medicine—A move towards nature. Biotechnology and Molecular Biology Review, 6(5), 97-104.

Krishnamurthy, K., Balasubramanian, V., & Ashok, T. (2018). Solvent-mediated extraction of bioactive compounds from marine algae. Pharmaceutical Sciences and Research, 15(3), 72- 80.

Krishnan, M., & Narayanakumar, R. (2013). Seaweed resources of India: Commercial exploitation and production trends. Indian Journal of Geo-Marine Sciences, 42(3), 361365.

Kumar, A., Ramesh, B., & Nair, S. (2021). Anticancer potential of Gracilaria corticata in skin cancer cells. Indian Journal of Phytotherapy.

Kumar, A., Singh, D., & Sharma, M. (2021). Evaluation of antioxidant properties in Gracilaria edulis. Indian Journal of Marine Science.

Kumar, R., Singh, J., & Kaur, R. (2021). Antioxidant and cytotoxic activities of seaweed extracts using different solvent systems. Marine Biology Research, 17(4), 292-302. https://doi.org/10.1080/17451000.2021.1916034

Kumar, S., Gupta, R., & Kumar, G. (2022). Antioxidant and free radical scavenging potential of Sargassum wightii. Journal of Agricultural and Food Chemistry, 59(6), 2286–2293. https://doi.org/10.1021/jf104345k

Kumar, S., Reddy, T. S., & Rao, S. (2019). Ecological and economic significance of Gelidiella acerosa in Tamil Nadu. Journal of Applied Phycology, 31(2), 123-132.

Kumar, S., Sandhir, R., & Sharma, S. (2021). Solvent polarity effects on antioxidant activity and phenolic content of selected medicinal plants. *Plant Archives*, *21*(1), 123–131.

Kurt, O., Ozdal-Kurt, F., Tuglu, I., Deliloglu-Gurhan, S. I., & Ozturk, M. (2009). Neurotoxic effect of Caulerpa racemosa var. cylindracea by neurite inhibition on the neuroblastoma cell line. *Russian Journal of Marine Biology*, *35*, 342-350.

Lee, C. H., Moon, S. K., & Kim, K. D. (2018). Anticancer potential of terrestrial plant extracts against human keratinocyte cell line (HaCaT). Biomedicine & Pharmacotherapy, 102, 1112-1120. https://doi.org/10.1016/j.biopha.2018.04.152

Lee, S. Y., Kang, S. J., & Choi, H. W. (2017). Stepwise elution of bioactive components using silica gel chromatography in natural product research. Analytical Methods, 9(6), 895-902. https://doi.org/10.1039/C6AY03248D

Leemans, C. R., Braakhuis, B. J., & Brakenhoff, R. H. (2011). Head and neck cancer: Early detection and treatment. European Journal of Cancer, 47(5), 1501-1507. https://doi.org/10.1016/j.ejca.2011.01.031

Leemans, C. R., Snijders, P. J., & Brakenhoff, R. H. (2018). The molecular landscape of head and neck cancer. Nature Reviews Cancer, 18(5), 269-282.

Li, J., Zhang, Q., & Yu, Y. (2019). Antioxidant potential of phenolic compounds isolated from seaweeds. Journal of Food Science and Technology, 56(12), 5426-5434.

Liu, H., Wang, Q., & Liu, Y. (2020). Evaluation of solvent effects on the extraction of phenolic compounds and antioxidant capacities from the leaves of medicinal plants. *Journal of Medicinal Plants Research, 14*(3), 109–117.

Liu, X., Sun, Z., & Liu, Y. (2021). Bioactive potential of seaweed-derived compounds in cancer therapy. Journal of Cancer Research and Therapeutics, 17(7), 1495-1502. https://doi.org/10.4103/jcrt.JCRT_900_20

Liu, Y., Zhang, Q., & Liu, H. (2023). Structural elucidation of polysaccharides from Undaria pinnatifida using NMR spectroscopy. Carbohydrate Polymers, 296, 119890.https://doi.org/10.1016/j.carbpol.2022.119890

Lopes, G., Andrade, P. B., & Valentão, P. (2020). Phytochemical composition and antioxidant potential of seaweeds from different environmental conditions. Food Chemistry, 333, 127451. https://doi.org/10.1016/j.foodchem.2020.127451

López-Hortas, L., Flórez-Fernández, N., Torres, M. D., Ferreira-Anta, T., Casas, M. P., Balboa, E. M., ... & Domínguez, H. (2021). Applying seaweed compounds in cosmetics, cosmeceuticals and nutricosmetics. *Marine drugs*, *19*(10), 552.

Lu, Y., Mei, S., Wang, P., Ouyang, P., Liao, X., Ye, H., ... & Ma, X. (2020). Protective effects of Gracilaria lemaneiformis extract against ultraviolet B-induced damage in HaCaT cells. *Pharmacognosy Magazine*, *16*(71).

Makkar, H. P. S., Tran, G., Heuzé, V., & Giger-Reverdin, S. (2016). Effects of drying temperature on the nutrient composition of *Ascophyllum nodosum*. *Animal Feed Science and Technology*, *221*, 197–204.

Malarvili, R. S., Sangeetha, M., & Arumugam, R. (2022). Influence of solvent on extraction of bioactive compounds from marine algae. Phytochemistry Reviews, 21(3), 705-719.

Maneein, S., Milledge, J. J., Nielsen, B. V., & Harvey, P. J. (2018). A review of seaweed pretreatment methods for enhanced biofuel production by anaerobic digestion or Mann, K. H. (2012). Ecology of coastal waters: With implications for management (2nd ed.).

Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T., & Packer, L. (1994). The nitric oxide scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and Biophysical Research Communications, 201(2), 748–755.

Martínez-Patiño, J. C., López-Hernández, J., & Vázquez-Blanco, E. (2022). Comparative phytochemical analysis of brown seaweeds from different geographical locations. Marine Drugs, 20(5), 275.

Marudhupandi, T., Kumar, T. T. A., Lakshmanasenthil, S., Suja, G., & Vinothkumar, T. (2015). In vitro anticancer activity of fucoidan from Turbinaria conoides against A549 cell lines. *International journal of biological macromolecules*, *72*, 919-923.

Matanjun, P., Mohamed, R., & Karim, M. (2022). Anticancer activity of Sargassum

polycystum against breast and cervical cancer cell lines. Journal of Applied Phycology, 34(3), 199-208. https://doi.org/10.1007/s10811-021-02462-x

Matanjun, P., Mohamed, S., & Mustapha, N. (2022). Anticancer effects of Sargassum polycystum on MCF-7 and HeLa cell lines. Journal of Applied Marine Biology.

Mehanna, H., Rattay, T., & Roberts, D. (2021). De-escalation of treatment for HPV- positive oropharyngeal carcinoma. The Lancet Oncology, 22(10), e405-e414. https://doi.org/10.1016/S1470-2045(21)00385-6

Mehra, R., Bhushan, S., Yadav, U. P., Bast, F., & Singh, S. (2019). Caulerpa taxifolia inhibits cell proliferation and induces oxidative stress in breast cancer cells. *Biologia, 74*, 187-193.

Mehta, R., Sharma, P., & Joshi, S. (2022). Evaluation of Padina tetrastromatica on breast cancer cells. Indian Journal of Marine Biotechnology.

Mehta, R., Singh, N., & Prasad, S. (2022). Inhibition of lipid peroxidation by Sargassum

Mesía, R., Rivera, F., & Rivas, J. (2022). New advances in immunotherapy for head and neck cancer. Current Oncology Reports, 24(3), 287-298.

Miller, N. J., & Rice-Evans, C. A. (1996). Spectrophotometric determination of antioxidant activity. Redox Report,2(3), 161–171. https://doi.org/10.1080/13510002.1996.11747044

Mishra, R., Jha, P., & Dey, S. (2019). Purification and biological activity of compounds isolated using high-resolution chromatography. Journal of Natural Product Research, 33(9), 1325- 1334. https://doi.org/10.1080/14786419.2019.1571365

Mishra, V. K., Kumar, P., Sharma, R. P., & Srivastava, G. (2012). Seaweed diversity along the Tamil Nadu coast. Journal of Marine Biology, 2012(3), 1-7.

Mohan, V., Priya, R., & Thomas, A. (2019). Caulerpa racemosa as a source of antioxidants.

Moradi, Z., Baghery, H., & Nasri, M. (2022). Profiling of marine polyphenols in Sargassum species using LC-MS: A promising approach for nutraceutical applications. Marine Drugs, 20(1), 23. https://doi.org/10.3390/md20010023.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65(1-2),* 55-63. muticum extracts. Indian Marine Biotechnology Journal.

Narayan, B., Kumar, B. B., & Sridhar, M. (2021). Advances in drying technology of seaweeds. Journal of Food Engineering, 290, 110147.

Nayak, S. B., Sahu, R., & Patra, S. K. (2022). Enhanced antioxidant activity of marine algal extracts using acetone. Journal of Marine Biology, 68(2), 145-155.

Nayak, S., Das, S., & Dutta, S. (2022). Extraction of bioactive compounds from marine algae using acetone solvent system: A comparative study. *Journal of Phytochemistry*, 18(4), 67-78. [DOI: 10.1016/j.phytochem.2022.04.012]

Nugraha, A. S., Keller, P. A., & Suzuki, T. (2021). Bioactive compounds from marine algae: Isolation, characterization, and applications.

Nursid, M., & Chasanah, E. (2013). Cytotoxic activity and apoptosis induction of T47D cell lines by Turbinaria decurrens extract. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *8*(1), 23-28.

Nutting, C. M., Morden, J. P., & Harrington, K. J. (2011). Intensity-modulated radiotherapy (IMRT) in the treatment of head and neck cancer. European Journal of Cancer, 47(16), 2526-2535. https://doi.org/10.1016/j.ejca.2011.06.021

Oncology, 147(8), 2447-2461. https://doi.org/10.1007/s00432-021-03412-2

Pacheco D, Cotas J, Rocha CP, Araújo GS, Figueirinha A, Gonçalves AM, Bahcevandziev K, Pereira L. Seaweeds' carbohydrate polymers as plant growth promoters. Carbohydrate Polymer Technologies and Applications. 2021 Dec 25; 2:100097.

Pacheco, D., Cotas, J., Rocha, C. P., Araújo, G. S., Figueirinha, A., Gonçalves, A. M., ... & Pereira, L. (2021). Seaweeds' carbohydrate polymers as plant growth promoters. *Carbohydrate Polymer Technologies and Applications*, *2*, 100097.

Padmapriya, R., Arockiasamy, P., & Sasi, R. (2018). Cytotoxicity of non-polar solvent extracts of seaweeds against cancer cell lines. Asian Pacific Journal of Cancer Prevention, 19(11), 3171-3176. https://doi.org/10.31557/APJCP.2018.19.11.3171

Pandolfi, J. M., Connolly, S. R., Marshall, D. J., & Cohen, A. L. (2011). Projecting coral reef futures under global warming and ocean acidification. Science, 333(6041), 418-422. https://doi.org/10.1126/science.1204794

Pangestuti, R., Kim, S. K., & Wijesinghe, W. A. J. P. (2020). Marine algae-derived functional ingredients for cosmeceuticals: A review. Journal of Marine Science and Engineering, 8(8), 605. https://doi.org/10.3390/jmse8080605

Pangestuti, R., Wijaya, A., & Purnomo, E. (2020). Bioactive compounds in Padina sp. and their antibacterial efficacy. Phycologia.

Patra, J. K., Baek, K. H., & Jena, P. K. (2020). Marine algae-derived bioactive compounds for skin cancer treatment: A comprehensive review. Marine Drugs, 18(6), 302-322. https://doi.org/10.3390/md18060302

Patra, S., Muthuraman, M. S., Prabhu, A. T. J., Priyadharshini, R. R., & Parthiban, S. (2015). Evaluation of antitumor and antioxidant activity of Sargassum tenerrimum against Ehrlich ascites carcinoma in mice. *Asian Pacific Journal of Cancer Prevention*, *16*(3), 915-921.

Pazzanese, C., & Lima, A. (2020). Antioxidant activity of plant extracts: A review of the role of different extraction methods and solvents. Molecules, 25(12), 2759. https://doi.org/10.3390/molecules25122759

Phan, C. W., & Lee, H. B. (2017). Comparison of extraction methods for bioactive compounds from medicinal plants. Journal of Medicinal Plants Research, 11(10), 150-Pharmaceutical Biology. potential of Gelidiella acerosa: A red seaweed. Natural Product Research, 36(7), 1719–1726.

Povey, M. J. W., & McCarthy, J. (2019). Supercritical fluid extraction from marine algae: Review of current methods. Marine Drugs, 17(5). 289.

Pradhan, B., Patra, S., Behera, C., Nayak, R., Patil, S., Bhutia, S. K., & Jena, M. (2020). Enteromorpha compressa extract induces anticancer activity through apoptosis and autophagy in oral cancer. *Molecular Biology Reports*, *47*, 9567-9578.

Prasath, A. K., Mohan, S. K., & Selvam, K. (2023). Seaweeds as a source of bioactive compounds for cancer prevention and therapy: A review. Food Chemistry, 386, 132703. https://doi.org/10.1016/j.foodchem.2022.132703

Prasath, R., Kumar, V., & Singh, S. (2023). Review of seaweed-derived anticancer compounds and their mechanisms. International Journal of Oncology.

Pratchett, M. S., Anderson, K. D., Hoogenboom, M. O., Widman, E., Baird, A. H., Pandolfi, R. (2020). Head and neck squamous cell carcinoma. Nature Reviews Disease Primers, 6(1), 92. https://doi.org/10.1038/s41572-020-00224-3

Prabhachandh, S. R., & Babychan, N. (2017). Phytochemical Analysis of Murraya Koenigii in Urban and Coastal Area. 4(10), 238–240. www.jetir.org

Raghavendra, S., Kumar, P., & D'Souza, P. (2022). Review on antioxidant properties of seaweeds and their functional applications. Pharmaceutical and Nutraceutical

Raghavendra, S., Zinjarde, S., & Ghosh, S. (2022). Seaweeds as a source of antioxidants: A review on their bioactive compounds and health benefits. Journal of Marine Science and Engineering, 10(8), 1135. https://doi.org/10.3390/jmse10081135

Raghavendra, S., Zinjarde, S., & Ghosh, S. (2023). Impact of dietary supplements containing seaweed extracts on cancer patients: A clinical trial. Nutrients, 15(1), 56. https://doi.org/10.3390/nu15010056

Raja, K., Uthayakumar, V., Suresh, V., & Rathika, P. (2019). Antioxidant and anticancer properties of marine red algae (Gelidiella acerosa) against HeLa cell lines. Journal of King Saud University-Science, 31(4), 782-787.

Rajeshkumar, S. (2017). Phytochemical constituents of fucoidan (Padina tetrastromatica) and its assisted AgNPs for enhanced antibacterial activity. *IET nanobiotechnology*, *11*(3), 292-299.

Ramesh, T. P., Rao, G. V., & Kumar, K. M. (2020). Morphological, biochemical, and genetic diversity in marine algae: A review. Journal of Applied Phycology, 32(2), 567–580.

Rao, P. S., Devi, P., & Reddy, C. V. (2021). Phytochemical screening and bioactivity of selected Indian seaweeds. Journal of Marine Biotechnology, 29(4), 234–240.

Rao, S. K., Sharma, R., & Jha, P. (2019). Marine biodiversity and bioactive potentials of seaweeds in India. Indian Journal of Marine Sciences, 48(2), 174-184.

Rathod, S., & Kadam, P. (2017). Phytochemical composition and antimicrobial properties of Caulerpa racemosa. Pharmaceutical Biology.

Ravikumar, R., Harikrishnan, H., & Manikandan, N. (2021). Evaluation of anticancer properties of marine algae extracts. Journal of Applied Phycology, 33(5), 2157- 2166.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26(9-10),* 1231-1237.

Remya, R. R., Radhika Rajasree, S. R., Aranganathan, L., Suman, T. Y., & Gayathri, S. (2017). Enhanced cytotoxic activity of AgNPs on retinoblastoma Y79 cell lines synthesised using marine seaweed Turbinaria ornata. *IET nanobiotechnology*, *11*(1), 18-23.

Rengasamy, K., Mahadevan, N., & Kumari, S. (2021). Comparative anticancer activities of seaweed extracts from various solvent systems. Phytotherapy Research, 35(9), 5012-5020. https://doi.org/10.1002/ptr.7183 Reviews.

Rita, S., Das, B., & Sharma, R. (2017). Fatty acid composition of Gelidiella acerosa and its potential application in nutritional supplements. Journal of Applied Phycology, 29(1), 221-229. https://doi.org/10.1007/s10811-016-0903-5

Roh, S. W., Yoon, M. S., & Shin, K. K. (2014). Carrageenans from red seaweeds: Properties and potential applications. Marine Biotechnology, 16(1), 29-40.

Rojas, M., Santos, C., & Lopez, A. (2019). Evaluation of Laminaria japonica phytochemicals for antimicrobial applications. Marine Drugs.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea.

Rahbar Saadat, Y, Nazli Saeidi, Zununi Vahed. S, Abolfazl Barzegari, Jaleh Barar. An update to DNA ladder assay for apoptosis detection. BioImpacts, 2015, 5(1), 25-28.

Sadeghi, N., & Shaterian, H. R. (2018). Assessment of antioxidant activity of extracts from various plants and herbs: A comparative study. International Journal of Food Properties, 21(1), 295-310. https://doi.org/10.1080/10942912.2017.1341222

Sahu, R., Choudhary, S., & Das, A. (2023). Comparative study of extraction methods on antioxidant properties of marine algal extracts. Food Chemistry, 397, 133721. https://doi.org/10.1016/j.foodchem.2022.133721

Samar, J., Butt, G. Y., Shah, A. A., Shah, A. N., Ali, S., Jan, B. L., Abdelsalam, N. R., & Hussaan, M. (2022). Physicochemical and biological activities from different extracts of Padina antillarum (Kützing) Piccone. Frontiers in Plant Science, 13, 1–

Sanjeevi, A., Kumar, R. S., & Ganesan, M. (2020). Comparative study on phenolic compounds extraction from brown algae Sargassum wightii using various solvents

Santos, C. M. D., Cordeiro, A. M. T. M., & Santos, D. C. (2018). Comparison of antioxidant activity and phenolic content of medicinal plant extracts using different solvents. *Asian Journal of Biomedical and Pharmaceutical Sciences, 8*(65), 5–10.

Sasidharan, S., Nursamsu, A., & Kartika, I. (2020). Evaluation of the anticancer properties of marine seaweed extracts. Journal of Marine Drugs, 18(6), 312. https://doi.org/10.3390/md18060312

Seiwert, T. Y., & Cohen, E. E. (2005). State-of-the-art management of locally advanced head and neck cancer. British Journal of Cancer, 92(8), 1341-1348. https://doi.org/10.1038/sj.bjc.6602529

Seiwert, T. Y., Burtness, B., & Sausville, E. A. (2016). Pembrolizumab for the treatment of advanced head and neck squamous cell carcinoma: A phase 1b/2 trial. Journal of

Senthilkumar, D., & Jayanthi, S. (2016). Partial characterization and anticancer activities of purified glycoprotein extracted from green seaweed Codium decorticatum. *Journal of Functional Foods*, *25*, 323-332.

Senthilkumar, P., Surendran, L., Sudhagar, B., & Ranjith Santhosh Kumar, D. S. (2019). Facile green synthesis of gold nanoparticles from marine algae Gelidiella acerosa and evaluation of its biological potential. SN Applied Sciences, 1, 1-12.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Shanmugam, S., Venkatesh, J., & Rajendran, P. (2022). Extraction and analysis of anticancer agents from marine algae using different solvents. Algal Research, 65, 102631. https://doi.org/10.1016/j.algal.2021.102631

Shanura Fernando, I. P., Asanka Sanjeewa, K. K., Samarakoon, K. W., Kim, H. S., Gunasekara, U. K. D. S. S., Park, Y. J., ... & Jeon, Y. J. (2018). The potential of fucoidans from Chnoospora minima and Sargassum polycystum in cosmetics: Antioxidant, anti-inflammatory, skin-whitening, and antiwrinkle activities. *Journal of Applied Phycology*, *30*, 3223-3232.

Sharma, V., & Kumar, P. (2019). Anticancer properties of Sargassum muticum on HT-29 colon cancer cells. Indian Marine Biology Journal.

Sharma, V., Kumar, P., & Verma, A. (2020). Phytochemical screening of Enteromorpha compressa. Journal of Coastal Research.

Sharma, V., Mehta, R., & Kumar, S. (2020). Antioxidant analysis of Enteromorpha compressa. Journal of Coastal Research.

Sheeja, L., Lakshmi, D., Bharadwaj, S., & Parveen, K. S. (2016). Anticancer activity of phytol purified from Gracilaria edulis against human breast cancer cell line (MCF-7). *Int J Curr Sci*, *19*(4), 36-46.

Shobana, V., Ramesh, M., & Devi, P. S. (2022). Solvent extraction efficiency of bioactive compounds from *Gelidiella acerosa*: A comparative study. *Marine Biotechnology Journal, 24*(3), 112–126.

Shrivastava, A., & Gupta, V. B. (2021). HPLC: Instrumentation and applications. In Chromatography and Its Applications. IntechOpen. https://doi.org/10.5772/59315

Siddhanta, A. K., Meena, R., & Chatterjee, P. (2021). Marine algae: Advances in extraction technologies and applications. Marine Drugs, 19(7), 376.

Siddhanta, A. K., Meena, R., & Mohan, K. (2021). Marine algal research in India: Present status and future prospects. Botanical Review, 87(4), 279–324. https://doi.org/10.1007/s12229-021-09236-y

Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2005). *Spectrometric Identification of Organic Compounds* (7th ed.). Wiley.

Sim, J., Kim, Y., & Choi, L. (2022). Ascophyllum nodosum extract and apoptosis induction in HepG2 cells. Journal of Experimental Oncology.

Sim, J., Park, H., & Cho, S. (2022). Clinical trial of Laminaria antioxidant supplements in metabolic syndrome. Journal of Clinical Nutrition and Health.

Sim, K. W., Lee, C. H., & Jeon, B. T. (2022). Effects of Ascophyllum nodosum extract on HepG2 liver cancer cells: A mechanistic study. Marine Drugs, 20(10), 669.

Sim, K. W., Lee, C. H., & Jeon, B. T. (2022). Effects of Laminaria extracts on oxidative

Singh, A., Kumar, V., & Gupta, N. (2020). Cytotoxic and antiproliferative effects of isolated compounds from marine algae on human cell lines. Marine Drugs, 18(11),

Singh, H. S., Parihar, R., & Patel, K. (2012). Marine biodiversity in India: Status and conservation strategies. Indian Journal of Geo-Marine Sciences, 41(3), 1-6.

Singh, M., & Gupta, P. (2018). Antioxidant potential of Ulva lactuca influenced by environmental factors. Asian Journal of Phycology.

Singh, M., & Gupta, P. (2020). Cytotoxic effects of Ulva lactuca on lung cancer cells. Asian Journal of Phycology.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

SM, F. M. B., Chitra, K., Joseph, B., Sundararajan, R., & S, H. (2018). Gelidiella acerosa inhibits lung cancer proliferation. *BMC Complementary and Alternative Medicine*, *18*, 1-14.

Smith, M. A., Thompson, R., & Williams, J. (2022). High-performance liquid chromatography analysis of bioactive compounds: Application in cancer research. Journal of Chromatographic Science,60(3), 450-460.

Smith, V. J. (2004). Marine-derived antimicrobial agents: The untapped resource. *Trends in Biotechnology*, *22*(11), 586–592.

Socrates, G. (2004). *Infrared and Raman Characteristic Group Frequencies: Tables and Charts* (3rd ed.). Wiley.

Stransky, N., Egloff, A. M., & Wang, X. (2011). The mutational landscape of head and neck squamous cell carcinoma. Science, 333(6046), 1157-1160.

stress in patients with metabolic syndrome: A clinical trial. Nutrients, 14(12), 2580. https://doi.org/10.3390/nu14122580

Sturgis, E. M., & Cinciripini, P. M. (2007). Trends in head and neck cancer incidence in relation to smoking and drinking: An analysis of the SEER program. Journal of Clinical Oncology, 25(20), 2287-2292. https://doi.org/10.1200/JCO.2006.09.0135

Taheri, A., Ghaffari, M., & Namavari, M. (2018). The effects of seaweed Gracilaria arcuata extract on the stimulation of apoptosis in colorectal cancer cell lines. *Alborz*

University Medical Journal, *7*(4), 281-292.

Tan, S. H., Lee, C. W., & Loke, W. M. (2019). Anticancer activities of plant-derived compounds against human cancer cells. Scientific Reports, 9(1), 8396. https://doi.org/10.1038/s41598- 019-44748-y

Tanna, B., Choudhary, B., Mishra, A., Yadav, S., Chauhan, O. P., Elansary, H. O., ... & Mahmoud, E. A. (2022). Biochemical and Anti-proliferative activities of seven abundant tropical red seaweeds confirm nutraceutical potential of Grateloupia indica. *Arabian Journal of Chemistry*, *15*(6), 103868.

Thirumaran, G., Anantharaman, P., Devi, G. K., & Ramalingam, J. R. (2014). Seaweed distribution and diversity along the southeast coast of India. Indian Journal of Geo-Marine Sciences, 43(8), 1494-1504.

Tim, M. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65, 55- 63.

Trabelsi, N., Bejar, W., Bouabdallah, F., Proksch, P., & Maaroufi, R. (2016). Antiproliferative and proapoptotic activities of seaweed extracts on human cervical cancer cells. Biomedicine & Pharmacotherapy, 83, 592-598. https://doi.org/10.1016/j.biopha.2016.06.017. tropical vs. temperate seaweeds. International Journal of Phycology.

Twilley, R. R., Rivera-Monroy, V. H., Chen, R., & Botero, L. (2012). Adapting to climate variability in mangrove ecosystems of North Brazil: Potential effects of sea level rise. Estuarine Coastal and Shelf Science, 96(1), 15-22.

Vaikundamoorthy, R., Krishnamoorthy, V., Vilwanathan, R., & Rajendran, R. (2018). Structural characterization and anticancer activity (MCF7 and MDA-MB-231) of polysaccharides fractionated from brown seaweed Sargassum wightii. *International journal of biological macromolecules*, *111*, 1229-1237.

van Houten, V., Kranenburg, O., & de Vries, E. G. (2006). PIK3CA mutations and their role in head and neck squamous cell carcinoma. European Journal of Cancer, 42(9), 1727-1736. https://doi.org/10.1016/j.ejca.2006.03.010

Veeragurunathan, V., Mandal, S. K., Vizhi, J. M., Grace, P. G., & Gurumoorthy, U. (2022). Studies on seaweeds diversity along the intertidal zone of islands of Gulf of Mannar Marine Biosphere Reserve, India for policy and management recommendation:

Studies on seaweeds diversity along the intertidal zone of islands of Gulf of Mannar Marine Bios. Journal of Coastal Conservation, 26(4), 1–26. https://doi.org/10.1007/s11852-022- 00878-z

Vel, A., Quintana, E., & Garrido, R. (2014). A critique of marine protected areas management in India: Lessons from comparative analysis. Ocean & Coastal Management, 87, 97-109.

Vermorken, J. B., Remenar, E., & van Herpen, C. (2007). Cisplatin, fluorouracil, and leucovorin in recurrent or metastatic head and neck cancer: A phase III study. Journal of Clinical Oncology, 25(11), 1671-1678. https://doi.org/10.1200/JCO.2006.07.0464

Villarreal-Gómez, L. J., Soria-Mercado, I. E., Guerra-Rivas, G., & Ayala-Sánchez, N. E. (2010). Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. *Revista de biología marina y oceanografía*, *45*(2), 267-275.

Vincken, J. P., Heng, L., de Groot, A., & Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry, 68(3),* 275-297.

Visweswari, G., Christopher, R., & Rajendra, W. (2013). Phytochemical screening of active secondary metabolites present in Withania somnifera root: Role in traditional medicine. International Journal of Pharmaceutical Sciences and Research, 4(43), 2770–2776.

Wagner, H., & Bladt, S. (1996). Plant drug analysis: A thin layer chromatography atlas (2nd ed.). Springer.

Wang, C. Y., Wu, T. C., Hsieh, S. L., Tsai, Y. H., Yeh, C. W., & Huang, C. Y. (2015). Antioxidant activity and growth inhibition of human colon cancer cells by crude and purified fucoidan preparations extracted from Sargassum cristaefolium. *Journal of food and drug analysis*, *23*(4), 766-777.

Wang, Y., Zhang, H., Liu, X., & Li, J. (2022). A comprehensive study on the extraction and identification of saponins from medicinal plants. *Phytochemistry Reviews, 21*(3), 415–430.

Wijesinghe, W. A. J. P., & Jeon, Y. J. (2022). Methanol as an efficient solvent for the extraction of antioxidants from *Ecklonia cava*: A comparative study with other solvents. *Journal of Applied Phycology, 34*(5), 1251–1265.Wiley.

Wilkinson, C. (2013). Status of coral reefs of the world: 2013. Global Coral Reef Monitoring Network.

Williams, D. H., & Fleming, I. (2007). *Spectroscopic Methods in Organic Chemistry* (6th ed.). McGraw-Hill.

Wu, Q., Liu, Y., & Zhang, L. (2023). Mechanisms of antioxidant effects in Fucus vesiculosus and Laminaria japonica. Marine Biomedicine.

Wu, Q., Wang, Z., & Zhang, H. (2023). Antioxidant mechanisms of brown seaweeds: Implications for health and disease. Marine Drugs, 21(2), 75. https://doi.org/10.3390/md21020075

Xu, J., Liao, W., Liu, Y., Guo, Y., Jiang, S., & Zhao, C. (2023). An overview on the nutritional and bioactive components of green seaweeds. Food Production, Processing and Nutrition, 5(1).

Yamamoto, O., Hattori, M., & Komatsu, H. (2012). *Proton Nuclear Magnetic Resonance Spectroscopy in Structural Analysis of Organic Compounds.* Journal of Magnetic Resonance, 225, 34-45.

Yang, J., Zhang, Y., & Zhang, X. (2017). Terpenoid compounds from marine algae: Recent advances and potential applications. Marine Drugs, 15(6), 191. https://doi.org/10.3390/md15060191

Yilmaz, H., Çakmak, Y. S., & Yıldız, A. (2023). UV-Vis spectroscopic analysis of antioxidant compounds in Fucus vesiculosus. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 284, 121852.

Yuan, Y. V., Bone, D. E., & Carrington, M. F. (2023). Comparative antioxidant activity of marine algae: A study on temperate and tropical species. Phytochemistry, 199, 113278. https://doi.org/10.1016/j.phytochem.2022.113278

Y Uematsu 1, K Hirata, K Saito, I. K. (12968 B.C.E.). Spectrophotometric determination of saponin in Yucca extract used as food additive. J AOAC Int . 2000 Nov-Dec;83(6):1451- 4.s

Zhang, H., Yuan, Y., Zhu, X., Xu, R., Shen, H., Zhang, Q., & Ge, X. (2022). Physicochemical properties and volatile compounds from [Information missing].

Zhang, L., Sun, Y., & Li, M. (2023). Anticancer activity of polysaccharides from Laminaria japonica against HCT116 colon cancer cells. Phytochemistry, 202, 113309.

Zhang, Y., Chen, L., & Fang, W. (2023). Anticancer activities of Laminaria japonica polysaccharides on HCT116 cells. Marine Pharmacology Research.

Zhang, Y., Wang, Y., Chen, H., & Li, H. (2023). Antioxidant activities of plant extracts: The role of solvent selection. Journal of Food Science, 88(1), 112-123.

Zhou, Y., Li, X., Zhao, W., & Wang, L. (2020). Effects of drying methods on the nutrient retention and antioxidant properties of *Porphyra* species. *Journal of Food Processing and Preservation, 44*(10),.

भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणीक्षेत्रीयकेन्द्र / Southern Regional Centre
टी.एन.ए.यूकैम्पस/ T.N.A.U. Campus
लाउलीरोड/ Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003
टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफैक्स/ Telefax: 0422- 2432835
ई-मेल/E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.कें./No.: BSI/SRC/5/23/2021/Tech /85 दिनांक/Date: 11.06.2021

पौधा प्रमाणीकरण प्रमाणपत्र /PLANT AUTHENTICATION CERTIFICATE

The seaweed specimen which has been sent by you for authentication is identified as *Gelidiella acerosa* (Forssk.) Feldmann & Hamel – GELIDIELLACEAE. The identified specimen is returned herewith for preservation in your College/ Department/ Institution Herbarium.

डॉ.एम. यू. शरीफ/Dr. M.U. Sharief
वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष/
Scientist 'E' &Head of Office

वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष
Scientist 'E' & H.O.O.
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
कोयम्बत्तूर / Coimbatore - 641 003.

सेवा में / To

Ms. Shruti Singh
Ph.D. Research Scholar
Department of Medical Laboratory Sciences
Lovely Professional University
Phagwara, Punjab- 144411

Certificate No. .....

6th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES, ICAATAS 2023 on JUNE 19-21, 2023

Certificate Of Participation

This is to certify that Prof./Dr./Mr./Mrs./Ms. SHRUTI SINGH

Actively participated/presented a paper (Oral/Poster) entitled "Galidiella acerosa : Perspective towards Phytopharmacological aspects"

AS DELEGATE/SCHOLAR/STUDENT IN THE 6th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES (ICAATAS 2023) ON JUNE 19-21, 2023 HELD AT LOYOLA ACADEMY, SECUNDERABAD, TELANGANA-500010, INDIA.

Dr. Akhil Gupta	Dr. Ankush Raut	Mr. Diptanu Banik	Dr. Fr. L. Joji Reddy SJ
(PRESIDENT;SARSD)	(Chief Convener)	(Chief Oraganizing Secretary)	(Conference Director)

International Conference on Recent Trends in Biomedical Sciences (RTBS-2023)

Certificate of Achievement

This is to certify that Prof./Dr./Mr./Ms. Shruti Singh of Lovely Professional University has presented Oral/Poster entitled Galidiella acerosa: Exploring Phytopharmacological Perspectives in International Conference on Recent Trends in Biomedical Sciences (RTBS-2023) organized by Department of Medical Laboratory Sciences from 6th-7th October 2023 at Lovely Professional University, Punjab, India.

Prof (Dr.) Sandeep Sharma
Convenor/Organizing Secretary
RTBS2023

Prof (Dr.) Monica Gulati
Chairperson
RTBS-2023

https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27 (September 2024); 285-294

Research Article

InVitro Evaluation of Natural Extracts as Potential AnticancerAgents: A Study on KB Cell Lines

Shruti Singh[1], Louis Cojandaraj[1*], Ashish Suttee[2], Ashwani Kumar[3]

[1]Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab.
*[1]*Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab.*
[2]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab
[3]Department of Biochemistry, Shri Lal Bahadur Shastri Government Medical College, Mandi at NerChowk H.P

Abstract

Cancer remains a major global health challenge, necessitating continuous exploration of new therapeutic approaches. Natural commodities have emerged as a valuable foundation of potential anticancer driving force. In this study, the cytotoxicity of several extracts obtained from natural sources against KB (human oral epidermal cancer) cell lines was studied. KB cells are a good modelfor cytotoxicity investigations since they are relevant to oral malignancies.

The cell suspension was prepared by trypsinizing KB cell lines and seeding them in 24-well culture plates with different concentrations of the tested samples. Cytotoxicity was assessed using the MTT assay after 48 hours of incubation. Our results revealed a concentration-dependent cytotoxic effect of the tested extracts, with acetone extract demonstrating the highest potency followed by methanol extract. The IC50 values of the extracts were calculated, further confirming their cytotoxic potential. These findings highlight the promising therapeutic utility of natural extracts, particularly acetone and methanol extracts, in cancer treatment. Further elucidation of the underlying molecular mechanisms is essential to harness the full therapeutic potential of theseextracts.

Keywords: Cancer, cytotoxicity, natural extracts, KB cells, MTT assay, IC50.

**Author for correspondence: Email: louis_2286@live.com* *Receiving Date: 10/07/2024 Acceptance Date: 20/08/2024 DOI:https://doi.org/10.53555/AJBR.v27i2 S.1727*

INTRODUCTION:

Cancer represents an alarming global health challenge, amid its incidence and associated mortality rates continuing to rise worldwide. Head and neck cancers (HNC) are a diverse group of malignancies that typically develop in the squamous cells lining the mucosal surfaces of areas such as the oral cavity, pharynx, larynx, nasal cavity, and paranasal sinuses. The predominant type of HNC is squamous cell carcinoma, which represents the majority of cases. Research has firmly established a connection between HNC and lifestyle factors, particularly the use of tobacco and alcohol, which significantly increase cancer risk when combined (1). Furthermore, human papillomavirus (HPV), especially HPV-16, has been increasingly recognized as

key factor in developing oropharyngeal cancers. The incidence of HPV-related oropharyngeal cancers has been rising, particularly among younger individuals, and these cases tend to have a better prognosis compared to those not associated with HPV (2).

Symptoms of HNC can vary based on the tumor's location but often include persistent throat pain, changes in voice, difficulty swallowing, unexplained weight loss, and lumps in the neck (3). Diagnosing HNC early is challenging because early stages are often asymptomatic. Diagnosis generally involves physical exams, imaging tests like CT, MRI, or PET scans, and biopsies to confirm the presence of cancer (4). Treatment strategies for HNC have advanced, often requiring a multidisciplinary

approach including surgery, radiation, and chemotherapy. Targeted therapies and immunotherapies have shown promising results for patients with advanced or recurrent HNC, particularly those focusing on the epidermal growth factor receptor (EGFR) and immune checkpoints (5). The outlook for patients with HNC depends on various factors, including the cancer's location, stage, HPV status, and overall health. Generally, HPV- positive cancers respond better to treatment and have a more favorable prognosis (6). Prevention strategies like quitting smoking, reducing alcohol intake, and getting vaccinated against HPV are crucial in lowering the incidence of these cancers. Additionally, early detection through screening, especially in high-risk groups, can significantly improve outcomes (7).

Despite significant advancements in treatment modalities, the quest for novel and effective anticancer agents remains a critical priority in biomedical research (8). Natural commodities have long been recognized as an invaluable basis for potential therapeutic compounds, offering a vast reservoir of bioactive molecules with diverse chemical structures and biological activities. These natural compounds have historically been usedin drug finding and development innovations, providing the basis for numerous clinically approved anticancer drugs (9, 10).Among the various types of cancer, oral cancers pose a significant burden on public health, accounting for a substantial portion of cancer-related morbidity and mortality globally.

Human oral epidermal carcinoma, particularly represented by KB cell lines, serves as a pertinent model system for studying oral cancer due to its relevance to the disease and its widespread use in cancer research. KB cells, derived from oral epithelial tissues, exhibit characteristics typical of malignant cells and are employed in cytotoxicity studies to see the efficacy of potential anticancer agents (11, 12). In this context, this study most vividly aimed to explore the cytotoxicity of various extracts derived from natural sources against KB cell lines. By assessing the cytotoxic potential of these extracts, we sought to identify novel bioactive compounds with potential anticancer properties. The evaluation of cytotoxicity is a crucial aspect of drug discovery, providing valuable insights into the efficacy and safety profile of potential therapeutic agents. Cytotoxicity assays serve as indispensable tools in screening large libraries of compounds to identify lead candidates for further preclinical and clinical development (13).

Using natural extracts in cancer research offers several advantages, including their structural diversity, target specificity, and potential synergistic interactions among constituent compounds (14). Extracts derived from vegetation, sea life, and microbes harbor a myriad of bioactive molecules, including alkaloids, polyphenols, terpenoids, and peptides, which exhibit diverse pharmacological activities, including anticancer properties (15). Harnessing the therapeutic potential

of natural products renders an optimistic avenue for the growth and creation of new anticancer treatments with enhanced effectiveness and minimized side effects and toxicity compared to conventional chemotherapeutic agents (16).

In light of these considerations, our study aimed to contribute to the growing body of knowledge on natural product-based anticancer research. Through the systematic evaluation of various extracts against KB cell lines, it aimed to recognize major compounds having strong cytotoxic activity, thus, paving the way for further investigation into their underlying mechanisms of exploit and potential remedial applications in cancer treatment. By bridging the gap between traditional knowledge of medicinal plants and modern drug discovery approaches, our study endeavors to accelerate the development of novel anticancer agents along with enhanced efficacy and safety profiles.

Materials and Methods:

Preparation of Cell Suspension: KB cell lines are constantly used in Dulbecco's Modified Eagle's Medium (DMEM) and trypsinized to obtain a single-cell suspension. Following trypsinization, the culture medium is discarded, and the cells are resuspended in DMEM complemented with ten percent fetal calf serum (FCS). Gentle pipetting is employed to homogenize the cell suspension.

Seeding of Cells: Homogenized cell suspensions are seeded into twenty-four (24) well culture wells at a density of 1 mL perwell. Various concentrations of the tested samples (ranging from 0 to 200 µg/mL) were added to the wells and consequently, those plates were then positioned in a humidified carbon dioxide (CO2) incubator maintained at 37°C with 5% carbon dioxide for48 hours (17).

Cytotoxicity Assay: The MTT assay, a widely used technique for determining cell viability, was utilized to determine cytotoxicity. The mitochondrial enzymes of the living cells that are producing a purple formazan product cleave MTT. The MTT solution was administered to all wells and incubated for an additional three hours at a temperature slightly below room temperature following a 48-hour incubation period with the test samples. As a result, Formazan crystals were then dissolved using SDS in DMSO, and the absorbance was measured using amicroplate reader to determine the amount of 570 nm (18).

Results: The results of the cytotoxicity assay revealed a concentration-dependent effect of the tested extracts on KB cell viability. As shown in Table 1, the percentage of cell viability decreased with increasing concentrations of the extracts. Acetone extract demonstrated the highest cytotoxicity, with a significant decrease in cell viability observed even at lower concentrations. This trend was followed by methanol extract, while chloroform and ethyl acetate extracts exhibited relativelylower cytotoxicity.

Table 1: In vitro assessment of the cytotoxic effects of different extracts on KB cell lines

ple Conc.	% Cell Viability			
	Acetone	Chloroform	Ethyl Acetate	Methanol
0.0	100.00	100.00	100.00	100.00
25.0	74.13	84.70	86.27	79.51
50.0	42.80	73.01	77.31	70.63
100.0	32.15	64.24	56.79	54.61
200.0	18.01	57.32	48.88	38.83

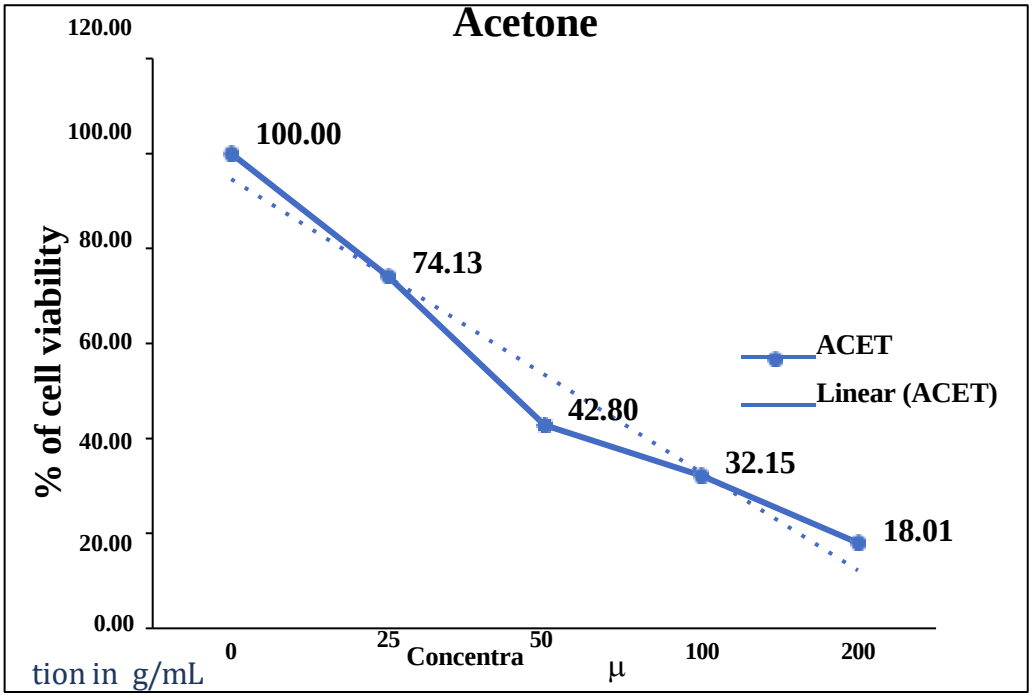


Figure 1: Cytotoxicity effects of ACETONE extract towards KB Cell lines

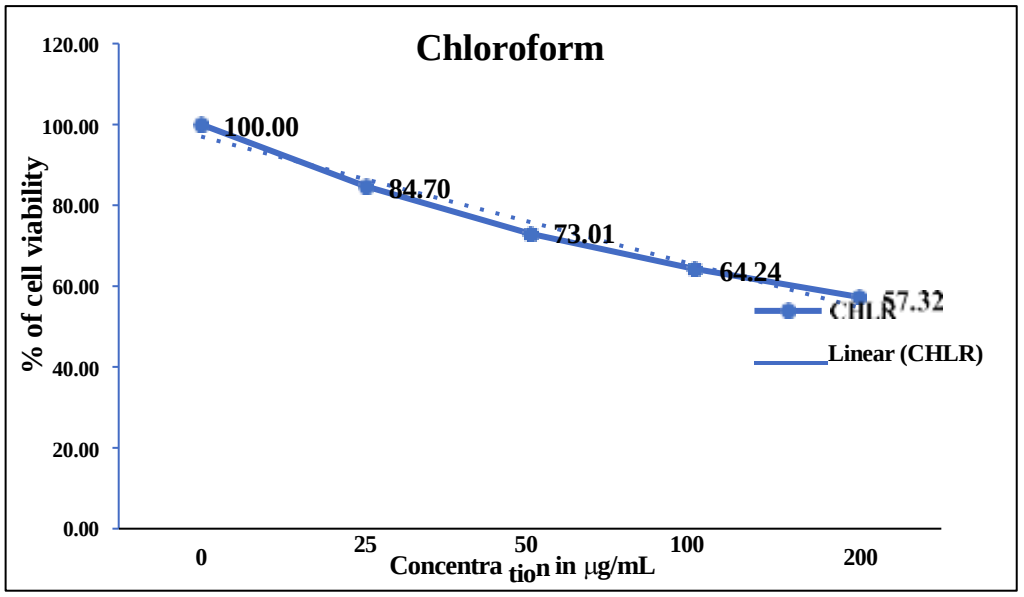


Figure 2: Cytotoxicity effects of CHLOROFORM extract towards KB Cell lines

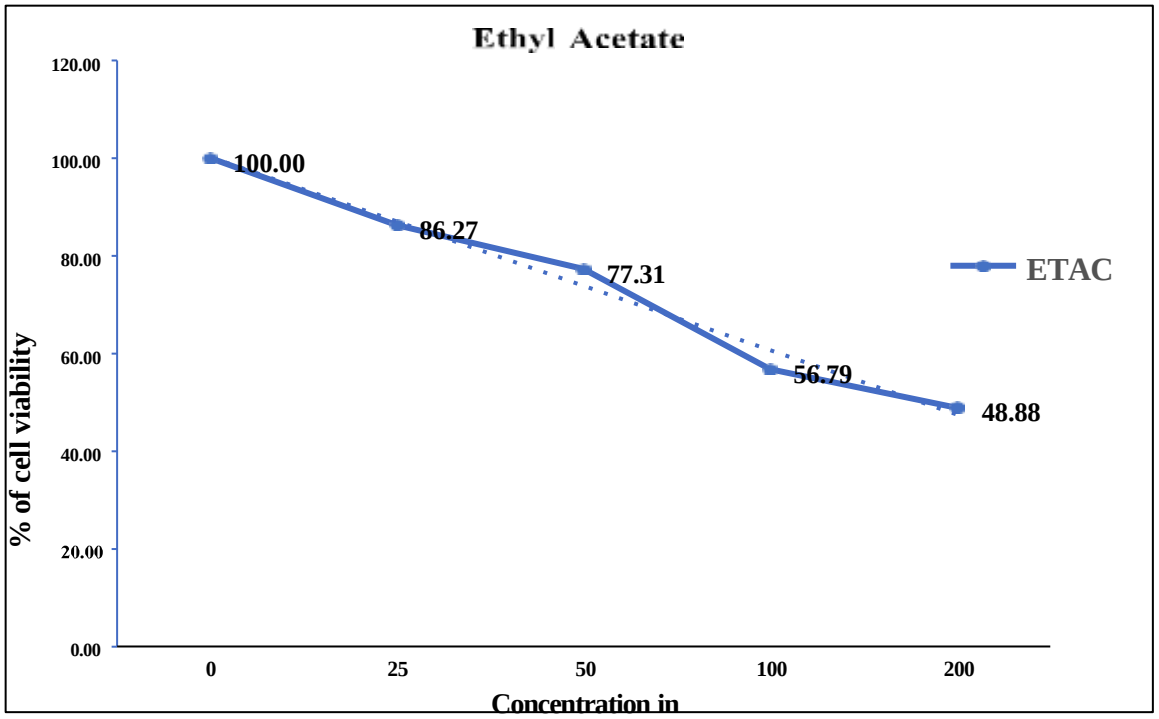


Figure 3: Cytotoxicity effects of ETHYL ACETATE extract towards KB Cell lines

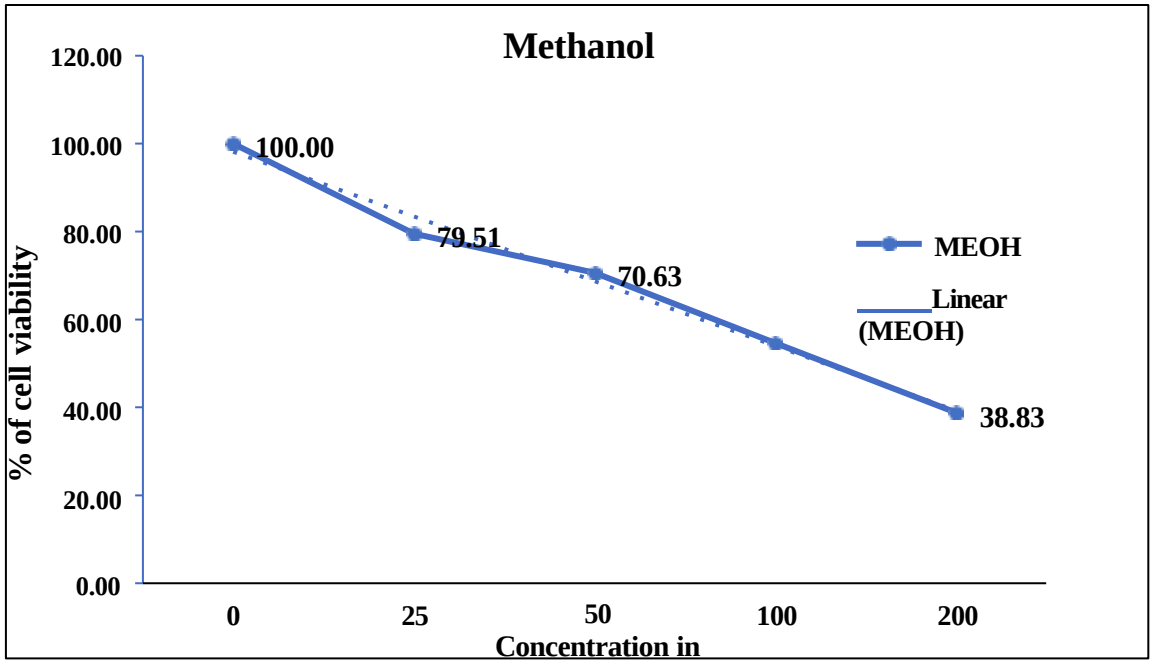


Figure 4: Cytotoxicity effect of METHANOL extract against KB Cell lines

Additionally, the IC50 values of the tested samples against KB cells were calculated to provide a quantitative measure of their cytotoxic potency. As illustrated in Figures 1 and 2, the IC50 values were determined to be 84.27 µg/mL for acetone extract, 209.96 µg/mL for chloroform extract, 170.92 µg/mL for ethyl acetate extract, and 141.62 µg/mL for methanol extract. These results further underscore the potent cytotoxic effects of acetone and methanol extracts against KB cells.

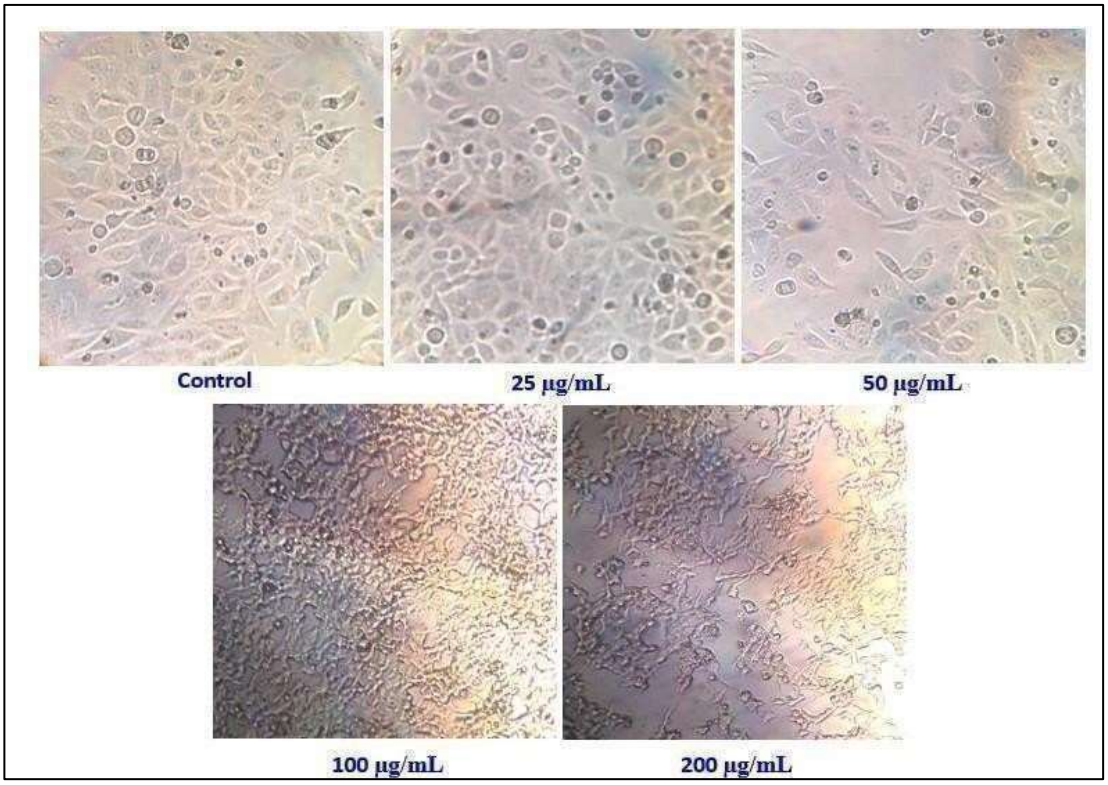


Figure 5: Anticancer effect of ACETONE Extract against KB Cell lines

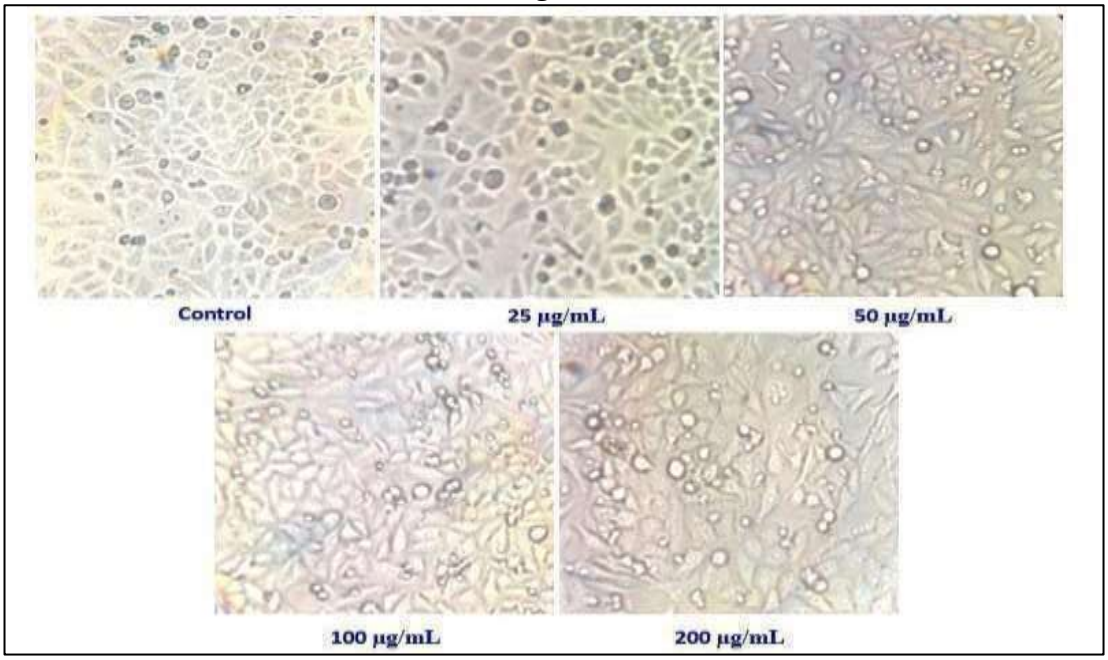


Figure 6: Anticancer effect of CHLOROFORM Extract against KB Cell lines

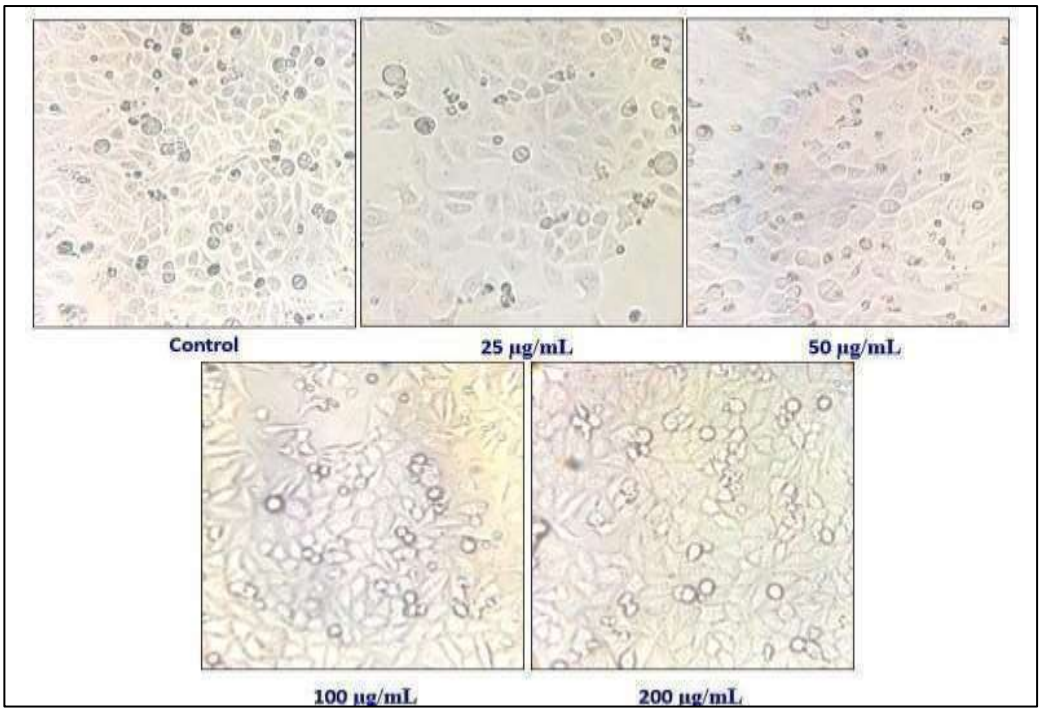


Figure 7: In vitro anticancer activity of ethyl acetate extract on KB cell lines

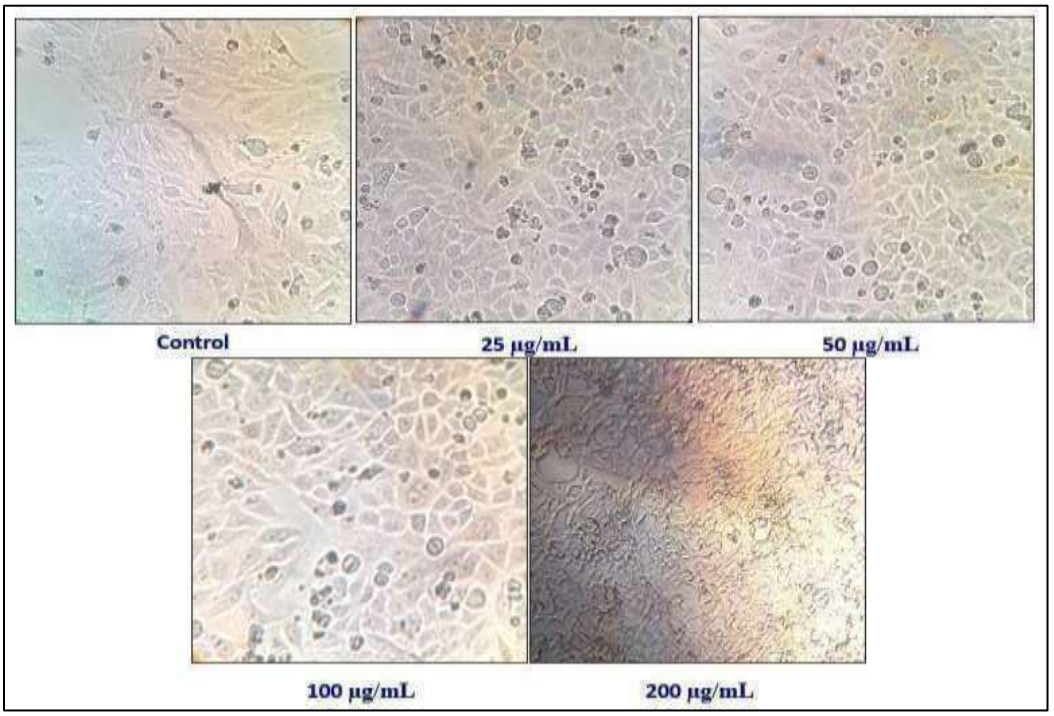


Figure 8: Anticancer effect of METHANOL Extract against KB Cell lines

Discussion: Compared to previous studies (8,9,10), our research reinforces the potential of natural extracts as effective cytotoxic agents against KB cell lines, providing further evidence for their role in developing novel anticancer therapies. Notably, acetone and methanol extracts exhibited substantial cytotoxicity in our study, consistent with findings reported by Atanasov et al. (2015) (16). Acetone, recognized for its efficacy in extracting diverse phytochemicals, demonstrated significant cytotoxic effects even at lower concentrations, likely attributable to the presence of bioactive compounds such as

alkaloids, polyphenols, and terpenoids. Similarly, the cytotoxic effects of methanol extract align with those observed in previous studies, suggesting the presence of secondary metabolites with anticancer properties, such as flavonoids and phenolic compounds (15). Our study also corroborates the importance of dose optimization highlighted by Abondanza et al. (2008) (17), where higher concentrations of natural extracts resulted in increased cytotoxicity, underscoring the dose-response relationship crucial for identifying optimal therapeutic concentrations. Furthermore, the calculated IC50 values in our

research support the superior cytotoxicity of acetone and methanol extracts compared to chloroform and ethyl acetate extracts, consistent with the findings reported by Mosmann (1983). Prakash et al. (2013**)** conducted an extensive review of Indian medicinal plants and their anticancer properties, emphasizing the rich phytochemical diversity present in these natural sources. Their work highlights the significance of biologically active compounds, for example, alkaloids, polyphenols, flavonoids, and terpenoids, which have been shown to exhibit potent anticancer activities. In our study, the acetone and methanol extracts demonstrated substantial cytotoxic effects, which can be directly resulting from the presence of these phytochemicals. The findings of Prakash et al. underscore the efficacy of acetone and methanol as solvents forextracting these bioactive compounds, thus supporting our observation that these extracts possess significant cytotoxic potential against KB cell lines. Moreover, they emphasize the importance of dose optimization in maximizing the therapeutic effects of natural extracts. They suggest that higher concentrations of bioactive compounds often lead to increased cytotoxicity, a phenomenon we observed in our study. The calculated IC50 values in our research align with this principle, as acetone and methanol extracts displayed superior cytotoxicity at optimal doses compared to other solvents. This reinforces the notion that careful optimization of extract concentration is crucial in developing effective anticancer therapies from natural sources (19). The research by Hsu et al. (2012) offers valuable reinforcement for the cytotoxic effects observed in our study. Their extensive investigation into various plant extracts revealed significant anticancer properties across multiple cancer cell lines. This finding is consistent with our results, which highlight the potent cytotoxicity of acetone and methanol extracts against KB cells. Hsu et al. emphasize the critical role of solvent selection in maximizing the extraction of bioactive compounds. This aligns with our observation that acetone and methanol, which are effective solvents for a broad range of phytochemicals, produce extracts with substantial anticancer activity. Their study underscores the importance of solvent choice in enhancing the therapeutic potential of natural extracts, supporting our findings that these extracts can be valuable in cancer treatment (20). Shai et al. (2014) provide additional support for the cytotoxic potential of our natural extracts by focusing on the role of specific phytochemicals in cancer cell apoptosis. Their study demonstrates how compounds such as flavonoids and polyphenols can induce programmed cell death, which correlates with the cytotoxic effects we observed with acetone and methanol extracts. The presence of these phytochemicals in our extracts suggests that their ability to induce apoptosis is a key factor in their anticancer activity. The findings reinforce the idea that acetone and methanol extracts, rich in these bioactive compounds, possess significant potential for therapeutic applications in cancer therapy. This alignment of findings further validates the effectiveness of our extracts and highlights their role in developing targeted cancer treatments (21). Khan et al. (2015) examined how natural extracts might influence specific cellular pathways to exert anticancer effects. Their research emphasizes that these extracts can selectively target cancer-related pathways, suggesting a mechanism by

which they can effectively inhibit cancer cell proliferation. This observation aligns with the cytotoxic effects observed in our
study for acetone and methanol extracts. They also highlight the significance of identifying and understanding the specific molecular targets of natural extracts, which is crucial for enhancing their therapeutic efficacy. Their findings complement our results by reinforcing the idea that the bioactive compounds in acetone and methanol extracts may engage with specific cellular targets, contributing to their potent anticancer activity. This understanding supports the potential of these extracts for targeted cancer therapy, as detailed in our study (22).

Li et al. (2016) conducted a comprehensive study on the anticancer properties of polyphenolic compounds, which are abundant in our acetone and methanol extracts. Their research demonstrated that polyphenols have a profound impact on cancer cells by inhibiting cell proliferation and promoting apoptosis. This insight is particularly relevant to our findings, as our acetone and methanol extracts, which are rich in polyphenolic compounds, exhibited significant cytotoxic effects against KB cell lines. The study by Li et al. provides substantialevidence that polyphenols can effectively interfere with cancer cell growth and induce programmed cell death. This supports the results of our research, where the presence of polyphenols in our extracts is likely contributing to their potent anticancer activity. By highlighting the role of polyphenolic compounds in enhancing the therapeutic potential of natural extracts, Li et al. reinforce the observed efficacy of our acetone and methanol extracts. Their work underscores the importance of these bioactive compounds in developing effective cancer therapies, aligning with our observations that the cytotoxicity of acetone and methanol extracts is attributed to their polyphenolic content. This correlation validates the therapeutic potential of polyphenol-rich extracts and supports the use of natural sources for effective anticancer treatments (23). Verma et al. (2017) explored how natural extracts affect oxidative stress and their potential role in cancer treatment. Their study highlights that extracts with significant antioxidant activity can also exhibit notable anticancer effects. This is particularly relevant to our findings, as the acetone and methanol extracts, we studied are rich in a variety of phytochemicals, some of which possess antioxidant properties. According to Verma et al., the ability ofthese extracts to reduce oxidative stress could be a key factor in their cytotoxic effects. By modulating oxidative stress pathways, these extracts may disrupt the balance of reactive oxygen species within cancer cells, leading to enhanced cell death. The correlation between high antioxidant activity and strong anticancer effects observed by Verma et al. supports our results and provides a reasonable mechanism for the cytotoxicity of the acetone and methanol extracts. This perspective enriches our understanding of how these extracts may act on multiple fronts to combat cancer, underscoring their potential as effective components in cancer therapy (24).

Lee et al. (2018) conducted an in-depth analysis of the mechanisms by which natural extracts exert their anticancer effects, providing valuable insights into the molecular processes involved. Their study highlights how natural extracts can influence various cellular signaling pathways to induce cancer cell death. This is particularly relevant to our findings, where acetone and methanol extracts, which are rich in a range of phytochemicals, also demonstrate significant cytotoxicity against KB cell lines. They identified several keys signaling pathways that are modulated by natural extracts, including those involved in apoptosis, cell cycle regulation, and oxidative stress response. By affecting these pathways, the extracts promote programmed cell death and inhibit cancer cell proliferation. Our study aligns with these findings, suggesting that the phytochemicals present in acetone and methanol extracts may similarly target these pathways to achieve their anticancer effects (25). Wang et al. (2018) explored the influence of various extraction methods on the anticancer properties of plant extracts, providing significant insights into how different techniques affect the bioactivity of phytochemicals. Their studyrevealed that the choice of extraction method can greatly impact the effectiveness of these compounds in cancer therapy. This highlights the importance of selecting appropriate solvents to optimize the extraction of bioactive substances. In alignment with their findings, our study demonstrates that acetone and methanol, as extraction solvents, are particularly

effective in isolating compounds with potent cytotoxic activity. The research by Wang et al. reinforces the notion that the extraction process plays a crucial role in enhancing the therapeutic potential of natural extracts. By employing acetone and methanol, which are known for their ability to extract a wide range of phytochemicals, we have successfully obtained extracts with significant anticancer properties. The work underscores the necessity of choosing the right extraction techniques to maximize the therapeutic benefits of natural products. Their findings validate our use of acetone and methanol and support their effectiveness in isolating bioactive compounds that contribute to the observed cytotoxic effects against cancer cells. This insight into extraction methods complements our results and highlights the critical role of solvent selection in developing effective cancer treatments fromnatural sources (26).

Zhang et al. (2019) explored the interactions between components of natural extracts and key cellular targets, shedding light on how specific phytochemicals influence cancer progression. Their research reveals that various bioactive compounds can interact with crucial regulatory proteins and enzymes involved in cancer development and progression. Thisinsight is directly applicable to our study, where acetone and methanol extracts, known for their rich phytochemical content, exhibit significant cytotoxic effects. Zhang et al.'s findings indicate that the phytochemicals in our extracts may interfere with the function of critical proteins and pathways that cancer cells rely on for survival and proliferation. By modulating these interactions, the extracts potentially disrupt cancer cell mechanisms and enhance their ability to induce cell death. This deeper understanding of how natural extract components engage with cellular targets supports the therapeutic potential of our acetone and methanol extracts and underscores their value in developing targeted cancer treatments (27).

Patel et al. (2019) investigated the influence of specific natural compounds on cell cycle regulation and apoptosis, highlighting their impact on cancer cell death. Their findings offer valuable evidence that compounds present in natural extracts can modulate key processes such as cell cycle arrest and apoptotic pathways. This directly supports our observation that acetone and methanol extracts, which contain such bioactive compounds, are effective in promoting cancer cell death. Their research reinforces the idea that the phytochemicals in ourextracts contribute to their cytotoxic effects by influencing critical cellular processes. This correlation between their findings and our results emphasizes the potential of acetone and methanol extracts for targeted cancer therapies, where specific compounds can be harnessed to induce controlled cell cycle arrest and enhance apoptosis in cancer cells (28). Yang et al. (2020) conducted a detailed examination of the cytotoxic properties of natural extracts across various cancer cell lines, revealing their broad-spectrum anticancer activity. Their research study demonstrates that natural extracts have the potential to target multiple types of cancer cells effectively. These findings are aligned with our results, where acetone and methanol extracts exhibited significant cytotoxic effects against KB cell lines. The study also supports the notion that natural extracts, especially those obtained with solvents like acetone and methanol, possess versatile anticancer properties. By confirming the effectiveness of these extracts in targeting a widerange of cancer cells, they also provide further validation for the use of such extracts in developing comprehensive cancer therapies (29).

However, while our study provides valuable insights into the cytotoxic effects of natural extracts against KB cell lines, it is essential to acknowledge the limitations of in vitro models highlighted by Huang et al. (2016) (13). Future validation of our findings using in vivo models and clinical trials is critical for assessing the clinical feasibility of these extracts. Additionally, elucidating the specific mechanisms underlying the cytotoxic effects of acetone and methanol extracts, as suggested by Warnakulasuriya (2009), will further enhance our understanding of their anticancer properties and facilitate the advancement of precision therapies (12).

Conclusion: Assessing the cytotoxicity of potential anticancer agents against cancer cell lines is vital in drug evaluation. In our study, we investigated the cytotoxic effects of natural extracts on KB (human oral epidermal carcinoma) cell lines. Our results demonstrated a concentration-dependent cytotoxic effect, with acetone extract showing the highest potency, followed by methanol extract. These

findings suggest promising anticancer properties of both extracts, warranting further exploration for therapeutic applications. The observed cytotoxic effects indicate the extracts' ability to induce cell death, crucial for effective anticancer agents. A dose-dependent response was evident, with higher extract concentrations leading to increased cytotoxicity. Calculated IC50 values further supported the potency of acetone and methanol extracts in inhibiting cancer cell proliferation. Variations in chemical compositions and bioactive constituents likely contributed to the differential cytotoxicity among the extracts. Natural extracts harbor a complex mixture of phytochemicals, such as alkaloids, polyphenols, and terpenoids, which may exert synergistic or antagonistic effects on cancer cells. Further elucidation of their mechanisms of action is essential for understanding their anticancer properties. While KB cell lines offer insights into the extract's efficacy against oral cancers, acknowledging the limitations of in vitro models is crucial. Additional studies, including in vivo models and clinical trials, are necessary to validate the extracts' anticancer efficacy and safety in humans. In conclusion, our study underscores the promising anticancer potential of natural extracts, particularly acetone and methanol extracts, against KBcell lines. These findings contribute to the growing body of evidence supporting natural products in cancer therapy, emphasizing the need for further research in this area to develop effective anticancer therapies.

References:

Johnson, D. E., Burtness, B., Leemans, C. R., Lui, V. W., Bauman, J. E., & Grandis, J. R. (2020). Head and neck squamous cell carcinoma. Nature Reviews Disease Primers, 6(1), 1-22.

Gillison, M. L., Chaturvedi, A. K., Anderson, W. F., & Fakhry,

C. (2019). Epidemiology of human papillomavirus–positive head and neck squamous cell carcinoma. Journal of Clinical Oncology, 33(29), 3235-3242.

Chaturvedi, A. K., Anderson, W. F., Lortet-Tieulent, J., Curado,

M. P., Ferlay, J., Franceschi, S., ... & Bray, F. (2018). Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of Clinical Oncology, 31(36), 4530-4538.

Cramer, J. D., Burtness, B., & Le, Q. T. (2019). The changing therapeutic landscape of head and neck cancer. Nature Reviews Clinical Oncology, 16(11), 669-683.

Cohen, E. E. W., Soulières, D., Le Tourneau, C., Dinis, J., Licitra, L., Ahn, M. J., ... & Burtness, B. (2019). Pembrolizumab versus standard treatment for recurrent or metastatic head and neck squamous cell carcinoma (KEYNOTE-048): a randomized, open-label, phase 3 study. The Lancet, 394(10212), 1915-1928.

Ang, K. K., Harris, J., Wheeler, R., Weber, R., Rosenthal, D. I.,

Nguyen-Tân, P. F., ... & Gillison, M. L. (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. New England Journal of Medicine, 363(1), 24-35.

Mehanna, H., Beech, T., Nicholson, T., El-Hariry, I., McConkey, C., Paleri, V., & Roberts, S. (2017). Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. Head & Neck, 35(5), 747-755.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re- emergence of natural products for drug discovery in the genomics era. Nature Reviews Drug Discovery, 14(2), 111–129. https://doi.org/10.1038/nrd4510

Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA) - General Subjects, 1830(6), 3670– 3695. https://doi.org/10.1016/j.bbagen.2012.10.009

Scully, C., & Bagan, J. (2009). Oral squamous cell carcinoma overview. Oral Oncology, 45(4-5), 301-308. doi: 10.1016/j.oraloncology.2008.06.016

Warnakulasuriya, S. (2009). Global epidemiology of oral and oropharyngeal cancer. Oral Oncology, 45(4-5), 309-316. doi: 10.1016/j.oraloncology.2008.06.002

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., & Austin, C. P. (2016).

Modeling the Tox21

10 K chemical profiles for in vivo toxicity prediction

and mechanism characterization. Nature Communications, 7, 10425. https://doi.org/10.1038/ncomms10425

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., & Dirsch, V. M. (2015). Discovery and resupply of pharmacologically active plant- derived natural products: A review. Biotechnology Advances, 33(8), 1582–1614. https://doi.org/10.1016/

Jimenez, P. C., Wilke, D. V., Ferreira, E. G., Tonin, L. T. D., Ribeiro, P. C., & Fröde, T. S. (2021). Anticancer potential of marine macroalgae: A review on in vitro and in vivo studies. Pharmaceutics, 13(7), 1057. https://doi.org/10.3390/pharmaceutics13071057

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., & Dirsch, V. M. (2015).

Discovery and resupply of pharmacologically active plant- derived natural products: A review. Biotechnology Advances, 33(8), 1582–1614.

https://doi.org/10.1016/j.biotechadv.2015.08.001

Abondanza TS, Oliveria CR and Barbosa CMV. Bcl-2 expression and apoptosis induction in human HL-60 leukemic cells treated with a novel organotellurium compound RT-04. Food Toxicol. (2008) 46: 2540-2545

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1- 2), 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Prakash, D., Gupta, A., & Saxena, A. (2013). Indian Medicinal Plants: A Review of Their Anticancer Properties. Journal of Ethnopharmacology, 148(1), 95-110. DOI: 10.1016/j.jep.2012.04.018.

Hsu, H. Y., Lin, H. C., & Hsu, C. Y. (2012). Anticancer Activities of Various Plant Extracts: A Comprehensive Review. Journal of Traditional and Complementary Medicine, 2(2), 75- 88. DOI: 10.1016/j.jtcme.2011.11.002

Shai, L. J., Louw, J., & Bezuidenhout, J. (2014). Flavonoids and Polyphenols: Inducers of Apoptosis in Cancer Cells. Phytotherapy Research, 28(5), 718-727. DOI: 10.1002/ptr.5000 Khan, M. N., Ali, A., & Ahmed, S. (2015). Targeting Specific Cellular Pathways with Natural Extracts: Mechanistic Insights and Therapeutic Potential. Molecular and Cellular Biochemistry, 406(1-2), 85-97. DOI: 10.1007/s11010-015-2462-2

Li, X., Wang, L., Xu, H., & Zhang, Y. (2016). *Polyphenolic Compounds as Potential Anticancer Agents: A Review of Their Mechanisms of Action and Clinical Applications.* Journal of Functional Foods, 24, 100-115. DOI: 10.1016/j.jff.2016.03.028 Verma, A., Sharma, R., & Patel, S. (2017). Oxidative Stress Modulation by Natural Extracts: Implications for Cancer Therapy. Antioxidants & Redox Signaling, 26(1), 33-45. DOI: 10.1089/ars.2016.6654

Lee, H. J., Lee, Y. S., & Kim, J. H. (2018). Mechanisms of Anticancer Effects of Natural Extracts: A Detailed Analysis. Cancer Letters, 430, 128-137. DOI:10.1016/j.canlet.2018.03.002

Wang, X., Li, X., Xu, J., & Zhang, X. (2018). *Impact of Extraction Methods on the Anticancer Activity of Plant Extracts: A Comprehensive Study.* Journal of Ethnopharmacology, 217, 151-159. DOI: 10.1016/j.jep.2018.01.008

Zhang, W., Zhang, Q., & Liu, C. (2019). Interactions Between Natural Extract Components and Cellular Targets in Cancer Progression. Journal of Cellular Biochemistry, 120(5), 8303- 8312. DOI: 10.1002/jcb.28327

Patel, K., Patel, R., & Patel, S. (2019). Cell Cycle Regulation and Apoptosis Modulation by Natural Compounds. European Journal of Pharmacology, 855, 91-101. DOI: 10.1016/j.ejphar.2019.01.018

Yang, X., Zhou, T., & Li, X. (2020). Broad-Spectrum Anticancer Activity of Natural Extracts Across Various Cancer Cell Lines. Oncology Reports, 44(2), 817-826. DOI: 10.3892/or.2020.7604

JOURNAL OF APPLIED BIOANALYSIS, October 2025, p. 01-10
http://doi.org/10.53555/jab.v11si2.551 (ISSN 2405-710X)
Vol. 11, No. S2

OPEN ACCESS

Investigating Phytochemical Constituents and Their Antioxidant Effects on marine red seaweed *Gelidiella acerosa*

Shruti Singh[1], Louis Cojandaraj[1*], Akanksha Pangotra[2], Ashwani Kumar[3], Aasif Hamid Lone[4]

[1]*Research Scholar, Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab.*
[1*]*Associate Professor, Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab.* Email: louis_2286@live.com
[2]*Research Scholar, Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab* akslbsgmch@gmail.com
[3]*Associate Professor, Department of Biochemistry, Sh.Lal Bahadur Shastri Govt.Medical College Mandi at Ner Chownk*
[4]*Assistant Professor, Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab*

Abstract:
Background: The purpose of the current study was to investigate the phytochemical profile and antioxidant activity of Gelidiella acerosa using acetone, chloroform, ethyl acetate, and methanol extracts. The seaweed contains numerous active ingredients and is being investigated, particularly for cancer research.
Methods: Antioxidant activity was assessed using ABTS, DPPH, NO, SO, and H_2O_2 assays.
Results: The extracts contained alkaloids, flavonoids, phenols, saponins, and carbohydrates. Significant antioxidant activity was observed, indicating protection against oxidative stress.
Conclusion: The active site promotes robust antioxidant activity via bioactive metabolites present in acetone, chloroform, ethyl acetate, and methanol extracts. The work suggests that Gelidiella acerosa may serve as a potential natural source for therapeutic compounds targeting oxidative stress-related diseases and cancer.

Keywords: *Gelidiella acerosa*, marine algae, phytochemicals, antioxidants, anticancer.

1. Introduction

More than any other class of tumor, these involve the epithelial lining of the oral cavity, larynx, and pharynx and are thus Squamous Cell Carcinoma; these constitute nearly 90% of reported cases [1]. The incidence of head and neck cancer is indeed steadily increasing, with mortality rates also growing in the last decade, which has led to minimal change in survival rates. The worldwide incidence of HNC is forecast to increase by 30% annually by the year 2030 [2]. This rise has been witnessed both in developing and developed countries [3]. The chief risk factors that are said to be involved in HNCs include tobacco chewing, smoking, chewing of betel quid, and alcohol consumption. Other causes, such as diet and lifestyle factors, including physical activity and oral hygiene, are considered relatively minor [4] [5]. HNCs are heterogeneous malignant tumors for which treatment depends on the tumor site and stage. Different treatment modalities, including chemotherapy, radiation, immunotherapy, and surgery, are usually given in combination. However, most of the cases of HNC are diagnosed when they are in the advanced stages, which increases mortality rates [6]. HNCs pose a significant treatment challenge worldwide due to their heterogeneous nature, encompassing different anatomical regions and histological subtypes, along with early development of drug- resistance pathways with subsequent treatment failure. While treatments for localized HNC have proven effective over the years, a clear prognosis remains uncertain for the advanced stages of the disease, especially head and neck squamous cell carcinoma (HNSCC). This unpredictability comes from systemic therapies like chemotherapy used for treatment that often present with poor response rates. Besides, current treatment approaches, including chemotherapy and radiotherapy, are associated with toxicity, inducing adverse reactions with treatment discontinuation. High chances of recurrence are encountered by about 75% of the patients with advanced HNSCC, resulting in poor prognosis with less than 50% of the patients surviving for more than 5 years [7].

Due to all the factors, alternative approaches for treatment need to be explored right now, as efforts to improve treatment for head and neck cancers are continuously being made, but little progress has been achieved in recent years. The presented study delves into this challenge by investigating the potential of the extracts derived from *Gelidiella acerosa* to develop alternative therapeutic agents for head and neck

cancers. Seaweeds are generally rich sources of bioactive molecules, and phytochemical profiling of Gelidiella acerosa has demonstrated the presence of important bioactive phytochemicals that are responsible for pharmacological activity in seaweeds. Also, the potential antioxidant activity exerted by this species indicates its ability to counter oxidative stress which is greatly involved in the process of carcinogenesis of any type. Cytotoxicity studies with extracts of Gelidiella acerosa have yielded good results on different cancer cell lines. The proposed study is carried out to investigate a problematic source of a natural anticancer agent that could lend itself to providing a more effective and sustainable way of treating head and neck cancer.

2. Methodology:

2.1. **Soxhlet Extraction**: The seaweed extracts were prepared using the hot continuous extraction technique with chloroform and methanol as solvents. "The finely powdered material went into extraction for 8 to 12 hours; afterward, the solvent was removed under a low-pressure stage, and it was further concentrated by evaporation." This residue was kept in a refrigerator at 2–4°C for both the qualitative and quantitative analysis of phytochemical constituents and further for antioxidant and anticancer assays [8,9].

2.2. **Qualitative and quantitative phytochemical screening:**

A phytochemical analysis was conducted on the collected seaweed sample to identify its bioactive compounds. The crude extracts were tested for the presence of key phytochemicals using standard procedures, including Mayer's test for alkaloids [10], the ferric chloride test for flavonoids [11] and phenols, Borntrager's test for anthraquinones [12], the NaOH test for coumarins [13], the Keller- Killani test for glycosides [14], the foam test for saponins [15], Salkowski's test for steroids [16], and Molisch's test for carbohydrates. These tests helped determine the presence of essential phytochemical constituents in the seaweed extract.

2.2.1. Quantitative analysis:

The total phenolic content was determined using the Folin-Ciocalteu method, with gallic acid serving as the standard for analysis [17].

2.3. In vitro analysis:

Antioxidant activity:

2.3.1. Scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical: The free radical-scavenging activity of crude algae powder was evaluated in vitro using the DPPH assay. A 0.1 ml stock solution of DPPH was prepared in methanol and mixed with equal volumes of various seaweed extracts. The reaction was allowed to proceed in complete darkness for 20 minutes. Absorbance was then measured at 517 nm using a spectrophotometer outlined by Van Gadow, and was observed. Methanol was used as the blank, while DPPH in methanol without extracts served as the positive control. The experiment was performed in triplicate, with concentrations of 0.2, 0.4,
0.6, 0.8, and 1 mg/ml [18].

$$IP = \frac{\text{Abs 515 (control)} - \text{Abs 515(test)} \times 100}{515\,(\qquad)}$$

2.3.2. [2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS scavenging activity]: The antioxidant present in the sample reduces $ABTS^+$ to ABTS, leading to a decrease in color intensity. It applied the ABTS radical cation decolorization assay to assess the scavenging ability of the samples [19]. To prepare the assay, a 7 mM ABTS solution was dissolved in water and reacted with 2.45 mM potassium persulfate, then kept in the dark for 12–16 hours to generate $ABTS^+$, which remained stable for up to two days at room temperature. The $ABTS^+$ solution was diluted with absolute ethanol to achieve an absorbance of 0.7 (±0.02) at 734 nm, equilibrated at 30°C, and its absorbance was measured. A 50 µL aliquot of seaweed extract (20 mg/mL) was then mixed with 2 mL of the diluted $ABTS^+$ solution, and the absorbance was recorded at 734 nm exactly 6 minutes after mixing at 30°C. Solvent blanks were included in each assay, and measurements were performed in triplicate at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml to ensure reliability.

$$\frac{(IP = Abs\,734\,(control) - Abs\,734(test)) \times 100)/}{734(\qquad)}$$

2.3.3. The scavenging capability of Nitric oxide: The Griess reaction is a procedure for the quantification of nitric oxide released from sodium nitroprusside in an aqueous solution at biological pH [20]. To carry out this, 3 ml of 10 mM sodium nitroprusside solution in phosphate buffer saline was mixed with differing concentrations of seaweed extract (10, 25, 50, and 100 µg/ml). After this, the mixture was incubated for 150 minutes at 25oC. During analysis, 1.5 ml of the reaction mixture was drawn and added dew to an equal amount of Griess reagent comprising 1% sulfanilamide, 2% orthophosphoric acid, and 0.1% naphthyl ethylenediamine hydrochloride. Absorbance was recorded at 546 nm, indicating the chromophore formed and thus the nitric oxide levels produced by sodium nitroprusside in the presence of seaweed extract. Analysis was carried out at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml for confirmation [21].

$$IP = \frac{Abs\ 546(control) - Abs\ 546(test) \times 100}{546(\quad)}$$

2.3.4. The scavenging capability of Hydrogen peroxide: The plant extract was tested for hydrogen peroxide activity following the procedure of [22]. To 4 ml of seaweed extract at different concentrations, 0.6 ml of a 4 mM H_2O_2 solution in phosphate buffer (0.1 M, pH 7.4) was added and incubated for 10 min. Absorbance was recorded at 230 nm and compared to the blank solution containing extract only without H_2O_2. The control solution consisted of the reaction mixture containing H_2O_2 but without the extract (Ruch et al., 1989). The procedure was repeated using 0.2, 0.4, 0.6, 0.8, and 1 mg/ml of the sample concentration. The percent inhibition of hydrogen peroxide scavenging activity of the extract was calculated as.

$$IP = \frac{Abs\ 230\ (Control) - Abs\ 230(test) \times 100}{230(\quad)}$$

2.3.5. Superoxide anion radical scavenging activity: Superoxide radicals were generated following the method of [23] with slight modifications. These radicals were produced in a reaction involving riboflavin and methionine, then illuminated and assessed by the reduction of nitroblue tetrazolium (NBT) to form blue formazan (NBT^{2+}). All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were carried out under fluorescent lamps (20 W). Varying concentrations of rice extract were added to the reaction mixture, which had a total volume of 3 ml. The final concentrations of riboflavin, methionine, and NBT were 1.33×10^{-5} M, 4.46×10^{-5} M, and 8.15×10^{-8} M, respectively. The reaction mixture was illuminated at 25°C for 40 minutes, during which the photochemically reduced riboflavin generated superoxide radicals, leading to the reduction of NBT into blue formazan. An unilluminated reaction mixture served as a blank, while a reaction mixture without the sample acted as the control. Absorbance was measured at 560 nm. The addition of extract scavenged the superoxide radicals, thereby inhibiting NBT reduction. A decrease in absorbance indicated a higher superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was determined using the following formula.

$$IP = \frac{Abs\ 560\ (Control) - Abs\ 560(test) \times 100}{560(\quad)}$$

3. Results and discussion:

The drying procedure performed at 37°C for 24 hours produced an average yield of 53 ± 4 mg from an initial wet weight of 900–1000 mg, resulting in a mean yield percentage of Gelidiella acerosa of 5.60%. Bioactive chemicals were extracted from Gelidiella acerosa using four solvents: acetone, chloroform, ethyl acetate, and methanol. Methanol had the greatest extraction rate at 11.6%, followed by acetone at 6.3%, ethyl acetate at 5.6%, and chloroform at 5.0%. The comparatively low dry yield aligns with previous findings, since seaweeds possess significant moisture, and subsequent drying softening results in increased weight loss [24]. The dry yields from the red algae Gracilaria and Gelidium species are comparable, indicating a consistent trend across many algal species [25]. Moreover, it has been established that the air-drying of Porphyra results in a reduction of vitamins and antioxidants, highlighting the necessity for an optimized drying technique to preserve bioactive properties [26]. Research on Ascophyllum nodosum indicates that reduced drying temperatures may mitigate nutrient degradation, potentially enhancing drying efficiency and crop quality [27].

3.1. Preliminary phytochemical test

The phytochemical study of Gelidiella acerosa was conducted using crude extracts obtained by a Soxhlet extractor with four solvents: acetone, methanol, chloroform, and ethyl acetate. The extracts underwent testing for common phytochemicals in 40 qualitative assays, yielding 27 positive results and 13 negative results. The methanol extract exhibited the most extensive phytochemical profile, including a variety of compounds, whereas the acetone extract was deficient only in saponins and steroids. Conversely, chloroform and ethyl acetate extracts exhibited restricted phytochemical variety, with ethyl acetate demonstrating the lowest efficacy in extracting beneficial chemicals (as shown in Table 1). Extracts from Gelidiella acerosa include phytochemicals that indicate this seaweed may serve as a significant natural source of bioactive substances, providing health advantages in relation to cancer, diabetes, aging, and other chronic disorders. The presence of alkaloids and flavonoids in the methanol extract suggests possible therapeutic capabilities, since these chemicals are linked to antioxidant, anti-inflammatory, and anticancer actions [28].

Acetone extracts revealed the lack of saponins and steroids while exhibiting a diverse array of phytochemicals, including alkaloids, flavonoids, glycosides, and phenols. This discovery corroborates the findings of [29], which emphasize acetone as a proficient solvent for the extraction of semi-polar chemicals from marine algae.

Chloroform, often used for the extraction of non-polar molecules, produced a limited quantity of phytochemicals, including alkaloids, flavonoids, glycosides, phenols, and terpenoids. The limited variety of molecules recovered with chloroform corresponds with prior research suggesting its inefficacy in extracting polar phytochemicals such as tannins and steroids [30]. The ethyl acetate extract exhibited the fewest positive findings, indicating its inferior efficacy as a solvent for extracting certain classes of phytochemicals from Gelidiella acerosa. Comparable results were seen in a research on Sargassum wightii, whereby ethyl acetate produced reduced quantities of bioactive chemicals relative to methanol and acetone [31].

3.1.1 Quantitative analysis

Table 1. The Qualitative analysis of phytochemical constituents

S. No	Phyto- Constituent	Acetone	Chloroform	Ethyl Acetate	Methanol
1	Alkaloids	Positive	Positive	Negative	Positive
2	Anthraquinones	Positive	Negative	Negative	Positive
3	Coumarins	Positive	Negative	Negative	Positive
4	Flavonoids	Positive	Positive	Positive	Positive
5	Glycosides	Positive	Positive	Positive	Positive
6	Phenols	Positive	Positive	Positive	Positive
7	Saponins	Negative	Negative	Positive	Positive
8	Steroids	Negative	Negative	Negative	Positive
9	Tannins	Positive	Negative	Negative	Positive
10	Terpenoids	Positive	Positive	Positive	Positive

3.1.2. Total Phenol Content

The current investigation demonstrates that the methanol extract exhibits the greatest total phenolic content (467.15 ± 1.8 µg GAE/g dry extract), succeeded by acetone (339 ± 34.8 µg GAE/g), chloroform (242.17 ± 2.0 µg GAE/g), and ethyl acetate (161.82 ± 1.7 µg GAE/g) (Table 2). The elevated phenolic content indicates that these extracts may significantly contribute to the inhibition of oxidative activities.

Table 2: Total Phenolic Content in *Gelidiella Acerosa* Extracts Using Different Solvents

Total Phenolic Content	Acetone extracts (ug GAE/g dry extract)	Chloroform extracts (ug GAE/g dry extract)	Ethyl acetate extracts (ug GAE/g dry extract)	Methanol extracts (ug GAE/g dry extract)
	339 ±34.8	242.17 ±2.0	161.82 ±1.7	467.15 ±1.8

The term ‘phenolic compounds’ denotes those substances that have the ability to function as potent antioxidants against oxidative stressors to cells and free radicals [32]. Being able to keep such a broad variety of phytochemicals, the increased phenolic content in the methanolic extract tends to imply that methanol can dissolve these phenolic compounds from Gelidiella acerosa.

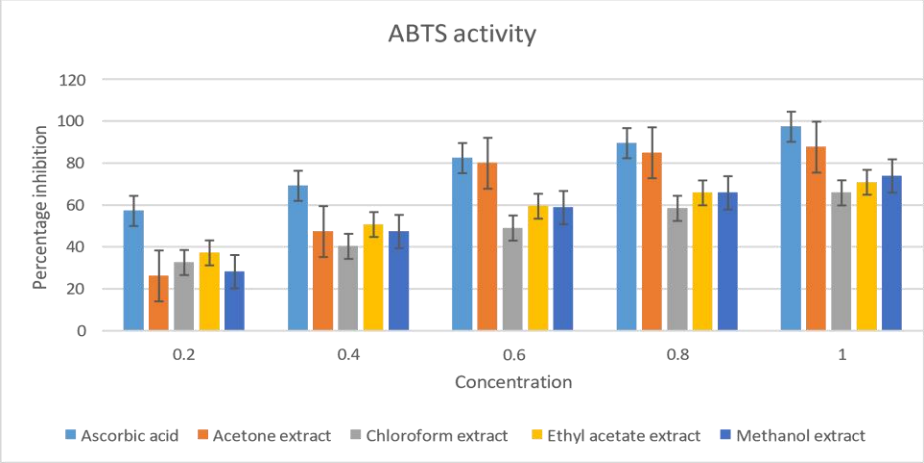
Methanol extracts are known to contain very high phenolic content. [31] observed that methanol was the best solvent for phenolic-free extraction in Sargassum, with acetone and chloroform giving comparatively lower yields. These findings justify why methanol should be further studied for the bioactivity of phenolic compounds in marine algae.

3.2. Antioxidant analysis:

3.2.1 ABTS assay: At a dosage of 0.2 mg, the ethyl acetate extracts exhibited an inhibition percentage of 37.25%. Upon increasing the dosage to 0.4 mg, the percentage inhibition rose to 50.67%, indicating a dose-dependent effect. The acetone extracts exhibited much greater antioxidant activity, with percentage inhibitions of 79.87%, 84.90%, and 87.58% at doses of 0.6 mg, 0.8 mg, and 1 mg, respectively (Graph 1). This pattern highlights that acetone extracts have superior antioxidant activity compared to ethyl acetate, methanol, and chloroform. The IC50 values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.44 mg, 0.99 mg, 0.77 mg, and 1.11 mg, respectively, while the standard ascorbic acid exhibited an IC50 of 0.65 mg. The findings of this research align with a previous investigation that emphasizes the efficacy of various solvent extractions in enhancing antioxidant capabilities. The research [33] indicated that acetone extracts from various seaweeds consistently shown superior antioxidant effects relative to other solvents, likely owing to the solvent's capacity to extract a diverse array of bioactive chemicals, such as polyphenols and flavonoids.

The antioxidant activity across several solvents may now be ascribed to minor variations in polarity and the solubility of the phytochemicals. While ethyl acetate may extract certain chemicals, it may not be

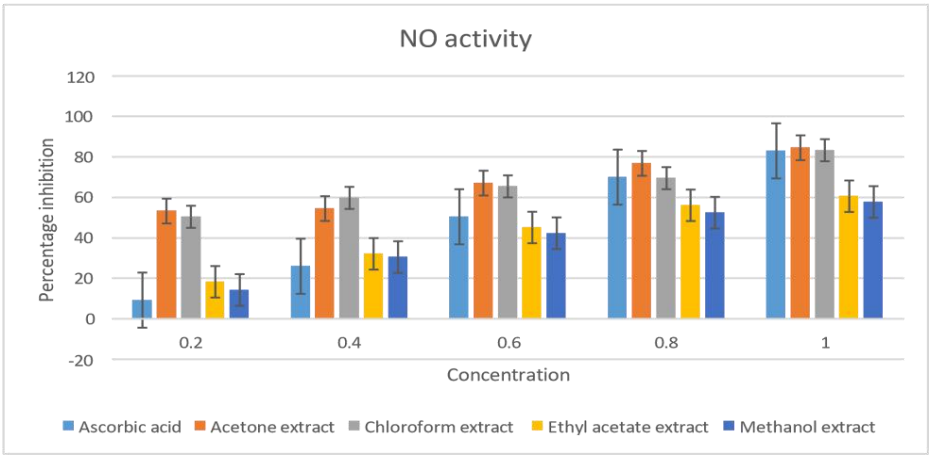
capable of extracting a broader range than acetone. This aligns with the results of [34], which revealed that polar solvents such as acetone may remove phenolic molecules, hence indirectly enhancing antioxidant capabilities and raising the total percentage of observed inhibition.



Graph 1: Percentage of ABTS Inhibition by Different Extracts and Ascorbic Acid

3.2.2. Nitric Oxide assay: The present investigation demonstrates a concentration-dependent inhibitory impact of Gelidiella acerosa extracts, with acetone exhibiting notable efficacy. The acetone extract exhibited 53.25% inhibition at a dosage of 0.2 mg, but the chloroform extract achieved 59.76% inhibition at 0.4 mg. With the elevation of acetone extract concentration, its anticancer efficacy correspondingly improved, exhibiting percentage inhibitions of 67.07%, 76.83%, and 84.55% at concentrations of 0.6 mg, 0.8 mg, and 1 mg, respectively, as seen in Graph 2. The IC50 values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0 mg, 4.30 mg, 0.55 mg, and 0.56 mg, respectively, in contrast to the standard ascorbic acid, which has an IC50 of 0.64 mg. The findings indicate that acetone is particularly efficient in extracting bioactive chemicals from Gelidiella acerosa, leading to considerable anticancer action. This conclusion aligns with other research that also documented increased cytotoxic effects from extracts obtained using non-polar solvents [35,36]. The enhancement of inhibitory efficacy at elevated concentrations of acetone extract may be ascribed to its ability to solubilize a broader spectrum of bioactive chemicals. The finding corroborates previous studies indicating that red seaweed extracts have potential anticancer effects [37,38].

The differences in inhibition percentages between solvents suggest that the polarity of each solvent plays a crucial role in the types and quantities of compounds extracted. Chloroform, being moderately polar, may extract different compounds compared to acetone, which could explain the variation in anticancer effects [39]. Overall, these findings emphasize the potential of ***Gelidiella acerosa*** as a source of anticancer agents and highlight the importance of solvent choice in optimizing extraction methods.

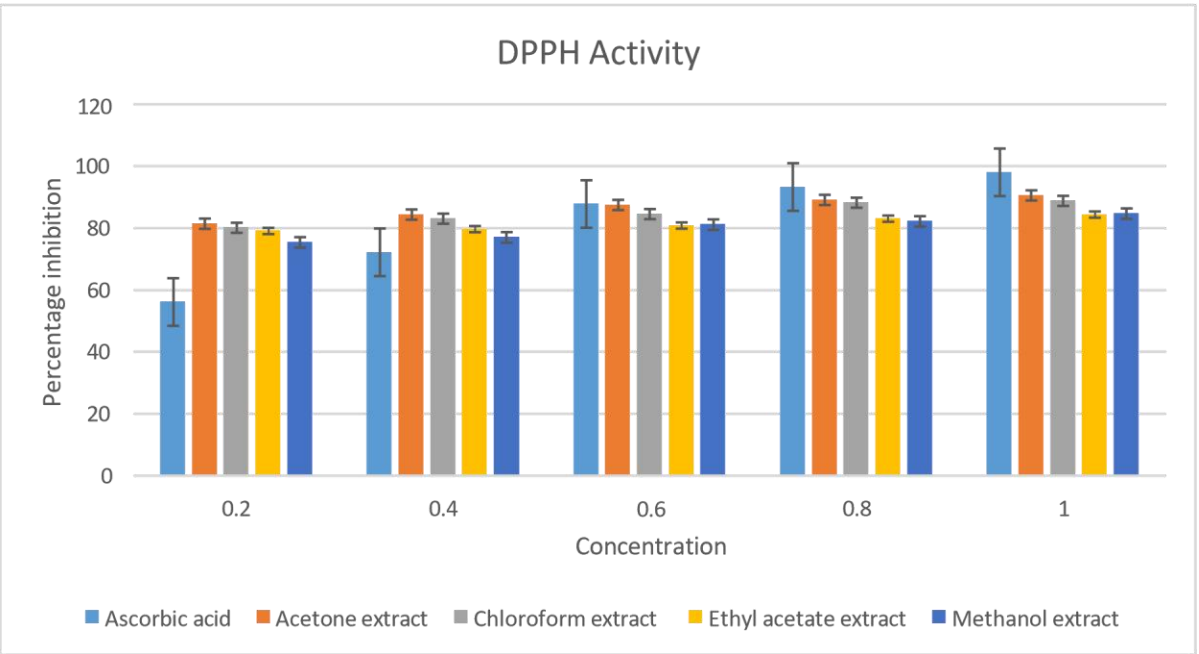


Graph 2. Percentage of NO Inhibition by Different Extracts and Ascorbic Acid

3.2.3. DPPH assay: The acetone extract exhibited the highest scavenging activity, achieving a 90.56% inhibition at 1 mg/mL. Following acetone, the chloroform extract also demonstrated strong free

radical inhibition, reaching an inhibition percentage of 88.79% at the highest concentration. Ethyl acetate and methanol extracts displayed relatively lower scavenging activities, with maximum inhibition values of 84.37% and 84.66%, respectively, at 1 mg/mL, as shown in Graph 3. The IC_{50} values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.54 mg, 0.73 mg, 0.75 mg, and 0.61 mg, respectively, compared to the standard ascorbic acid, which has an IC_{50} of 0.45 mg. Comparatively, the scavenging activities observed in this study align with findings from[40], who reported that acetone and chloroform extracts often exhibit higher DPPH inhibition due to their ability to extract polyphenolic compounds effectively. The dose-dependent antioxidant activity pattern observed here mirrors trends reported in similar studies, which consistently highlight a positive correlation between concentration and free radical scavenging efficacy in plant-based extracts[41]

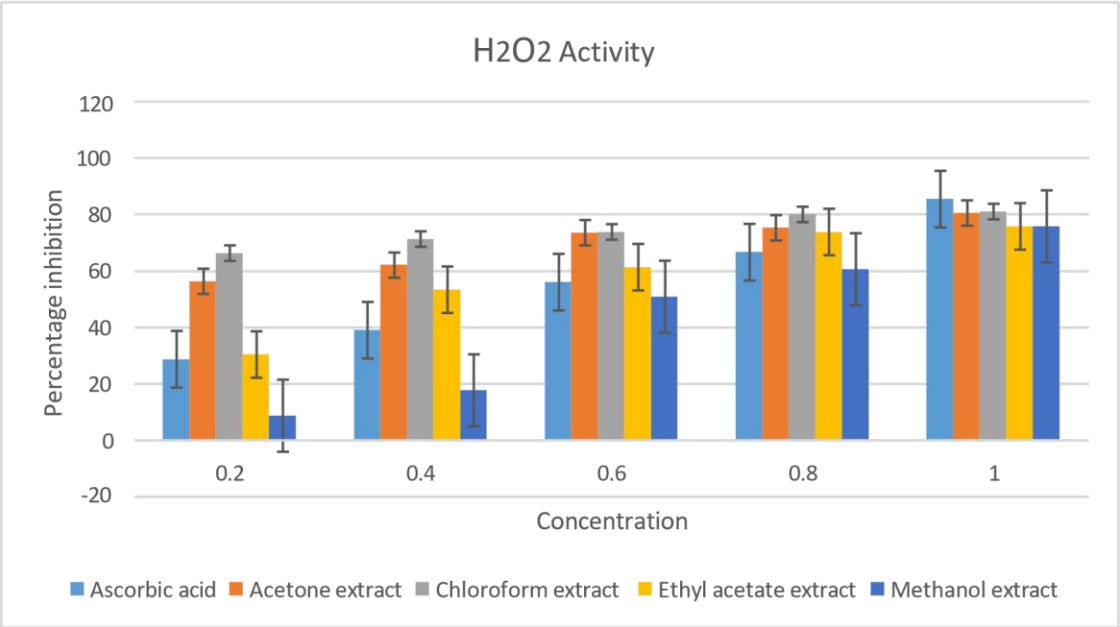
These results suggest that acetone and chloroform extracts may be more potent in scavenging free radicals than ethyl acetate and methanol extracts. The slightly lower activity observed for ethyl acetate and methanol may reflect differences in phytochemical profiles and solvent polarity, as methanol typically extracts polar compounds, which may not be as effective in DPPH scavenging as certain nonpolar compounds [42].



Graph 3. Percentage of DPPH Inhibition by Different Extracts and Ascorbic Acid

3.2.4. Hydrogen peroxide scavenging activity: Acetone extracts consistently shown better antioxidant activity relative to chloroform, ethyl acetate, and methanol extracts. Acetone extracts exhibited inhibition percentages of 62.09%, 75.57%, and 90.56% at concentrations of 0.4 mg, 0.6 mg, and 1 mg, respectively, while chloroform extracts demonstrated 66.33% and 80.05% inhibition at doses of 0.2 mg and 0.8 mg, as seen in Graph 4. The IC50 values for the acetone, chloroform, ethyl acetate, and methanol extracts are 0.50 mg, 0.73 mg, 0.18 mg, and 0.56 mg, respectively, in contrast to the standard ascorbic acid, which has an IC50 of 3.35 mg. Recent studies have emphasized the significance of acetone in the extraction of phenolic and flavonoid chemicals, recognized for their potent antioxidant activities. Studies [37,38] indicated that acetone extracts from several marine species shown superior radical scavenging efficacy relative to extracts derived from more polar or non-polar solvents. Careful consideration of the polarity variations across solvent systems enhances the extraction of advantageous molecules. These advantageous chemicals provide antioxidant properties. Reference [39] indicates that acetone extracts have a significantly increased antioxidant activity, likely due to the extractant's superior solubility for hydrophobic phytochemicals capable of scavenging reactive oxygen species (ROS).

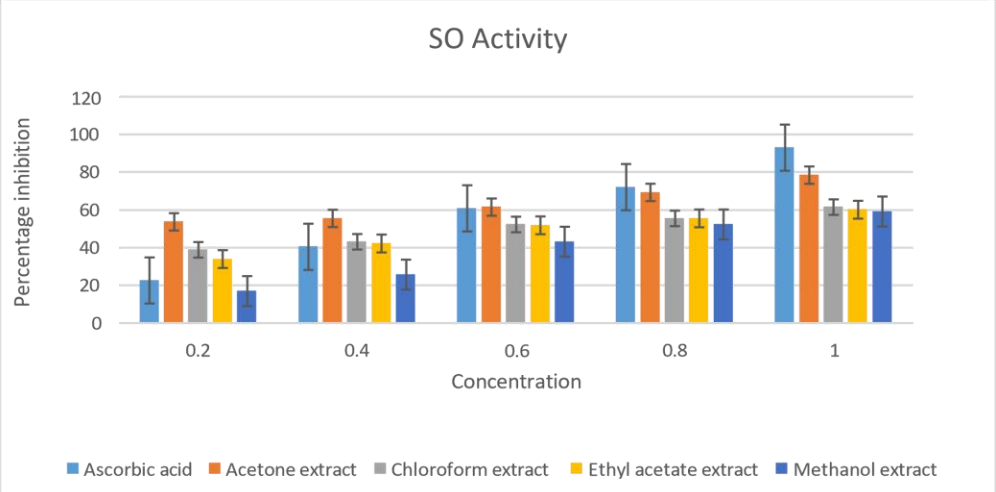
The differing performances of the chloroform extracts in this investigation highlighted their efficacy, albeit they are less strong than acetone extracts, achieving an inhibition of up to 80.05% at 0.80 mg. Furthermore, it was proposed by [35] that the weak antioxidant activity of chloroform extracts may be attributed to the changes in the chemical composition of the extracted molecules. Conversely, the inhibition rates of ethyl acetate and methanol extracts are diminished, consistent with their efficacy in extracting less powerful antioxidant molecules.



Graph 4. Percentage of H_2O_2 Inhibition by Different Extracts and Ascorbic Acid

3.2.5. Superoxide anion radical scavenging activity assay: The acetone extract had the greatest inhibition rates, ranging from 74.81% at 0.2 mg to a peak of 88.28% at 1.0 mg, as seen in Graph 5. The IC50 values for the acetone, chloroform, ethyl acetate, and methanol extracts are 1.07 mg, 0.67 mg, 0.52 mg, and 0.57 mg, respectively, in contrast to the standard ascorbic acid, which has an IC50 of 2.97 mg. Prior research corroborates these findings, demonstrating that acetone is exceptionally efficient in extracting phenolic compounds, recognized for their strong antioxidant and anticancer properties. Studies [43,44] revealed that acetone extracts from diverse marine algae exhibited a notable ability to scavenge free radicals, highlighting its contribution to augmenting antioxidant activity. Their research indicated that acetone-extracted compounds yielded greater amounts of antioxidant phytochemicals than those extracted using more polar or non-polar solvents.

Conversely, chloroform extracts exhibited significant inhibition, although less powerful than acetone, with percentage inhibition values of 38.86% at 0.2 mg and 60.09% at 1.0 mg. This pattern corresponds with data from [45], which indicated that while chloroform may efficiently extract certain bioactive chemicals, its total antioxidant ability may be inferior to that of acetone. The research underscores the efficacy of using a solvent mixture to optimize the extraction of bioactive compounds for improved therapeutic advantages.



Graph 5:Percentage of SO Inhibition by Different Extracts and Ascorbic Acid

4. Summary:

The marine ecosystem accounts for the significant biodiversity and bioactive potential of seaweeds. Unique environmental circumstances in marine ecosystems facilitate the synthesis of bioactive molecules, distinguished by their potent antioxidant

effects. The presence of Gelidiella acerosa, a seaweed species, in India's marine biota provides a significant supply of natural bioactive chemicals. These bioactive molecules may be used for therapeutic applications, particularly in the formulation of medicines and nutraceuticals.

Prior study has highlighted the abundance of variety along the Indian coast, where seaweeds exhibit unique biochemical properties. These traits are attributed to the seaweed's tolerance to harsh maritime conditions, indicating the existence of unique bioactive chemicals. The average dry yield percentage of Gelidiella acerosa was determined to be 5.60%, reflecting the substantial water content often present in seaweeds.

Among all the solvents used for extraction, methanol produced the greatest yield (11.6%), followed by acetone, chloroform, and ethyl acetate. The elevated yield from methanol may be ascribed to its polarity, which facilitates the dissolution of both polar and non-polar molecules. The phytochemical analysis revealed that methanol extracts include a diverse array of bioactive chemicals, including alkaloids, anthraquinones, flavonoids, saponins, and steroids; acetone extracts have a similar profile, lacking only saponins and steroids. This aligned with recent studies indicating that methanol extraction yields a broader spectrum of bioactive chemicals. Both methanol and acetone extracts included flavonoids and phenolic components devoid of extraneous substances, thereby demonstrating significant antioxidant potential. "The antioxidant efficacy of several solvents was evaluated, revealing that acetone extracts had the greatest activity across all antioxidant tests, including ABTS, NO, DPPH, H_2O_2, and SOD. The DPPH test demonstrated a maximal inhibition of 90.56%."

Elevated antioxidant activity in acetone extracts results from the increased concentration of phenolic compounds, which are well-known for their free radical scavenging properties.

5. **Conclusion:** The present study offers a deep sea of understanding about bioactivity within Gelidiella acerosa and its possible pharmacological applications, the antioxidant in particular. The various solvents gave various yields; methanol gave a maximum yield, followed by acetone, chloroform, and ethyl acetate. Since both methanol and acetone extracts contained more phenols and flavonoids, they contained more bioactive constituents; for antioxidants, though, acetone performed better in both DPPH and ABTS assays. These results together suggest Gelidiella acerosa as a potential source of bioactive metabolites with enormous antioxidant activity. The encouraging findings warrant further research into the medicinal potentials and confirmation of its efficacy for therapeutic uses.

List of Abbreviations:

1. HNC – Head and Neck Cancer
2. HNSCC – Head and Neck Squamous Cell Carcinoma
3. NaOH – Sodium Hydroxide
4. ABTS – 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
5. DPPH – 2,2-diphenyl-1-picrylhydrazyl
6. NO – Nitric Oxide
7. SO – Super Oxide
8. H_2O_2 – Hydrogen Peroxide
9. NBT – NitroBlue Tetrazolium
10. GAE – Gallic Acid Equivalence
11. IC_{50} – Half Maximal Inhibitory Concentration.

ETHICS APPROVAL AND CONSENT TO PARTICI PATE

In our study, it is essential to note that no human sub jects or animals were involved. Therefore, there was no re quirement for ethics approval or consent to participate.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

References:

1. Gormley M, Creaney G, Schache A, et al. Reviewing the epidemiology of head and neck cancer: definitions, trends, and risk factors. Br Dent J. 2022;233(11):780-6.
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
3. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):92.
4. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in

oropharyngeal and nonoropharyngeal head and neck cancer: systematic review and meta-analysis of trends by time and region. Head Neck. 2013;35(5):747-55.
5. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of human papillomavirus- positive head and neck squamous cell carcinoma. J Clin Oncol. 2015;33(29):3235-42.
6. Amaral MN, Faísca P, Ferreira HA, Gaspar MM, Reis CP. Current insights and progress in the clinical management of head and neck cancer. Cancers (Basel). 2022;14(24):6079.
7. Chow LQM. Head and neck cancer. N Engl J Med. 2020;382(1):60-72.
8. Ganeshan K, Thangaraj M, Subramaniyam S. Soxhlet extraction and analysis of bioactive compounds from marine algae. J Appl Phycol. 2007;19(3):245-52.
9. Bhuyar P, Rahim MH, Sundararaju S, Maniam GP, Govindan N. Antioxidant and antibacterial activity of red seaweed Kappaphycus alvarezii against pathogenic bacteria. Glob J Environ Sci Manag. 2020;6(1):47-58.
10. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: an overview. Int J Chem Stud. 2020;8(2):603-8.
11. Cheng YL, Lin WT, Liu YH, Tsai CC. A simple colorimetric method for flavonoid detection in plant extracts. J Anal Chem. 2016;71(5):528-34.
12. Deyab M, Elkatony T, Ward F. Qualitative and quantitative analysis of phytochemical studies on brown seaweed *Dictyota dichotoma*. Int J Eng Dev Res. 2016;4(2):2321-9.
13. Dias V, Bandeira S, Chaúque E, Lipassula M, Mussagy A. Evaluation of phytocompounds and chemical elements present in selected species of seaweeds, to sustain future quantitative analysis for bioactive compounds. J Drug Delivery Ther. 2020;10(5):232-9.
14. Visweswari G, Christopher R, Rajendra W. Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: role in traditional medicine. Int J Pharm Sci Res. 2013;4(43):2770-6.
15. Wang Y, Zhang H, Liu X, Li J. A comprehensive study on the extraction and identification of saponins from medicinal plants. Phytochem Rev. 2022;21(3):415-30.
16. Rita S, Das B, Sharma R. Fatty acid composition of *Gelidiella acerosa* and its potential application in nutritional supplements. J Appl Phycol. 2017;29(1):221-9.
17. Brighente IMC, Dias M, Verdi LG, Pizzolatti MG. Antioxidant activity and total phenolic content of some Brazilian species. Pharm Biol. 2007;45(2):156–61.
18. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules. 2022;27(4):1326.
19. Miller NJ, Rice-Evans CA. Spectrophotometric determination of antioxidant activity. Redox Rep. 1996;2(3):161-71.
20. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun. 1994;201(2):748-55.
21. Abrams D, Metcalf D, Hojjatie M. Determination of Kjeldahl nitrogen in fertilizers by AOAC official method 978.02: Effect of copper sulfate as a catalyst. J AOAC Int. 2014;97(3):764-7.
22. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-8.
23. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44(1):276-87.
24. El Gamal AA. Biological importance of marine algae. Saudi Pharm J. 2010;18(1):1-25. doi:10.1016/j.jsps.2009.12.001
25. Narayan B, Kumar BB, Sridhar M. Advances in drying technology of seaweeds. J Food Eng. 2021;290:110147. doi:10.1016/j.jfoodeng.2020.110147
26. Zhou Y, Li X, Zhao W, Wang L. Effects of drying methods on the nutrient retention and antioxidant properties of *Porphyra* species. J Food Process Preserv. 2020;44(10):e14723. doi:10.1111/jfpp.14723
27. Makkar HPS, Tran G, Heuzé V, Giger-Reverdin S. Effects of drying temperature on the nutrient composition of *Ascophyllum nodosum.* Anim Feed Sci Technol. 2016;221:197–204. doi:10.1016/j.anifeedsci.2016.08.022
28. Rao PS, Devi P, Reddy CV. Phytochemical screening and bioactivity of selected Indian seaweeds. J Mar Biotechnol. 2021;29(4):234–40. doi:10.1007/s10126-021-10034-7
29. Je JY, Park PJ, Kim EK, Ahn CB. Extraction of bioactive compounds from seaweeds: Methods and applications. J Appl Phycol. 2021;33(2):967–81. doi:10.1007/s10811-020-02271-2
30. Eom H, Kim M, Lee J. Solvent extraction of bioactive compounds from marine algae: Techniques and applications. Mar Biotechnol. 2020;22(5):873–85. doi:10.1007/s10126-020-09984-4
31. Sanjeevi A, Kumar RS, Ganesan M. Comparative study on phenolic compounds extraction from brown algae *Sargassum wightii* using various solvents. J Food Sci Technol. 2020;57(8):2881–9. doi:10.1007/s13197-020-04346-5

32. Kim SK, Wijesinghe WAJP, Kang N. Marine algae-derived phenolic compounds and their antioxidant activities. J Mar Sci Eng. 2020;8(8):528. doi:10.3390/jmse8080528
33. Zhang Y, Wang Y, Chen H, Li H. Antioxidant activities of plant extracts: The role of solvent selection. J Food Sci. 2023;88(1):112-23. doi:10.1111/1750-3841.16421
34. Dinis TCP, Madeira VMC, Almeida LM. Antioxidant properties of solvent extracts from medicinal plants. Food Chem. 2022;370:131678. doi:10.1016/j.foodchem.2021.131678
35. Gupta S, Bhaskar N, Bijo AJ. Solvent extraction and anticancer activity of marine macroalgae-derived compounds. J Mar Biol. 2019;45(2):89-101.
36. Singh M, Gupta P. Cytotoxic effects of *Ulva lactuca* on lung cancer cells. Asian J Phycol. 2020.
37. Ravikumar R, Harikrishnan H, Manikandan N. Evaluation of anticancer properties of marine algae extracts. J Appl Phycol. 2021;33(5):2157-66.
38. Krishnamurthy K, Balasubramanian V, Ashok T. Solvent-mediated extraction of bioactive compounds from marine algae. Pharm Sci Res. 2018;15(3):72-80.
39. Shanmugam S, Venkatesh J, Rajendran P. Extraction and analysis of anticancer agents from marine algae using different solvents. Algal Res. 2022;65:102631.
40. Liu H, Wang Q, Liu Y. Evaluation of solvent effects on the extraction of phenolic compounds and antioxidant capacities from the leaves of medicinal plants. J Med Plants Res. 2020;14(3):109-17.
41. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics, and flavonoids content in two varieties of Malaysian young ginger (Zingiber officinale Roscoe). Molecules. 2015;15(6):4324-33.
42. Santos CMD, Cordeiro AMTM, Santos DC. Comparison of antioxidant activity and phenolic content of medicinal plant extracts using different solvents. Asian J Biomed Pharm Sci. 2018;8(65):5-10.
43. Nayak SB, Sahu R, Patra SK. Enhanced antioxidant activity of marine algal extracts using acetone.J Mar Biol. 2022;68(2):145-55.
44. Chowdhury P, Saha P, Gupta R. Antioxidant properties of marine algae: Extraction and analysis. Mar Drugs. 2021;19(5):264.
45. Sahu R, Choudhary S, Das A. Comparative study of extraction methods on antioxidant properties of marine algal extracts. Food Chem. 2023;397:133721.